Sensitive Detection of High Explosives Using Electrogenerated Chemiluminescence

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SENSITIVE DETECTION OF HIGH EXPLOSIVES USING ELECTROGENERATED CHEMILUMINESCENCE

by

Suman Parajuli

Abstract of a Dissertation
Submitted to the Graduate School
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ABSTRACT

SENSITIVE DETECTION OF HIGH EXPLOSIVES USING ELECTROGENERATED CHEMILUMINESCENCE

by Suman Parajuli

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The core of this dissertation lies in the search of analytical tools which can be used to detect and quantify the high explosives confiscated from suspects in transportation hubs and from soil and water bodies where these explosives pose a greater threat to public health and safety. High explosives, namely, hexamethylene triperoxide diamine (HMTD), triacetone triperoxide (TATP), trinitrotoluene (TNT), and pentaerythritol tetranitrate (PETN), were detected and quantified by electrochemical methods such as electrogenerated chemiluminescence (ECL) and cyclic voltammetry (CV).

Sensitive detection and quantification of HMTD, one commonly used explosive by terrorists, was presented first in this dissertation on the basis of ECL technology coupled with silver nitrate (AgNO₃) enhancement in acetonitrile (MeCN) at a Pt electrode. Upon the anodic potential scanning, HMTD irreversibly oxidized at ~1.70 V vs Ag/Ag⁺ (10 mM) at a scan rate of 50 mV/s, and the ECL profile was coincident with the oxidation potential of HMTD in the presence of tris(2,2'-bipyridine)ruthenium(II) cation (Ru(bpy)₃²⁺) luminophore species which showed a half-wave potential of 0.96 V vs Ag/Ag⁺. The addition of small amounts of AgNO₃ (0.50 to 7.0 mM) into the HMTD/Ru(bpy)₃²⁺ system resulted in significant enhancement in HMTD ECL production (up to 27 times). This enhancement was found to be largely associated with
NO$_3^-$ and was linearly proportional to the concentrations of NO$_3^-$ and Ag$^+$ in solution. Homogeneous chemical oxidations of HMTD by electrogenerated NO$_3^\bullet$ and Ag(II) species proximity to the electrode were proposed to be responsible for the ECL enhancement. On the basis of experimentally obtained CV data and theoretical CV digital simulations, standard potential values of 1.79 V vs Ag/Ag$^+$ (or 1.98 V vs NHE) and 1.82 V vs Ag/Ag$^+$ (or 2.01 V vs NHE) were estimated for Ag(II)/Ag(I) and NO$_3^\bullet$/NO$_3^-$ couples, respectively. A limit of detection of 50 μM of HMTD was achieved with the current technique, which was 10 times lower than that reported previously based on a liquid chromatography separation (HPLC) and Fourier transform infrared (FT-IR) detection method.

Detection of TATP and the differentiation of TATP from HMTD were accomplished subsequently with ECL at glassy carbon electrode in water-MeCN mixture solvents. In the presence of Ru(bpy)$_3^{2+}$, TATP or hydrogen peroxide (H$_2$O$_2$) derived from TATP via UV irradiation or acid treatment produced ECL emissions upon cathodic potential scanning. Interference of H$_2$O$_2$ on TATP detection was eliminated by pre-treatment of the analyte with catalase enzyme. Selective detection of TATP from HMTD was realized by scanning the electrode potential positively as well as negatively; HMTD showed ECL emissions at both directions. The hydroxyl radical formed after the electrochemical reduction of TATP was believed to be the key intermediate for ECL production, and its stability was strongly dependent on the solution composition, which was verified with electron paramagnetic resonance spectroscopy. A detection limit of 2.5 μM TATP was obtained from direct electrochemical reduction of the explosive or H$_2$O$_2$ derived from
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ECL quenching method can also be used to detect explosive compounds where the explosive of interest can quench ECL response either by excited state quenching or quenching by depletion of the precursors of the excited state species in the test solution. In this investigation, ECL quenching behavior of the Ru(bpy)$_3^{2+}$/ tri-$n$-propylamine (TPrA) system with TNT at a Pt electrode in MeCN was explored. Effective ECL quenching of the system upon the addition of TNT was observed, with a Stern-Volmer constant of $2 \times 10^4$ M$^{-1}$. The apparent ECL quenching constant calculated from the Stern-Volmer plot was found to be $3.5 \times 10^{10}$ M$^{-1}$ s$^{-1}$, which suggests the efficient quenching of ECL by TNT. The consumption of the TPrA$'$ free radicals and Ru(bpy)$_3^{+}$ species (produced as a result of reduction of Ru(bpy)$_3^{2+}$ by TPrA$'$) by TNT could be the main reason of this quenching, as both TPrA$'$ and Ru(bpy)$_3^{+}$ species are precursors of the excited state Ru(bpy)$_3^{2+}$ species. The present technique can sensitively detect TNT as low as 4.4 µM.

Electrochemical detection of PETN was studied in MeCN with a Ag wire as the working electrode, where an irreversible reduction wave at -0.9 V vs Ag/Ag$^+$ was observed. The reduction of PETN probably involved the formation of alcohol and nitrite ion with the trace amount of water present in the solvent. The limit of detection of PETN by simple electrochemical CV method was 25 µM.
The University of Southern Mississippi

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A Dissertation
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CHAPTER I
INTRODUCTION

The main inspiration of this dissertation is to find the way for the detection and quantification of explosive materials using electrochemical methods such as electrogenerated chemiluminescence (ECL) and cyclic voltammetry (CV). This study involves the use of peroxide-based explosives such as hexamethylene triperoxide diamine (HMTD) and triacetone triperoxide (TATP) as ECL coreactant since they contain either amine and/or peroxide functional groups with tris(2,2'-bipyridine)ruthenium(II) cation (Ru(bpy)$_3^{2+}$) as ECL luminophore. The ECL quenching for the well-known Ru(bpy)$_3^{2+}$/TPrA system has also been utilized in the study for trinitrotoluene (TNT) detection.

This chapter is divided into two sections: the first section is a general introduction to commonly used electrochemical and ECL technologies, and the second section is a brief literature review of trace detection of high explosives with, e.g., nanomaterials.

Electroanalytical Chemistry

Electroanalytical chemistry is a branch of chemical analysis which employs electrochemical methods in order to gain information related to quantity and properties of analyte of interest. These methods deal with the electrochemical process incorporating phenomena involving charge transfers (such as redox processes, ion separations, etc.) and the electrical phenomena for determination of analyte.$^{1-5}$ Electroanalytical chemistry, which is different from physical electrochemistry with primary focus in theory of electrode processes and their applications, is generally classified as a subdivision of analytical chemistry. Electroanalytical chemistry does not
depend on the properties of the electrode and the solvent, and is focused on the properties of the analyte of interest. Electroanalytical chemistry can be subdivided into techniques based on the type of measurements carried out, normally with a three-electrode system (working, reference, and counter electrodes) in an electrochemical cell, as described below.

Electroanalytical Techniques

Electrochemical techniques are associated with the interactions of electricity and chemical species from the test solutions, especially the measurements of electrical quantities dealing with the potential, current, or charge, and their relations to chemical parameters. There is a wide range of applications of analytical techniques because of the advances made in this field. These techniques are very sensitive and can detect analytes at their micro molar concentration levels. They are popular among scientific communities because of the use of inexpensive instruments for analysis and providing important information about the quantity and redox behavior of the analyte in a short time.

Cyclic voltammetry (CV). Cyclic voltammetry (CV) is a versatile electroanalytical technique which can be used to acquire information of the basic electrochemical reaction. The CV is a useful technique in understanding redox behavior of chemical processes occurred mainly at the electrode surface for various types of electroactive species including organometallic complexes and conductive polymers. CV is also a simple but powerful technique which can be employed to obtain qualitative as well as quantitative information of the redox process under study. In a typical CV experiment, the potential is applied to the electrode kept in an unstirred solution of an analyte with the supporting electrolyte against the reference electrode such as silver/silver chloride (Ag/
AgCl/Cl\textsuperscript{−}), silver/silver ion (Ag/Ag\textsuperscript{+}), or saturated calomel electrode (SCE). As shown in Figure 1.1, the potential is scanned from an initial value ($E_{\text{initial}}$) to the final value ($E_{\text{final}}$) (“forward scan”) and back to the initial potential (“reverse scan”) at a constant scan rate, completing a cycle. These scans can be repeated so as to obtain successive second, third, cycles. The cyclic voltammogram (CV) can be obtained with the current response in Y-axis and the potential in X-axis as shown in Figure 1.2.

The typical cyclic voltammogram shown in Figure 1.2 was obtained from a solution of 6.0 mM K\textsubscript{3}Fe(CN)\textsubscript{6} in water with 1.0 M KNO\textsubscript{3} as a supporting electrolyte. The working, reference and counter electrodes used to obtain this CV were a Pt disk, a SCE, and a Pt wire, respectively. As shown in Figure 1.1, the potential was scanned from 0.8 V (as initial potential) in the negative direction to -0.15 V linearly and scanned back to 0.8 V, completing a cycle. At potential 0.8 V indicated by A, there is no current produced from the electroactive species, K\textsubscript{3}Fe(CN)\textsubscript{6}, indicating that no redox behavior from the analyte was observed at this potential. As the potential is scanned in cathodic direction, there is no reduction current produced from the analyte until the potential reaches to 0.35 V as shown in Figure 1.2 and marked by B. There is no redox reaction taking place from 0.8 V to 0.35 V. The reduction of K\textsubscript{3}Fe(CN)\textsubscript{6} starts at 0.35 V and the cathodic current increases rapidly (B to D) and reaches a maximum at D (0.19 V) where the surface concentration of Fe\textsuperscript{III}(CN)\textsubscript{6}\textsuperscript{3−} approaches zero and then the current decreases on further scanning to -0.15 V, meaning that the reducible species (Fe\textsuperscript{III}(CN)\textsubscript{6}\textsuperscript{3−}) is depleted. This process of reduction of analyte is represented by Eq. 1.1:

\[
[\text{Fe}^{\text{III}}(\text{CN})_6]^{3−} + e = [\text{Fe}^{\text{II}}(\text{CN})_6]^{4−}
\]  \hspace{1cm} 1.1

As the potential is switched to positive direction, i.e., anodic scanning, the cathodic
Figure 1.1. Cyclic voltammetric excitation signal.

Figure 1.2. Cyclic voltammogram (CV) obtained from a solution containing 6.0 mM K$_3$Fe(CN)$_6$ and 1.0 M KNO$_3$ at a 2.54 mm-diameter Pt working electrode at a scan rate of 50 mV/s. 19
current still persists because the electrode surface is still negative enough to reduce $\text{Fe}^{III}(\text{CN})_{6}^{3+}$. When the electrode surface becomes positive, oxidation of $\text{Fe}^{II}(\text{CN})_{6}^{4-}$ starts forming the original species ($\text{Fe}^{III}(\text{CN})_{6}^{3-}$). The anodic current increases as we scan further from 0.1 V rapidly for the oxidation of $\text{Fe}^{II}(\text{CN})_{6}^{4-}$ back to $\text{Fe}^{III}(\text{CN})_{6}^{3-}$ as indicated by H to J in the above voltammogram (Figure 1.2). So the reduced species gets oxidized back as:

$$[\text{Fe}^{III}(\text{CN})_{6}]^{4-} + e = [\text{Fe}^{II}(\text{CN})_{6}]^{3-} \quad 1.2$$

The anodic current reaches maximum at ~0.2 V and decreases for further scanning as the electroactive species for oxidation, $\text{Fe}^{II}(\text{CN})_{6}^{4-}$, decreases. Once the potential reaches at 0.8 V, the original condition is restored and can be further continued for successive cycles.

In the above CV, cathodic peak potential, cathodic peak current, anodic peak potential, and anodic peak current are represented by $E_{pc}$, $i_{pc}$, $E_{pa}$, and $i_{pa}$, respectively.

The detailed understanding of the changes of concentration of analyte which undergoes reversible changes (analyte reduced and the reduced species oxidized back to the original form) adjacent to the surface of electrode can be obtained by considering the Nernst equation:

$$E = E^{\circ}_{\text{Fe}^{II}(\text{CN})_{6}^{4-}/\text{Fe}^{III}(\text{CN})_{6}^{3-}} + \frac{0.059}{1} \log \left[ \frac{\text{Fe}^{III}(\text{CN})_{6}^{3-}}{\text{Fe}^{II}(\text{CN})_{6}^{4-}} \right] \quad \text{(at 25 °C)} \quad 1.3$$

where $E$ and $E^{\circ}$ are the measured and formal potentials respectively. At the beginning, the potential ($E_{\text{initial}}$) is much more positive than the formal potential since the concentration of $\text{Fe}^{III}(\text{CN})_{6}^{3-}$ is in majority. As we scan to the negative potential, $\text{Fe}^{III}(\text{CN})_{6}^{3-}$ is reduced to $\text{Fe}^{II}(\text{CN})_{6}^{4-}$ decreasing its concentration linearly. At equilibrium,
the ratio of \([\text{[Fe}^{III}(\text{CN})_6^{3-}]/\text{Fe}^{II}(\text{CN})_6^{4-}]\) becomes unity and the measured potential, \(E\), becomes equal to the formal potential, \(E^{\text{o'}}\).

For a reversible system, the formal potential \((E^{\text{0'}})\) lies midway from the cathodic peak potential \((E_{pc})\) and the anodic peak potential \((E_{pa})\), which can be expressed as:

\[
E^{\text{0'}} = \frac{E_{pa} + E_{pc}}{2}
\]

The separation of peak potentials (cathodic and anodic) is given by:

\[
\Delta E_p = E_{pa} - E_{pc} = \frac{0.057}{n} \text{ (V at 25 °C)}
\]

where \(n\) is the number of electron transfer involved in the redox process. For reduction of \(\text{Fe}^{III}(\text{CN})_6^{3-}\) to \(\text{Fe}^{II}(\text{CN})_6^{4-}\), this number \((n)\) is 1, thus \(\Delta E_p = 57 \text{ mV}\) is the expected value.

The Randles-Sevic equation for the reversible system, the peak current \((i_p)\) is given\(^3\)\(^,\)\(^{20}\) by

\[
i_p = (2.69 \times 10^5) n^{3/2}AD^{1/2}C^{1/2}\nu^{1/2}
\]

where \(n\) is the number of electrons transfer involved, \(A\) is the surface area of electrode \((\text{cm}^2)\), \(C\) is the concentration of the electroactive species \((\text{mol/cm}^3)\), \(D\) is the coefficient of diffusion \((\text{cm}^2/s)\), and \(\nu\) is the scan rate \((\text{V/s})\). Therefore, magnitude of peak current is proportional to the concentration of analyte and the square root of scan rate.

**Electrogenerated Chemiluminescence**

Electrogenerated chemiluminescence (also called as electrochemiluminescence and abbreviated as ECL) is the process where electrochemically generated species at the surface of electrode undergo electron transfer reactions to form the excited state that emits light.\(^{21-23}\) Bard and Hercules described the detailed studies in the middle of 1960s
Although emission of light during electrolysis were reported as early as 1920s. Currently, ECL has become a very powerful technique in analytical chemistry and has gained wide range of uses in the areas such as food and water testing, immunoassay based detections and biowarfare detection. ECL has also been exploited successfully as detector in other analytical techniques such as flow injection analysis (FIA), high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and micro total analysis (µTAS). A large number of reviews on ECL are available on different topics of ECL.

In a typical ECL system, the test solution contains species A and B (A and B could be the same species) with supporting electrolyte such as phosphate buffer in aqueous solution or tetra-n-butylammonium perchlorate (TBAP) in organic solution and three electrodes, namely, working, reference and counter electrodes.

There are different processes other than ECL which produce light, such as photoluminescence (PL) and chemiluminescence (CL). Both ECL and CL are forms of chemiluminescence where light is produced from the species which undergo highly energetic electron-transfer reactions. However, in CL, light is produced by mixing necessary reagents and careful manipulation of flow rate whereas in ECL the electroactive species are generated at the electrode surface by applying potential to the electrode.

ECL has a number of advantages over CL. In ECL, the electrochemical reaction allows a time and position for the light emitting reaction to be controlled which help in aligning the position of electrode to the detector thus increase sensitivity. ECL is more selective than CL since the generation of excited states can be selectively controlled by
varying the electrode potential. Usually, ECL is a nondestructive technique, because the ECL emitters can be regenerated after the experiment.

ECL also has many advantages over other light emitting techniques such as fluorescence.\textsuperscript{29,87} Compared with fluorescence methods, ECL does not involve a light source, so there is no problem of scattered light and luminescent impurities. Besides, ECL specificity is associated with the reaction of ECL label and the coreactant which reduces problem that may arise due to side reactions such as self quenching. Since ECL is a method of producing light at an electrode, it is also perceived as a bridge between electrochemical and spectroscopic methods.

ECL can be divided into ion annihilation ECL and coreactant ECL.

\textit{Ion Annihilation ECL}

The reactant R can be oxidized as well as reduced to form sufficiently stable radical cation (R\textsuperscript{+}) and anion (R\textsuperscript{-}) in annihilation ECL as shown in Scheme 1.1 below:

\begin{align*}
  &R - e \rightarrow R^+ \quad \text{(oxidation at electrode)} \quad 1.7 \\
  &R + e \rightarrow R^- \quad \text{(reduction at electrode)} \quad 1.8 \\
  &R^+ + R^- \rightarrow R + R^* \quad \text{(excited state formation)} \quad 1.9 \\
  &R^* \rightarrow R + h\nu \quad \text{(light emission)} \quad 1.10
\end{align*}

\textit{Scheme 1.1. Ion Annihilation ECL}

These radical ions then undergo annihilation process forming excited state (R\textsuperscript{*}) which emits light. Depending on the availability of energy in an annihilation in Eq. 1.9, the excited state species (R\textsuperscript{*}) could be either the lowest excited singlet state (\textsuperscript{1}R\textsuperscript{*}) or the triplet state (\textsuperscript{3}R\textsuperscript{*}). The energy available in Eq. 1.9 can be calculated from the redox potential for oxidation and reduction processes in Eqs. 1.7 and 1.8 and is given by:
\[-\Delta H_{\text{ann}} = E_p (R/R^{*+}) - E_p (R/R^{-}) - 0.16 \quad 1.11\]

where \(-\Delta H_{\text{ann}}\) (in eV) is the enthalpy for ion annihilation, \(E_p\) is the peak potential for electrochemical oxidation or reduction (in volts) for the formation of cation and anion radicals, and the numerical value (0.16) is the entropy approximation term (T\(\Delta S\)).\(^6\) If the energy \((-\Delta H_{\text{ann}})\) obtained from Eq. 1.11 is larger than the energy \(E_s\) required for the formation of the lowest excited singlet state \((^1R^*)\) from Eq.1.9, this system then is called energy-sufficient system, and the reaction is said to follow the S-route. A typical example of S-route is the DPA\(^{++}/\)DPA\(^{-}\) (DPA = 9,10-diphenylanthracene) system.\(^88,89\)

S-route

\[R^{*+} + R^{-} \rightarrow R + ^1R^* \quad \text{(excited singlet formation)} \quad 1.9a\]

In contrast, if the energy \((-\Delta H_{\text{ann}})\) is smaller than \(E_s\) but larger than the triplet state energy \((E_t)\), then \(^3R^*\) is initially formed which eventually produce \(^1R^*\) by triplet-triplet annihilation (TTA). This can be represented by Eqs. 1.9b and 1.12. This is called the energy-deficient system and the reaction is said to follow the T-route. A typical example of the T-route system is the TMPD\(^{++}/\)DPA\(^{-}\) (TMPD = \(N,N,N',N'^{-}\)-tetramethyl-p-phenylenediamine) system.\(^89,90\)

T-route

\[R^{*+} + R^{-} \rightarrow R + ^3R^* \quad 1.9b\]

\[^3R^* + ^3R^* \rightarrow R + ^1R^* \quad 1.12\]

The efficiency of direct emission of light from triplet form \((^3R^*)\) in solution phase is believed to be low due to the relatively long radiative life time of \(^3R^*\) as compared to \((^1R^*)\) and it is quenched by radical ions or other species such as molecular oxygen.
If neither of the above two cases persists, then the third route (ST-route) takes its course. If $-\Delta H_{\text{ann}}$ is nearly marginal to $E_s$, the T-route can contribute to the formation of $^1R^*$ in addition to the S-route, so the system is called as ST-route. A typical example of ST-route is rubrene cation-anion annihilation. In order for annihilation ECL to be produced, the large potential window of an electrochemical system must be used (~3.3 V to 2 V) so that sufficiently stable radical anions and cations can be produced which eventually produce ECL light. In non-aqueous media, an ion annihilation ECL is widely studied in MeCN with TBAP supporting electrolyte.

For efficient generation of ion annihilation ECL, certain conditions must be fulfilled which include:

(a) stable radical ions of the precursor molecules in the electrolyte of interest, which can be evaluated via cyclic voltammetric (CV) response;

(b) good photoluminescence efficiency of a product of the electron transfer reaction, which can be evaluated by fluorescent experiment; and

(c) sufficient energy in the electron transfer reaction to produce the excited state.

Coreactant ECL

In a coreactant ECL, the potential at the electrode is scanned in one direction where ECL luminophore can be oxidized or reduced in the presence of supporting electrolyte and at the same time coreactant species too will be oxidized or reduced depending upon the direction of scanning, producing cation or anion radicals which produces very strong reducing or oxidizing species to react with oxidized or reduced form of luminophore. This produces the excited state of luminophore which emits light. Highly reducing intermediates are generated from the electrochemical oxidation of co
reactant and highly oxidizing species are generated from the electrochemical reduction. The ECL produced from oxidation is referred to as “oxidative-reduction” ECL and that from the reduction is called “reductive-oxidation” ECL, respectively. The coreactant ECL is useful, especially when one of the cation or anion radicals is not stable enough for ECL reaction or when these radicals cannot be formed because of the narrow potential window of the solvent.

**Typical coreactant ECL systems and their mechanisms.** There are wide ranges of compounds which can produce ECL light, but majority of coreactant ECL are based on Ru(bpy)$_3^{2+}$ or its derivatives as the ECL emitters since they have excellent chemical, electrochemical and photochemical properties both in aqueous and non-aqueous medium. For the oxidative-reduction type coreactant ECL, the system often works well even in the presence of oxygen avoiding the need to purge the test solution before use.

(a) Ru(bpy)$_3^{2+}$/Tri-n-propylamine (TPrA) system. The most of the ECL applications reported so far involve Ru(bpy)$_3^{2+}$ or its derivatives as an emitter and TPrA as a coreactant, though there are other coreactans for ECL studies such as oxalate$^{97}$, persulfate$^{96}$, and hydrogen peroxide.$^{98}$ The Ru(bpy)$_3^{2+}$/TPrA system is an “oxidative-reduction” type of coreactant ECL. This system has shown the highest ECL efficiency known so far.$^{87,99}$ The ECL mechanism of Ru(bpy)$_3^{2+}$/TPrA system is complex and many workers have investigated it.$^{100-106}$ The system has been extensively used in commercial ECL systems for DNA analysis and immunoassays.$^{29}$ The ECL intensity of the Ru(bpy)$_3^{2+}$/TPrA system depends on several factors such as the concentration of both Ru(bpy)$_3^{2+}$ and TPrA, electrode materials, and the solution pH. The reason behind this pH dependence is not fully understood but it could be due to the formation and stability
of TPrA free radical or the solubility of TPrA in buffered solution. At glassy carbon (GC) and gold (Au) working electrode, this system shows two ECL waves when the Ru(bpy)$_3^{2+}$ concentration is kept low (in the order of nano molar to micro molar concentration, Figure 1.3).

![Figure 1.3](image)

*Figure 1.3.* (a) ECL and (b) CV of 1.0 nM Ru(bpy)$_3^{2+}$ in the presence of 0.10 M TPrA with 0.10 M tris/0.10 M LiClO$_4$ buffer (pH = 8) at a glassy carbon electrode (3 mm diameter) at a scan rate of 50 mV/s. (c) ECL with 1.0 μM Ru(bpy)$_3^{2+}$ and 0.10 M TPrA. The ECL intensity scale is given for (c) and should be multiplied by 100 for (a).$^{100}$

With the increase of Ru(bpy)$_3^{2+}$ concentration, the second ECL wave increases and the first wave become less prominent compared to the second wave because this wave is merged into the foot of the second wave as shown in Figure 1.4.

The first ECL wave arises due to the reduction of Ru(bpy)$_3^{2+}$ by electrochemically generated TPrA free radical (TPrA$^\cdot$) to Ru(bpy)$_3$ which is oxidized by the relatively long lived TPrA radical cation (TPrA$^{•+}$) to excited state Ru(bpy)$_3^{2+*}$. Here, Ru(bpy)$_3^{2+}$ does not participate in direct electrochemical oxidation. Hence, the first ECL wave can be
observed when potential is scanned from 0 to 0.9 V vs Ag/AgCl (Note: Ru(bpy)$_3^{2+}$ is oxidized at 1.1 V).

**Figure 1.4.** First and second ECL waves in 0.10 M TPrA (0.20 M PBS, pH 8.5) with different concentration of Ru(bpy)$_3^{2+}$: 1 mM (solid line), 0.50 mM (dashed line), 0.10 mM (dotted line) and 0.05 mM (dash-dotted line), at a 3 mm diameter glassy carbon electrode, scan rate of 100 mV/s.$^{100}$

[where TPrA$^{*+}$ = (CH$_3$CH$_2$CH$_2$)$_3$N$^{*+}$, TPrAH$^+$ = Pr$_3$NH$^+$, TPrA$^*$ = Pr$_2$NC$^*$HCH$_2$CH$_3$, P1 Pr$_2$N$^+$C=HCH$_2$CH$_3$]

**Scheme 1.2.** Mechanism for the first ECL wave of the Ru(bpy)$_3^{2+}$/TPrA system.$^{100}$
Scheme 1.3. Mechanism for the second ECL wave of the Ru(bpy)$_3^{2+}$/TPrA system.$^{100}$

The second ECL wave arises due to the direct oxidation of Ru(bpy)$_3^{2+}$ at the electrode surface producing Ru(bpy)$_3^{3+}$, and at the same time TPrA also gets oxidized to form TPrA$^{+}$. This radical cation readily deprotonates forming TPrA free radical (TPrA$^{•}$) which is a strong reducing agent and reduces Ru(bpy)$_3^{3+}$ to Ru(bpy)$_3^{2+\ast}$ excited state. This excited state emits light and comes back to the original state and further continues the process (Scheme 1.3).

Scheme 1.4. Mechanism for the second ECL wave of the Ru(bpy)$_3^{2+}$/TPrA system.$^{100}$

The second ECL wave can also be produced by the chemical reduction of Ru(bpy)$_3^{2+}$ by TPrA$^{•}$ to Ru(bpy)$_3^{+}$ and electrochemical oxidation of Ru(bpy)$_3^{2+}$ to
Ru(bpy)$_3$$^{3+}$. These oxidized and reduced species undergo chemical reaction to form excited state that emits light (Scheme 1.4).

(b) $\text{Ru(bpy)}_3$$^{2+}$/Peroxydisulphate (persulfate, $S_2O_8^{2-}$) system. This system was the first example of “reductive-oxidation” coreactant ECL reported in the literature.$^{96,107}$ Because of the solubility issue of the coreactant, $(\text{NH}_4)_2S_2O_8$, as it has a low solubility in MeCN and Ru(bpy)$_3$$^{3+}$ is unstable in aqueous medium so the mixed solvent (water and MeCN) was used to get intense ECL response. The potential was scanned in the negative direction where ECL luminophore as well as coreactant can be reduced electrochemically at the electrode. As shown in Scheme 1.5, persulfate ($S_2O_8^{2-}$) gets electrochemically reduced to radical anion ($S_2O_8^{*3-}$) and at same time Ru(bpy)$_3$$^{2+}$ also gets reduced to Ru(bpy)$_3$$^{3+}$. The $S_2O_8^{2-}$ can also oxidize the reduced Ru(bpy)$_3$$^{3+}$ back to the original state (Ru(bpy)$_3$$^{2+}$) whereby persulfate is chemically converted to radical anion ($S_2O_8^{*3-}$). The strong oxidizing radical ($SO_4^{*+}$) is formed from the decomposition of persulfate radical anion ($S_2O_8^{*3-}$) along with $SO_4^{2-}$. This sulfate radical either oxidizes Ru(bpy)$_3$$^{3+}$ to its excited state (Ru(bpy)$_3$$^{2+*}$) or oxidizes Ru(bpy)$_3$$^{2+}$ to Ru(bpy)$_3$$^{3+}$ which undergoes a chemical reaction with electrochemically generated Ru(bpy)$_3$$^{3+}$ to form the excited state that emits light (Scheme 1.5).

$$S_2O_8^{2-} + e \rightarrow S_2O_8^{*3-}$$ \hspace{1cm} 1.13

$$\text{Ru(bpy)}_3$$^{2+} + e \rightarrow \text{Ru(bpy)}_3$$^{3+}$$ \hspace{1cm} 1.14

$$\text{Ru(bpy)}_3$$^{3+} + S_2O_8^{2-} \rightarrow \text{Ru(bpy)}_3$$^{2+} + S_2O_8^{*3-}$$ \hspace{1cm} 1.15

$$S_2O_8^{*3-} \rightarrow SO_4^{2-} + SO_4^{*+}$$ \hspace{1cm} 1.16

$$\text{Ru(bpy)}_3$$^{3+} + SO_4^{*+} \rightarrow \text{Ru(bpy)}_3$$^{2+*} + SO_4^{2-}$$ \hspace{1cm} 1.17
\[
\text{Scheme 1.5. ECL mechanism for Ru(bpy)_3^{2+} / S_2O_8^{2-} system.}^{94}
\]

The next section provides a brief review of trace detection of high explosives.*

Detection and quantification of high explosives and related compounds have attracted much attention in recent years, due to the pressing needs associated with global security and growing environmental and health-related concerns.\textsuperscript{108-112} Such detection is necessary in a variety of complex environments, including mine fields, munitions storage facilities, ground and seawater samples, transportation areas, and blast sites. In each of these settings, sensitive and timely detection of explosive materials is required to ensure the safety and security of the surrounding area.

Explosive detection techniques can be broadly classified into two categories: bulk detection and trace detection.\textsuperscript{111,112} In bulk detection, a macroscopic mass of the explosive material is detected directly, usually by viewing images made by X-ray scanners or similar equipment such as millimeter-wave and tetrahertz imaging\textsuperscript{109} (Figure 1.5). Other recently developed bulk detection techniques include neutron techniques, nuclear quadrupole resonance (NQR), and laser techniques. In trace detection, the explosive is detected by chemical identification of microscopic residues of explosive

compound as the form of vapor or particulate. Table 1.1 summarizes commonly used trace detection methods of explosives and their features.

![Figure 1.5. X-ray imaging (B) reveals the explosives planted in a doll (A). Airport baggage scanners are programmed to display in red those materials with densities that match explosives.](image)

Additionally, integrated (fused) systems can be used, where two or more detection methods from the same or different types (e.g., two different bulk detection technologies or a bulk detection plus a trace detection technique) are combined. The integration can be carried out by using simultaneous detection or by a two-step detection method, for example, simultaneous operation of NQR and X-ray imaging or two-step operation of X-ray computed tomography (CT) and IMS. The strength of one technique may thus compensate for the weakness of the other, or the vulnerability of one detection device to a potential countermeasure could be compensated for by another detection device.

Chemical explosives commonly used by military and terrorists can be categorized into three groups (Table 1.2). The first group contains nitrated explosives that are generally used by military. On the basis of chemical structural and functional group properties, this group can be divided into three subgroups: (a) nitroaromatics (NACs), (b) nitroamines, and (c) nitrate esters. The second group of high explosives is peroxide based, which
includes hexamethylene triperoxide diamine (HMTD) and triacetone triperoxide (TATP).

HMTD and TATP have become popular with terrorists because they are easily prepared from readily obtainable ingredients, although the synthesis is fraught with danger. For example, TATP was used in the terrorist bombings on the London subway system in 2005 and by the infamous shoe bomber who tried to detonate his shoes on a trans-Atlantic flight in 2001. As HMTD and TATP contain three peroxide linkages per molecule, their explosive output is much higher than most organic peroxides. HMTD is estimated as 60% and TATP as 88% of TNT blast strength. Plastic explosives form the

Table 1.1 Commonly Used Trace Detection Methods of High Explosives and Their Features

<table>
<thead>
<tr>
<th>Method</th>
<th>Feature</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass spectrometry (MS) coupled with gas or high performance liquid chromatography (GC/MS, HPLC/MS)</td>
<td>Sensitive but expensive, not portable</td>
<td>108,113,115-126</td>
</tr>
<tr>
<td>Ion mobility spectrometry (IMS)</td>
<td>Sensitive but with matrix effect</td>
<td>127-133</td>
</tr>
<tr>
<td>Micro-mechanical sensors (e.g., micro-cantilevers)</td>
<td>Sensitive, low power consumption and real-time operation, but mainly for vapors, with moisture effect, less selective</td>
<td>134-138</td>
</tr>
<tr>
<td>Electrochemical methods</td>
<td>Inexpensive, fast, portable, less sensitive</td>
<td>139-144</td>
</tr>
<tr>
<td>Chemiluminescence (CL)</td>
<td>High sensitivity but poor selectivity</td>
<td>145-150</td>
</tr>
<tr>
<td>Colorimetric tests</td>
<td>Simple, inexpensive, fast but less sensitive and low specificity</td>
<td>151,152</td>
</tr>
<tr>
<td>Fluorescence (FL) spectroscopy</td>
<td>Sensitive with interferences effect</td>
<td>153-160</td>
</tr>
<tr>
<td>Surface plasmon resonance</td>
<td>Label-free, sensitive but subject to external contamination</td>
<td>161-165</td>
</tr>
<tr>
<td>Surface enhanced Raman scattering spectroscopy (SERS)</td>
<td>Sensitive, but complicated technique and difficult to operate</td>
<td>166-171</td>
</tr>
</tbody>
</table>
third group of explosives, in which one or more of the first group explosives are plasticized to make a mouldable material, such as C-4 and Semtex H. In order to retain the best explosive output, the inert plasticizers are usually added less than 10-15% of the overall weight. Plastic explosives were originally developed for convenient use in military demolitions but have since been widely used in terrorist bombs. Figure 1.6 shows the structures and abbreviations of explosives listed in Table 1.2.

**Table 1.2** Commonly Used High Explosives and Their Chemical Properties

<table>
<thead>
<tr>
<th>Explosive</th>
<th>Name/contents</th>
<th>Formula</th>
<th>d g/cm³</th>
<th>N %</th>
<th>O %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Nitrated explosives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Nitroaromatics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNT</td>
<td>2,4,6-Trinitrotoluene</td>
<td>C₇H₅N₃O₆</td>
<td>1.65</td>
<td>18.5</td>
<td>42.3</td>
</tr>
<tr>
<td>Picric acid</td>
<td>2,4,6-trinitro-1-phenol</td>
<td>C₆H₃N₃O₇</td>
<td>1.77</td>
<td>18.3</td>
<td>48.9</td>
</tr>
<tr>
<td>Tetryl</td>
<td>N-methyl-N,2,4,6-tetranitroaniline</td>
<td>C₇H₅N₅O₈</td>
<td>1.73</td>
<td>24.4</td>
<td>44.6</td>
</tr>
<tr>
<td>(b) Nitramines</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RDX</td>
<td>1,3,5-Trinitro-1,3,5-triazacyclohexane</td>
<td>C₃H₆N₆O₆</td>
<td>1.82</td>
<td>37.8</td>
<td>43.2</td>
</tr>
<tr>
<td>HMX</td>
<td>1,3,5,7-tetranitro-1,3,5,7-tetrazocane</td>
<td>C₄H₈N₈O₈</td>
<td>1.96</td>
<td>37.8</td>
<td>43.2</td>
</tr>
<tr>
<td>CL 20</td>
<td>hexanitrohexaazaisowurtzitane</td>
<td>C₆H₆N₁₂O₁₂</td>
<td>38.4</td>
<td>43.8</td>
<td></td>
</tr>
<tr>
<td>(c) Nitrate esters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PETN</td>
<td>Pentaerythritol tetranitrate</td>
<td>C₅H₈N₄O₁₂</td>
<td>1.76</td>
<td>17.7</td>
<td>60.7</td>
</tr>
<tr>
<td>Nitrocellulose</td>
<td>Cellulose nitrate</td>
<td>C₆H₇N₃O₁₁</td>
<td>1.2</td>
<td>14.1</td>
<td>59.2</td>
</tr>
<tr>
<td>(B) Peroxide-based explosives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HMTD</td>
<td>hexamethylene triperoxide diamine</td>
<td>C₆H₁₂N₂O₆</td>
<td>1.6</td>
<td>13.5</td>
<td>46.1</td>
</tr>
<tr>
<td>TATP</td>
<td>Triacetone triperoxide</td>
<td>C₉H₁₆O₆</td>
<td>1.2</td>
<td>0</td>
<td>43.2</td>
</tr>
<tr>
<td>(C) Plastic explosives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-4</td>
<td>RDX + plasticizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semtex H</td>
<td>RDX + PETN + plasticizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detasheet</td>
<td>PETN + plasticizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.6. Structures and abbreviations of commonly used high explosives. (a-c) Nitroaromatics, (d-f) Nitramines, (g-h) Nitrate esters, and (i-j) Peroxide-based explosive compounds.

Compared with other organic compounds, explosives show exceptionally high density (e.g., military explosives generally have a density greater than 1.6 g/cm³, Table 1.2), which indicates the high expulsive forces between atoms, leading to powerful explosion when explosives blow up. All explosives have very high oxygen and/or nitrogen contents, so that dramatically volume changes (from solid to gas) are expected when an explosion occurs. Many bulk detection methods are in fact based on the “bulk property” of high density and high oxygen and nitrogen contents of explosives.
Although a wide variety of explosive detection technologies are currently available, this section will mainly focus on trace detection methods involving nanomaterials. Such methods generally possess many advantages over traditional ones, which include high sensitivity, good selectivity, fast response, portability, and low cost. These features are clearly desirable for all analytical systems, and are essential in winning the war on explosives-based terrorism.

Typical Techniques for Trace Detection of High Explosives

*Electrochemistry.* The inherent redox properties of nitrated and peroxide-based explosives make them ideal candidates for electrochemical monitoring.\textsuperscript{142,144} Electrochemical sensors (ESs) for explosive detection provide several advantages over spectroscopic and spectrometric techniques such as SERS, MS and IMS. They are characterized by a reasonable sensitivity, low cost, and can be easily used as field detectors and remote control devices, due to the nature of the analytical signal.\textsuperscript{173-177} Various ESs for the detection of nitroaromatic compounds (NACs, Figure 1.6) have been reported using different sensing materials including bare carbon and Au as well as boron-doped diamond electrodes.\textsuperscript{173-176,178} A polyphenol-coated screen-printed carbon electrode was also used for highly sensitive voltammetric measurements of TNT in the presence of surface-active substances.\textsuperscript{179} Electrochemical responses of a number of NACs were compared at glassy carbon (GC), Pt, Ni, Au, and Ag electrodes, revealing that Au and Ag were suitable in capillary electrophoresis (CE) amperometric detection. A bimetal electrode, prepared by depositing Ag on Au, offered a superior performance by exploiting the sensitivity of Au while suppressing its response toward MeCN to achieve a 10-fold
lower detection limit of the explosive compounds (70-110 parts per billion (ppb)) than UV measurement.\textsuperscript{180}

\textit{Nanomaterial modified electrode.} Metallic and metal-oxide nanoparticles (NPs) are capable of increasing the activities for many chemical reactions due to the high ratio of surface atoms with free valences to the cluster of total atoms. In addition to a high surface area-to-volume ratio for NP derivatized materials, the size controllability, chemical stability, and surface tenability provide an ideal platform for exploiting such nanostructures in sensing/biosensing and catalytic applications. Using electrodes modified with NPs of transition metals and precious metals, which have specific properties compared to that of the bulk materials, opens new ways for their applications as ESs.\textsuperscript{181,182}

The modification of electrode surfaces with redox-active metal NPs has led to the development of various ESs. Filnovsky et al. found that the modification of carbon with NPs of noble metals is a promising approach for obtaining highly catalytically active electrodes for the detection of traces of aromatic compounds.\textsuperscript{183} Modification of the electrode was carried out with composites of nm-sized, mesoporous TiO$_2$, which acted as a support containing inserted or deposited NPs of Ru, Pt, or Au. Cyclic voltammetry shows that TNT can be reduced on thus-modified carbon-paper electrodes at potentials around -0.5 V (vs Ag/AgCl/Cl$^-$) in aqueous solutions. When the TiO$_2$/nano-Pt composites were used, remarkable electrochemical activities of the electrode toward the reduction of TNT were observed, suggesting that the composite material may play a specific role for facilitating the TNT reduction process. Modified electrodes based on mesoporous SiO$_2$-
MCM-41 coatings have been recently shown to be useful for enhancing the sensitivity through adsorptive accumulation of the target NACs explosives.\textsuperscript{184}

There are many reports in the literature using carbon nanotubes (CNTs) as the electrode modification material for improving the detection of explosives. Multi-walled CNTs (MWCNTs) modified GC electrodes offer a significant improvement in the electrochemical detection of TNT in seawater.\textsuperscript{185} Metal NPs (Pt, Au, or Cu) together with MWCNTs and single-walled CNTs (SWCNTs) have been used to form nanocomposites for modifying GC electrodes to improve their electroactivity and selectivity for TNT and several other NACs.\textsuperscript{186} The performance of the different metallic NPs in combination with both types of CNTs with respect to sensitivity, linear range, and selectivity toward NACs was evaluated and discussed. Among various combinations tested, the synergistic signal effect was observed for the nanocomposite modified GC electrode containing Cu NPs and SWCNTs solubilized in Nafion, in which combination provided the best sensitivity for detecting TNT and other NACs. Adsorptive stripping voltammetry for TNT resulted in a detection limit of 1 ppb, with linearity up to three orders of magnitude. Selectivity toward the number and position of the nitro groups in different NACs was found to be very reproducible and distinct. The Cu-SWCNT-modified GC electrode was demonstrated for analysis of TNT in tap water, river water, and contaminated soil.

\textit{Prussian-blue “artificial peroxidase” modified electrode}. Peroxide-based explosives, TATP and HMTD, are easy to synthesize from readily available precursor chemicals, but their detection is found to be very challenging, since they lack electrochemically reducible nitro groups, do not fluoresce and exhibit minimal UV absorption.\textsuperscript{187}
While TATP and HMTD can be measured using expensive instruments such as chemical-ionization MS or IR spectroscopy,\textsuperscript{123} these bulky instruments are not suitable for field screening scenarios or trace analysis of peroxide-based explosives. Accordingly, there are urgent needs for developing highly sensitive and yet small, easy-to-use, field deployable devices for on-site testing of peroxide explosives. Activity in this direction has focused primarily on enzymatic (peroxidase) based optical (fluorescent or colorimetric) assays of the hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) product of UV- or acid treatment\textsuperscript{187} of the peroxide explosives.

However, enzymatic assays often suffer from shortcomings associated with the limited stability and high cost of the biocatalyst. Surprisingly, little attention has been given to the development of electrochemical devices for monitoring peroxide explosives,\textsuperscript{188} despite the fact that these devices are uniquely qualified for meeting the size, cost, and low power requirements of field detection of TATP and HMTD.

Prussian blue (PB) polycrystal modified electrodes offer highly selective, low potential, and stable electrocatalytic detection for H\textsubscript{2}O\textsubscript{2}.\textsuperscript{189,190} A highly sensitive electrochemical assay of TATP and HMTD at such an electrode has been reported.\textsuperscript{140} The method involves UV light degradation of the peroxide explosives and a potential of (~0.0 V vs SCE) electrocatalytic amperometric sensing of the generated H\textsubscript{2}O\textsubscript{2} at the PB transducer and offers nanomolar detection limits following a short (15 s) irradiation times. Electrochemical detection based on direct reduction of the explosives at the electrode cannot be carried out, because the reduction of the explosives containing -O-O- peroxide groups is very ineffective. Although PB modified electrode is specific toward H\textsubscript{2}O\textsubscript{2} reduction at a low potential value (~0.0 V vs SCE) where unwanted reactions of co-
existing compounds are negligible,\textsuperscript{189-191} selective detection of TATP and HMTD is
difficult. The high catalytic activity of PB leads also to a very high sensitivity towards
H$_2$O$_2$. The behavior of PB-coated electrodes resembles that of peroxidase-based enzyme
electrodes, and hence PB has often been denoted as “artificial enzyme peroxidase.”\textsuperscript{192} PB
electrodes offer distinct stability and cost advantages over peroxidase biosensors, and
appear to be the most effective electrochemical transducer for H$_2$O$_2$. Besides, it offers a
substantial lowering of the overvoltage for the H$_2$O$_2$ redox process and permits a highly
selective and sensitive peroxide sensing. Such efficient H$_2$O$_2$ transducer facilitates the
rapid detection of peroxide explosives down to the nanomolar level.\textsuperscript{192}

Whenever needed, the PB film can be covered with a permselective (size-
exclusion) coating that can further enhance the sensor selectivity, stability and overall
performance.\textsuperscript{193} Also, relevant samples may be treated enzymatically (with catalase) to
remove the co-existing H$_2$O$_2$ that may originate from cleaning agents.\textsuperscript{194} The
electrochemical route can be further developed into disposable microsensors in
connection to single-use screen-printed electrode strips and a hand-held meter (similar to
those used for self testing of blood glucose). Preliminary data with such PB-coated
screen-printed electrodes are very encouraging. The PB-transducer can be readily adapted
for gas-phase electrochemical detection of trace TATP and HMTD in connection to
coverage with an appropriate solid electrolyte coating.\textsuperscript{140,141}

\textit{Fluorescence-Based Sensors}

Fluorescence (FL) sensors have been widely used for the detection of nitrated explosives.
The sensors are developed on the basis of either the FL quenching of the system or
competitive FL immunoassays. FL-quenching-based chemosensors, where analyte
binding produces attenuation in the light emission, are highly desirable for the detection of small molecular analytes in many challenging environments, due to the high signal output and detection simplicity. The operation mechanism of the chemosensors, for the detection of vaporous explosives such as dinitrotoluene (DNT) and TNT, is mainly based on the electron transfer from the electron-rich organic materials to those electron-deficient NACs leading to the fluorescence quenching of the organic materials. The exciton diffusion length and the surface areas of the sensing films are critical in detection sensitivity. FL-quenching-based detection represents one of the most sensitive and convenient methods that have been widely employed in explosives identification. Only the chromophore that interacts directly with the analyte molecule is quenched; the remaining chromophores continue to fluoresce. The basic sensor design (Figure 1.7) consists of a FL excitation source, such as a blue light emitting diode.

*Figure 1.7. Fluorescent polymer sensor design.*

Light passes through a lens and filter, allowing a narrow wavelength band (e.g., 430 nm) to impinge on the polymer film, which is coated on two sheets of glass. A pump pulls in air samples across the coated glass sheets. After exposing a glass slide coated
with the prepared nanocomposite film for a given period of time, the fluorescence spectra are immediately measured at an excitation wavelength. If the air sample contains explosive vapors, the PMT detector will sense a FL quenching in light intensity and trigger an alarm.

Fluorescent polymers chemical detectors. Among various photoluminescence materials, conjugated polymers have been most extensively explored as chemosensory materials for the FL detection of electron-deficient analytes such as NACs. Some conjugated polymers exhibit a high sensitivity to NACs explosives resulting in strong quenching of their emission. Swager and coworkers reported the amplified response to the analyte binding events in the aggregated systems and solid films of conjugated polymers by intermolecular exciton migration. Particularly, the multiphoton FL quenching has been observed with obvious advantages for the real-time detection of TNT. A recent report indicated that the molecular imprinting in the matrix of conjugated polymers can greatly improve their chemosensory selectivity to NACs. Other photoluminescent materials such as polytetraphenylsilole, polytetraphenylgermole, photoluminescent silica films, and silica microspheres with physisorbed dyes also exhibit the high FL response to the solution and vapor of NACs explosives at low-level concentrations.

Porous silicon (PSi) chemical and biological optical sensors have been intensively studied in the past because of the high surface area of PSi and the variety of optical transduction mechanisms upon exposure to different analytes. However, most of these sensors demonstrate no specificity for target molecules, and require the analytes (e.g., alcohols and saturated hydrocarbons) with high vapor pressures (~1-100×10⁻⁵ mm Hg) in order for the change in the reflectance or luminescence of PSi structures to be detected.
The detection of analytes with low vapor pressures (~10^{-5} mm Hg and lower) such as some nitrated explosives (e.g., TNT) is a challenge by these methods since nonspecific sorption coupled with low analyte concentration in the pores is not sufficient to uniquely alter PSi optical properties. Thus, entrapping polymers that exhibit a high sensitivity to NACs explosives inside the PSi microcavity (MC) significantly improve sensor efficiency due to specific binding of NACs to the sensory polymers, high quantum FL yield of the polymers (higher than PSi selfluminescence), amplification mechanism as a result of the energy migration,^{160} and the fine spectral patterning of the broad FL band induced by the MC structure. Also, sufficient changes in the MC reflectivity could result in the sensitive detection of explosive vapors. Based on this principle, Levitsky et al.\textsuperscript{196} have recently studied some fluorescent polymer-PSi MC devices, in which a conjugated chemosensitive polymer entrapped in PSi MC allows detection of vapors of explosive NACs via a modulation in both FL and reflectance signals. The MC resonant peak in the reflectance spectra is shifted upon vapor exposure. The broad polymer FL shows patterning by the narrow MC peak, which is also sensitive to the vapor exposure.

Many structure-property studies amplify the great potential of nanostructured materials fabricated on different length scales for practical applications. An important development in this area is the fabrication of materials with hierarchical porous structures, which combine the multiple benefits arising from the different pore size regimes.\textsuperscript{224-234} For instance, a material with interwoven meso- and macroporous structures can provide a high specific surface area and more interaction sites via small pores, whereas the presence of additional macropores can offer increased mass transport and easier accessibility to the active sites through the material. These features make such kinds of
materials highly suitable and promising for applications in catalysis, separation
technology, and sensor devices, especially if specific action sites or recognition units are
attached to these materials. A series of porphyrin or metalloporphyrin-doped silica films
with bimodal porous structures, fabricated using polystyrene spheres or evaporation
introduced self-assembly approach and a surfactant cetyltrimethylammonium bromide
(CTAB) as structure-directing agents, have been utilized for chemosensory applications
to detect trace amounts of vapors of explosives such as TNT, DNT, and nitrobenzene
(NB). The obtained results demonstrated that an appropriate combination of
macropores and mesopores can achieve high molecule permeability and high density of
interaction sites. As a result, silica films with bimodal porous structures exhibit much
more efficient FL response capability than single modal porous films. Films with
extremely high FL quenching efficiency towards TNT (10 ppb), close to 55% after 10 s
of exposure, were achieved, which is said to be nearly double those of conjugated
polymer based TNT sensor materials reported previously. Using toluene
washing, the sensory properties of the constructed films can be easily recovered. Besides
the remarkable TNT detecting capability, these hybrid films have several advantages over
other FL-based sensory materials, such as an easy preparation approach, inexpensive
materials, recognition ability of different NACs as well as stability of organic sensing
elements in inorganic matrices.

Fluorescent nanofibril film. One-dimensional crystalline structures of organic molecules
on the nanometer scale are good candidates for explosives detection because of the long
exciton diffusion length arising from crystalline structures and their intrinsic large
surface-to-volume ratio. The highly organized organic 1D nano- and supernanostructures
self-assembled with extended planar molecular surfaces enable both effective 1D \( \pi-\pi \) stacking favorable for exciton migration via cofacial intermolecular electronic coupling and flexibility in tuning morphologies on the nano- or microscopic scale. A fluorescent nanofibril film, fabricated from the alkoxy carbonyl- substituted, carbazole-cornered, arylen-ethynylene tetracycle (ACTC), was reported to be an efficient sensing film for detection of explosives. The incorporation of carbazole enhances the electron donating power of the molecule and thus increases the efficiency of FL quenching by oxidative explosives (e.g., NACs). The quenching response observed for the ACTC film is significantly faster than that previously observed for other organic materials, consistent with the fibril porous structure of the film, which facilitates both gaseous adsorption and exciton migration across the film. The quenching efficiency obtained for ACTC films is also higher than those previously reported for other explosive sensing materials at the same thickness. The porous film morphology and the extended one-dimensional \( \pi-\pi \) stacking facilitate the access of quencher molecules to the excited states, thereby resulting in effective FL quenching, which is little dependent on the film thickness as evidenced by the observations. This behavior is in contrast to what was usually observed for other organic film sensors, for which the emission quenching efficiency was inversely proportional to the film thickness owing to the diffusion limit of the exciton and the gaseous adsorbates.

Furthermore, porphyrin-doped nanocomposite fibers with a form of nanofibrous membrane were fabricated without the addition or help of polymers, and were demonstrated as novel FL-based chemosensors for the rapid detection of trace vapor (10 ppb) of explosive TNT. The research was based on sol-gel chemistry and the
electrospinning technique. Due to a larger surface area and good gas permeability, these fluorescent nanofibrous membranes exhibit remarkable sensitivity to trace TNT vapor compared to tightly cross-linked silica films, but their sensitivity is strongly dependent on the morphology and phase aggregation of the used nanofibers. Reducing the diameter and introducing a pore structure into nanofibers can considerably enhance the sensitivity of the resulting materials. Because of the well-known strong tendency to form hydrogen bonds between imino hydrogens of the used porphyrin molecule and nitro groups of NACs as well as π-stacking between porphyrins and NACs, porphyrin units have a relative large affinity for NACs molecules, which provides a strong driving force for fast FL quenching.

Several reasons are believed to be principally responsible for the remarkable observed sensing of the electrospun nanocomposite fibers towards trace TNT vapor. First, the unique bimodal porous structure provides a necessary condition for the facile diffusion of analytes to sensing elements, while the large surface area considerably enhances the interaction sites between analyte molecules and sensing elements, thereby further improves the detection sensitivity. Second, for a given analyte like TNT, strong binding strength and energy level matching are essential for obtaining high TNT quenching efficiency. Theoretical studies, which indicate that the FL quenching per unit time is affected by various factors, including the vapor pressure of analyte, the exergonicity of electron transfer, and the binding strength between sensing elements and analytes.

Quantum dots quenching sensor. Colloidal semiconductive nanocrystals/nanoparticles (also called quantum dots, QDs) are spherical particles in a size regime dominated by
strong quantum confinement of the charge carriers. This confinement lifts the degeneracy of the carrier states within the conduction and valence bands, and increases the effective band gap energy significantly with decreasing particle size, resulting in size dependence of several properties, such as absorption and photoluminescence spectra.\textsuperscript{239,240}

Luminescent QDs have the potential to circumvent some of the functional limitations encountered by organic dyes in biotechnological applications. Recently, QDs with high quantum yields have found a wider range of applications as a foundation of FL sensors.\textsuperscript{241-245} The photoluminescence of QDs is readily tunable within a large range of spectroscopy through the change of size or the introduction of dopant ions, which can potentially be utilized for obtaining a spectral response toward a particular target analyte.\textsuperscript{244} More importantly, QDs allow the chemical modification of functional groups and the installation of recognition receptors at their surfaces, providing the chemodetection selectivity to target species.\textsuperscript{243,245} Therefore, the FL chemosensors based on the “lab-on-QDs” concept have a remarkable advantage over other detection schemes in chemodetection sensitivity and selectivity.\textsuperscript{246} For example, Goldman and co-workers\textsuperscript{247,248} recently proposed a typical scheme of QDs-based chemosensors through the hybrid CdSe QDs of antibody segments and dye molecules. The specific detection toward TNT has been achieved through the fluorescence resonance energy transfer (FRET) between QDs and dye. As shown in Figure 1.8, the hybrid sensor consists of anti-TNT specific antibody fragments (receptors) attached to a hydrophilic QD via metal-affinity coordination. A dye-labeled TNT analogue (analog-quencher) pre-bound in the antibody binding site quenches the QD PL via proximity-induced FRET. Addition of
soluble TNT analyte displaces the dye-labeled analogue, eliminating FRET and resulting in a concentration-dependent recovery of QD PL.

A general strategy for FRET-based biosensor design and construction employing multifunctional surface-tethered components from the above research team has been proposed (Figure 1.9A) and used in the detection of TNT and related compounds.

Figure 1.8. Schematic of a hybrid QD-antibody fragment FRET-based TNT sensor.

The molecular biosensor consists of two modules: the biorecognition module and the modular arm. Both modules are specifically attached to a surface in a particular orientation. Choices for surface attachment include biotin-avidin chemistry, metal-affinity coordination, thiol bonding, hydrophobic interactions, DNA-directed immobilization, etc. The biorecognition module can consist of proteins (enzymes, receptors, bacterial periplasmic binding proteins (bPBPs), antibody fragments, peptides), aptamers, carbohydrates, DNA, PNA, RNA. This module is site-specifically dye-labeled in the current configuration. The modular arm may consist of flexible moieties such as DNA, PNA, RNA, peptides, polymers, etc. The modular arm is also site-specifically dye-labeled. An analogue of the primary analyte is attached to the distal end of the flexible
arm to act as the recognition analogue. Binding of this recognition element in the binding pocket of the biorecognition element assembles the sensor into the ground state by bringing both dyes into proximity, which establishes FRET.

Figure 1.9. (A) Schematic of the modular biosensor consisting of two modules: the biorecognition module and the modular arm (see main text for detailed description). (B) Schematic of the TNT targeting biosensor.

As shown in Figure 1.9, the dye-labeled anti-TNT scFv fragment (1, the biorecognition module) is attached to the surface with Bio-X-NTA (Bio: biotin; X: aminomethoxy spacer; NTA: nitrilotriacetic acid chelator) coordinating the 12 histidines (12-HIS) and orienting the protein on the NeutrAvidin (NA). The dye-labeled TNB (a TNT analogue 1,3,5-trinitrobenzene) DNA arm (2, modular arm) is attached to the NA via complementary hybridization to a biotinylated (B) flexible DNA linker. Both are added in equimolar amounts. ScFv binding of the TNB
analogue brings the protein located dye and DNA located dye into proximity
establishing FRET. Addition of TNT displaces the TNB analogue and DNA arm
disrupting FRET in a concentration-dependent manner. Addition of analyte competitively displaces the analogue and signal
transduction is designed to be sensitive to this displacement. FRET donor/acceptor
can be placed on either module. Mechanisms of controlling binding affinity include
stiffening the flexible arm or switching in of different affinity biorecognition elements.
As shown in Figure 1.9B, the sensor consists of a dye-labeled anti-TNT antibody
fragment (scFv) that interacts with a cofunctional surface-tethered DNA arm. The arm
consists of a flexible biotinylated DNA oligonucleotide base specifically modified
with a dye and terminating in a TNB recognition element, which is an analog of TNT,
1,3,5-trinitrobenzene. Both of these elements are tethered to a Neutravidin (NA)
surface with the TNB recognition element bound in the antibody fragment binding
site, bringing the two dyes into proximity and establishing a baseline level of FRET.
Addition of TNT, or related explosive compounds (e.g., RDX and DNT), to the sensor
environment alters FRET in a concentration-dependent manner. The sensor can be
regenerated repeatedly through washing away of analyte and specific reformation of
the sensor assembly, allowing for subsequent detection events. Sensor dynamic range
can be usefully altered through the addition of DNA oligonucleotide that hybridizes to
a portion of the cofunctional arm. Although the authors have used quenching of
organic dyes for biosensor signal generation, use of optical components such as QDs,
should be also possible.
Fluoroimmunoassays using QD-antibody conjugates. Goldman et al. developed a strategy based on the use of antibody-conjugated QDs in plate-based competitive immunoassays for the detection of ng quantities of the TNT surrogate, TNB fluorescein, and RDX in aqueous samples (Figure 1.10). The QD/antibody conjugates were formed by using either a molecular adaptor protein or using avidin to form QD/antibody conjugates. The analytes of interest compete with the surface-confined antigen for antibody-QDs, and the FL signal is measured from the plate after a washing step. A reverse relationship between the measured FL intensity and the analyte concentration is followed.

**Figure 1.10.** QD antibody conjugates prepared using molecular bridges. (A) Mixed surface conjugate after purification by cross-linked amylose affinity chromatography. (B) Schematic of competitive assay for the explosive RDX dissolved in water.

Displacement immunosensors. The displacement immunosensor is an improved, faster, and more efficient detection system with respect to a traditional immunoassay, and may be regarded as a category of chromatographic immunoassays. In the displacement immunosensor, immunoassays are coupled with a device that is set under continuous buffer flow, as illustrated in Figure 1.10. The key components of the system are antibodies (Ab) immobilized on a solid support (e.g., micro-sized beads, agarose gel,
membranes\textsuperscript{259}, antigen analogs that are labeled with a reporter molecule (e.g., a fluorophore) (Ag\textsuperscript{*}), and the associated hardware needed to establish a controlled flow system. Generally, monoclonal antibodies and reporter molecules are first linked to a prepared surface and a target antigen/analyte, respectively. The Ag\textsuperscript{*} is then allowed to react with the immobilized Ab until equilibrium is reached (e.g., 2\textendash{}15 h). To perform an assay, the solid support coated with the Ab/Ag\textsuperscript{*} complex is placed in a buffer flow. When a sample containing the analyte of interest is injected into the flow stream, the Ag\textsuperscript{*} molecules are displaced into the buffer and measured downstream using a FL detector. The measured FL intensity is proportional to the concentration of analyte molecules injected, within a predetermined linear range for each antibody.

\textbf{Figure 1.11.} Schematic of the displacement immunosensor method.\textsuperscript{254}

\textit{Metal Oxide Semiconductor (MOS) Nanoparticle Gas Sensors}

The metal oxide semiconductor (MOS) gas sensor is one class of the electronic noses\textsuperscript{260} and usually reported as detectors for volatile organic compounds because of its sensitivity, low-cost and easy manufacturing. Several reports on this topic for explosive detection have appeared. In the first study, sensing military grade TNT and some
substrates (air, sand and soil) was investigated by using TiO2 thin film sensors with a static headspace sampling, which indicated that using MOS sensors to detect solid explosive was a feasible method. For the detection of solid explosives, however, the low vapor concentration makes it an extremely difficult and challenging task. To solve this problem, some researchers suggested using Pt or Pd catalyst in the carrier gas line to increase the sensitivity of the MOS sensors, which could also allow the sampling of solids and liquids as well as gases with a gas sensor. In another study, several explosives (e.g., DNT, NH4NO3, and picric acid) have been investigated by using ZnO-doped nanoparticle sensors with additives of Sb2O3, TiO2, V2O5 and WO3.

**Surface-Enhanced Raman Scattering Spectroscopy**

Surface-enhanced Raman scattering spectroscopy (SERS) combines extremely high sensitivity, due to enhanced Raman cross-sections comparable or even better than fluorescence, with the observation of vibrational spectra of adsorbed species, providing one of the most incisive analytical methods for chemical and biochemical detection and analysis. The metallic NPs (e.g., Au and Ag NPs) that make SERS possible are of fundamental interest since they possess unique size-dependent properties. The use of SERS for trace explosive detection was first investigated during the late 1990s, and SERS detection of 2,4-DNT vapor to ~1 ppb was demonstrated. Subsequently, a field-portable unit had demonstrated a limit of detection of 5 ppb vapor DNT and the ability to locate buried land mines. More recently, nano-engineered SERS substrates have been employed, and ppb sensitivity for some nerve agent and explosive simulants has been demonstrated.

Surface-enhanced Raman scattering spectroscopy has also been used to detect
selectively functionalized TNT; additional enhancement due to the resonance Raman effect results in detection limits much better than 1 nM in solution.\textsuperscript{269} With the similar ideas, detection of explosive RDX via reduction and subsequent functionalization has been reported.\textsuperscript{270}

Conclusions

Seven types of techniques involving nanomaterials for trace detection and quantification of high explosives are reviewed. These techniques are based on (a) electrochemistry, (b) fluorescence, (c) metal oxide semiconductive nanoparticles, and (d) surface enhanced scattering spectroscopy. Most of the studies have been focused on nitroaromatics detection and a few are peroxide explosives related. Nanotechnology will continue to play an important role in developing new explosive sensors that have better sensitivity and higher selectivity toward analytes of interest, and are more portable for field testing, shorter response time, cheaper to maintain, and easier to operate.
References

(37) Bard, A. J.; Park, S. M. 1975; Academic; 305-326.
(49) Knight, A. W.; Greenway, G. M. *Analyst** **1994**, *119*, 879-890.
(50) Knight, A. W.; Greenway, G. M. *Analyst** **1996**, *121*, 101R-106R.


(69) Arnhold, J. 2001; Schweda-Werbedruck GmbH; 85-94.


(72) Gaffney, J. S.; Marley, N. A. 2002; American Meteorological Society; 1-6.


(84) Sherma, J. *J. AOAC Int.* **2004**, *87*, 20A-26A.


(169) Ruffin, P. B.; Brantley, C.; Edwards, E., San Diego, California, USA 2008; SPIE; 693102-693112.
(178) Kim, J.-W.; Kim, J-H.; Tung, S. Proc. 3rd IEEE Int. Conf. on Nano/Micro Engineered and Molecular Systems, Sanya, China, 2008; 630-632.


(205) Narayanan, A.; Varnavski, O. P.; Swager, T. M.; Goodson, T., III PMSE
Preprints 2007, 96, 678.
(210) Chan, S.; Horner, S. R.; Miller, B. L.; Fauchet, P. M. Materials Research Society
51-56.
(212) De Stefano, L.; Alfieri, D.; Rea, I.; Rotiroti, L.; Malecki, K.; Moretti, L.; Della
(213) De Stefano, L.; Moretti, L.; Rendina, I.; Rotiroti, L. Sens. Actuators, B 2005,
B111-B112, 522-525.
(214) De Stefano, L.; Rendina, I.; Moretti, L.; Rossi, A. M.; Lamberti, A.; Longo, O.;
43, 167-172.
(217) Mahmoudi, B.; Gabouze, N.; Guerbous, L.; Haddadi, M.; Cheraga, H.; Beldjilali,
1999, 2, 17-22.
(220) Rea, I.; Iodice, M.; Coppola, G.; Rendina, I.; Marino, A.; De Stefano, L. Sens.
Actuators, B 2009, B139, 39-43.
(221) Rotiroti, L.; De Stefano, L.; Rendina, I.; Moretti, L.; Rossi, A. M.; Piccolo, A.
(222) Saarinen, J. J.; Weiss, S. M.; Fauchet, P. M.; Sipe, J. E. Opt. Express 2005, 13,
3754-3764.
(225) Falcaro, P.; Malfatti, L.; Kidchob, T.; Giannini, G.; Falqui, A.; Casula, M. F.;
Amenitsch, H.; Marmiroli, B.; Grenci, G.; Innocenzi, P. Chem. Mater. 2009, 21,
2055-2061.
Mesoporous Mater. 2006, 96, 205-209.
10535.
(228) Li, Y.; Cai, W.; Cao, B.; Duan, G.; Sun, F.; Li, C.; Jia, L. Nanotechnology 2006,
17, 238-243.
2008, 18, 4985-4993.
(230) Yacou, C.; Fontaine, M.-L.; Ayral, A.; Lacroix-Desmazes, P.; Albouy, P.-A.;
(239) Mattoussi, H.; Radzilowski, L. H.; Dabbousi, B. O.; Thomas, E. L.; Bawendi, M.
407-414.
435-446.


CHAPTER II
SENSITIVE DETERMINATION OF HEXAMETHYLENE TRIPEROXIDE DIAMINE (HMTD) EXPLOSIVES USING ELECTROGENERATED CHEMILUMINESCENCE ENHANCED BY SILVER NITRATE*

Introduction

The legal authorities have witnessed increased number of threats of illegal use of peroxide-based explosive materials by the terrorists. These compounds are very popular among the terrorists because they can be synthesized readily from the commercially available chemicals.1 These explosives, also known as “unconventional explosives” since they have no use for military purposes because of their high instability and powerful initiating explosive capability, are basically linked to the terrorists.2 There is urgent need of detection of these “home-made” explosives especially in the checkpoints of the mass-transit facilities and other government and public facilities.3-6

Hexamethylene triperoxide diamine (HMTD) is a representative of the abovementioned peroxide-based explosives. It is a white solid with cyclic structure7 (Scheme 2.1), first synthesized by Legler in 18858 that is sensitive to friction, impact and electrical discharge.9 The friction sensitivity of HMTD is comparable to other well-known explosives like triacetone triperoxide (TATP) and trinitrotoluene (TNT) although its impact sensitivity is about a half of TATP.10-12

A number of analytical techniques have been used to detect peroxide explosives including HMTD. These techniques include separation-based gas chromatography

* Part of the results presented in this chapter have been published: Parajuli, S.; Miao, W. Anal. Chem. 2009, 81, 5267-5272.
coupled with mass spectrometry (GC/MS), liquid chromatography (LC)/MS, time-of-flight (TOF)/MS, desorption electrospray ionization (DESI)/MS, LC/FT-IR, and UV-Visible spectrometric based methods. MS-based methods are very sensitive and could detect peroxide explosives in nanogram to picogram levels but the instruments are generally very expensive and not portable for field tests. Chromatographic, UV-Visible and IR detection techniques are often time-consuming in sample preparation and insufficiently sensitive for trace amounts of explosive quantification.

Electrogenerated chemiluminescence (ECL), a process of light production as a result of electrochemical reactions at an electrode, has proved to be a powerful analytical technique due to its inherent features such as high sensitivity, good selectivity, low background, integrated versatility and fast sample analysis. ECL has been widely used for many kinds of targets detection and quantification under a broad variety of areas, which include DNA probe, immunoassay, pharmaceutical study, food and water testing, and biowarfare agent detection. Very recently, our research group reported an ultrasensitive detection of TNT, which was accomplished on the basis of sandwich-type TNT immunoassay combined with ECL technology. The limit of detection (≤ 0.10 ± 0.01 ppb) is about 600 times lower as compared with the most sensitive TNT detection method based on surface plasmon resonance in the literature, and the absolute detection limit in mass (~0.1 pg) is only ~0.5% of that from mass spectroscopy.

In this chapter, an ECL detection and quantification method for HMTD using AgNO₃ as an ECL enhancing agent will be reported. This method is based on the fact that HMTD contains tertiary amine moieties with α-C hydrogens (Scheme 2.1), which could act as an ECL coreactant like tri-ᵣ-propylamine (TPrA) in the presence of an ECL
luminophore such as Ru(bpy)$_3^{2+}$ species up on anodic potential scanning.$^{25}$ Remarkable enhancement by AgNO$_3$ for HMTD ECL generation and relevant electrochemical and ECL mechanisms will be described. The ECL enhancement strategy used in this chapter could be extended to other systems where the electrochemical oxidations of the analyte (coreactant) or the luminophore at an electrode are suppressed due to the nature of the working electrode$^{26}$ or “delayed” due to the slow heterogonous electron-transfer rate of the compound.

Experimental Section

Chemicals and Materials

Hexamethylenetetramine (99+%%) from Alfa Aesar (Ward Hill, MA), silver nitrate (99.5%) and tetra-$n$-butylammonium perchlorate (TBAP, 99+%, electrochemical grade) from Fluka (Milwaukee, WI), hydrogen peroxide (30%), anhydrous citric acid (>99.5%, ACS reagent), sulfuric acid (95-98%, ACS reagent), tris(2,2’-bipyridyl)-dichlororuthenium(II) hexahydrate (99.95%), silver benzoate (99%), silver tetrafluoroborate (98%), and acetonitrile (MeCN, 99.93+%, HPLC grade) from Sigma-Aldrich (Milwaukee, WI) were used as received.

Synthesis and Characterization of HMTD

As outlined in Scheme 2.1, the explosive compound, HMTD, had to be synthesized because it is not commercially available. We followed the standard procedure from the literature for its synthesis.$^{27}$ 7.0 g (50 mmole) of hexamethylenetetramine was dissolved in 20.5 mL of 30% hydrogen peroxide stirring mechanically in an ice bath (0 ºC). To this 10.5 g of anhydrous citric acid was slowly dissolved with constant stirring.$^{27}$ The mixture was stirred for 3 h and warmed at room temperature for 2 h. White crystals were washed with
deionized water (7 times), rinsed with methanol (3 times), air dried, and stored in a refrigerator at 4 °C prior to use.

**Scheme 2.1. Synthesis of hexamethylene triperoxide diamine (HMTD).**

HMTD was characterized by diamond crystal attenuated total reflection Fourier transform infrared (ATR-FTIR, Nicolet Nexus 470 FTIR spectrometer, Thermo Electron Corp., Madison, WI) and $^1$H NMR spectroscopy (Bruker 300 MHz NMR spectrometer). FTIR spectra (Figure 2.1a) shows a characteristic C-O stretch band at 1225 cm$^{-1}$ which along with all other peaks, is consistent with the literature values.$^{28}$

**Figure 2.1.** (a) ATR-FTIR and (b) $^1$H NMR Spectra of HMTD.

The $^1$H NMR spectra shown in Figure 2.1b reveals a singlet peak at 4.82 ppm as expected because all protons in the compound are chemically equivalent. This chemical shift is close to 4.60 ppm estimated theoretically using ChemBioDraw Ultra 11.
Note that $^1$H NMR spectra alone should not be used to verify the synthesis of HMTD, because similar $^1$H NMR spectra can be also obtained from the reaction precursor hexamethylenetetramine. Small $^1$H NMR peaks at 1.60 ppm and 7.26 ppm are ascribed to trace amounts of water contained in the solvent and the incomplete deuteration of the solvent (CDCl$_3$) used.$^{29}$ [CAUTION: HMTD is very sensitive to explosion when present as a dry solid. It should be handled carefully with appropriate precautions and should not be kept in a large quantity. For long-term storage, HMTD should be dissolved or kept wet.]

Electrochemical and ECL Studies

Cyclic voltammetry (CV) was carried out with a model 660A electrochemical workstation (CH Instruments, Austin, TX). The ECL signals along with the CV responses were measured simultaneously with a homemade ECL instrument as described previously.$^{19,30}$ This instrument combined the 660A electrochemical workstation with a photomultiplier tube (Hamamatsu R928, Japan, biased at -700 V DC) installed under a conventional three-electrode cell, in which a Pt wire was used as the counter electrode, a Ag/Ag$^+$ (10 mM AgNO$_3$ and 0.10 M TBAP in MeCN) as the reference electrode (~0.186 V vs NHE), and either a glassy carbon (GC, 3-mm diameter), a Pt (2-mm diameter), or a gold (Au, 2-mm diameter) disk as the working electrode. The working electrode was polished with 0.3-0.05-$\mu$m alumina slurry, thoroughly rinsed with water, and dried with the Kim wipes facial tissue and then an air blower before each experiment. The internal electrolyte solution and the Vycor tip of the reference electrode were changed periodically to eliminate possible contaminations of other species within the cell. No
degassing was needed because all electrochemical scans were conducted in the positive potential region.

Results and Discussion

Cyclic voltammetric (CV) and ECL studies of HMTD

The CV and ECL responses of a MeCN solution consisting of 1.0 mM HMTD, 0.70 mM Ru(bpy)$_3$Cl$_2$, and 0.10 M TBAP electrolyte are shown in Figure 2.2.

Figure 2.2. CV (black line) and ECL (blue line) responses obtained from 1.0 mM HMTD- 0.70 mM Ru(bpy)$_3$Cl$_2$-0.10 M TBAP in MeCN at a 2-mm diameter Pt electrode with a scan rate of 50 mV/s.

In the anodic potential scanning range of 0 to 2.0 V vs Ag/Ag$^+$, Ru(bpy)$_3$$^{2+}$ oxidizes reversibly to form Ru(bpy)$_3$$^{3+}$ at a Pt electrode with a half-wave potential of 0.96 V vs Ag/Ag$^+$, whereas HMTD displays an irreversible oxidation at ~1.7 V as shown in Figure 2.3. Because two identical tertiary amine moieties exist in HMTD (Scheme 2.1), an overall two-electron transfer oxidation process is expected, which is consistent with the
oxidation current ratio of HMTD to Ru(bpy)$_3^{2+}$. ECL response of the Ru(bpy)$_3^{2+}$/HMTD system, which appears at ~1.45 V and reaches the maximum at ~1.76 V, is coincident with the oxidation potential of HMTD. Scheme 2.2 summarizes the proposed ECL mechanism of this system, in which the ECL contribution associated with HMTD dication species, [HMTD$'^{2+}$ (Eq. 2.2), to the overall ECL emissions (Eq. 2.3) could be relatively small, because the oxidation of HMTD$'^{+}$ to [HMTD$'^{2+}$ is most likely the rate-determining step after the one-electron electro-oxidation of HMTD (Eq. 2.1).

![Figure 2.3](image_url)

*Figure 2.3. CVs of (a) 1.0 mM HMTD, (b) 0.7 mM Ru(bpy)$_3$Cl$_2$, and (c) 0.10 M TBAP MeCN blank at a 2-mm diameter Pt with a scan rate of 50 mV/s.*

The ECL intensity of the Ru(bpy)$_3^{2+}$/HMTD system depends on the added Ru(bpy)$_3^{2+}$ concentration as well as the nature of the working electrode. As revealed in Figure 2.4, for 1.0 mM HMTD, the maximum ECL appears at 0.70 mM Ru(bpy)$_3$Cl$_2$ for
all three working electrodes studied, in which the Pt electrode shows slightly stronger ECL responses than the Au electrode does, and the GC electrode gives relatively poor ECL signals. Similar behavior has been reported previously for the Ru(bpy)$_3$$^{2+}$/TPra system in MeCN.$^{31}$

\[
\begin{align*}
\text{HMTD} & \rightarrow \text{HMTD}^{*+} \rightarrow \text{HMTD}^* \rightarrow \text{Ru}^{III} + P_1, \\
\text{HMTD}^{*+} \rightarrow [\text{HMTD}^*]^{2+} \rightarrow \text{HMTD}^{*+} \rightarrow \text{HMTD}^* \rightarrow \text{Ru}^{III} + P_2, \\
\text{Ru}^{II*} & \rightarrow \text{Ru}^{II} + hv
\end{align*}
\]

where HMTD = hexamethylene triperoxide diamine, Ru$^{II} = \text{Ru(bpy)}_3^{2+}$, Ru$^{II} = \text{Ru(bpy)}_3^{3+}$, Ru$^{II*} =$ excited state Ru(bpy)$_3$$^{2+*}$, P$_1$ and P$_2$ = oxidation products of HMTD free radicals.

Scheme 2.2. Proposed ECL mechanism of the Ru(bpy)$_3$$^{2+}$/HMTD system in MeCN upon the anodic potential scanning.

**ECL Enhancement with AgNO$_3$**

As shown in Figures 2.5A and 2.6, after addition of AgNO$_3$ into a MeCN solution containing HMTD and Ru(bpy)$_3$Cl$_2$, significant enhancement in ECL signal is observed. For example, in 1.0 mM HMTD-0.70 mM Ru(bpy)$_3$Cl$_2$-0.10 M TBAP MeCN, about 9 and 27 times increases in ECL intensity were obtained in the presence of 2.0 mM and 7.0 mM AgNO$_3$ with respect to that in the absence of added AgNO$_3$, respectively. The ECL peak intensity is linearly proportional to the concentration of added AgNO$_3$ over a concentration range of 0 to 7.0 mM (Figure 2.6a). In the absence of HMTD, no ECL concentration range of 0 to 7.0 mM (Figure 2.6a). In the absence of HMTD, no ECL background (When $[\text{AgNO}_3] \leq 2.0$ mM) or values of less than 10% of ECL observed in
Figure 2.4. Effect of Ru(bpy)$_3$Cl$_2$ concentration and working electrode material on ECL intensity of the Ru(bpy)$_3$$^{2+}$/HMTD system in 0.10 M TBAP MeCN. Scan rate: 50 mV/s, [HMTD] = 1.0 mM.

Figure 2.5. (A) CV (black line) and ECL (blue line) responses obtained from 1.0 mM HMTD- 0.70 mM Ru(bpy)$_3$Cl$_2$-0.10 M TBAP in MeCN in the presence of 7.0 mM AgNO$_3$, and (B) CV of 7.0 mM AgNO$_3$ in MeCN containing 0.10 M TBAP at a 2-mm diameter Pt electrode with a scan rate of 50 mV/s.
the presence of HMTD (When 3.0 mM < [AgNO₃] < 9.0 mM) generated from 0.70 mM Ru(bpy)₃Cl₂-0.10 M TBAP MeCN (AgNO₃) is found (Figure 2.6b). As a result, the ECL enhancement must be related to both HMTD and AgNO₃. By comparing Figure 2.2 with Figure 2.5A, one can notice that the enhanced ECL signal is corresponded to the increase of CV currents in the potential region of HMTD oxidation.

![Graph](image)

**Figure 2.6.** (a) Effect of AgNO₃ on ECL intensity of 1.0 mM HMTD- 0.70 mM Ru(bpy)₃Cl₂-0.10 M TBAP in MeCN. (b) ECL background produced from 0.70 mM Ru(bpy)₃Cl₂-0.10 M TBAP with added AgNO₃ in MeCN in the absence of HMTD. Other experimental conditions were the same as in Figure 2.5.

A CV study of AgNO₃ in MeCN (0.10 M TBAP) at a Pt electrode (Figure 2.5B) indicates that AgNO₃ oxidizes irreversibly with an anodic peak potential of 1.72 V vs Ag/Ag⁺, which is in good agreement with an earlier report,³² and is coincident with the electrochemical oxidation potentials of HMTD at Pt. Such electrochemical oxidations could lead to the formation of strong oxidizing species of Ag(II) ($E^\circ$(Ag(II)/Ag(I)) = 1.98 V vs NHE³³ or ~1.79 V vs Ag/Ag⁺) and NO₃⁻ radicals.³²,³⁴,³⁷ The standard reduction
potential of \( \text{NO}_3^- \) to \( \text{NO}_3^\cdot \) in aqueous media was estimated to be as high as \( 2.48\pm0.03 \) V vs NHE (~2.29 V vs Ag/Ag⁺) on the basis of a series of rate and equilibrium constant measurements as well as computer modeling.\(^{38,39}\) However, this estimated value apparently is too high compared with the data obtained from various electrochemical measurements in MeCN, where the oxidation of \( \text{NO}_3^- \) at approximately 1.66-1.9 V vs Ag/Ag⁺ was observed at a Pt electrode.\(^{32,34-37}\) Previous electrochemical data also suggested that the formation of Ag(II) from AgNO₃ in MeCN solution resulted from a reaction between Ag(I) ions and electrochemically generated \( \text{NO}_3^\cdot \) radicals.\(^{32,35}\) That is, the direct electrode oxidation of Ag(I) to Ag(II) could be a relatively slow kinetic (Scheme 2.3) process. By using Scheme 2.3 with all known experimental conditions along with estimated CV and chemical parameters, digital simulations of the CV shown in Figure 2.5B were conducted (DigiSim 3.0, Bioanalytical Systems, Inc., West Lafayette, IN),\(^{40,41}\) which resulted in \( E^\circ(\text{Ag(II)/Ag(I)}) = 1.79 \) V vs Ag/Ag⁺ (or 1.98 V vs NHE) and \( E^\circ(\text{NO}_3^-/\text{NO}_3^\cdot) = 1.82 \) V vs Ag/Ag⁺ (or 2.01 V vs NHE). Thus, our simulated standard reduction potential for the \( \text{NO}_3^\cdot/\text{NO}_3^- \) couple is about 470 mV less positive than that obtained from non-electrochemical based experiments. Although precise fitting of the experimentally obtained CV (Figure 2.5B) with the simulated one (Figure 2.7) was difficult due to large amounts of non-Faradaic current responses of the blank solution (Figure 2.3c), major features of the voltammograms fit well. In addition to AgNO₃, NaNO₃ also demonstrates significant ECL enhancement for the HMTD/Ru(bpy)₃²⁺ system (Figure 2.8). However, in this case, the enhancement from NaNO₃ (by adding a few μL of a high concentration of aqueous NaNO₃ into MeCN solutions) is slightly
smaller than AgNO\textsubscript{3} when [AgNO\textsubscript{3}] > 1.4 mM. These data suggest that the ECL enhancement is primarily due to NO\textsubscript{3}\textsuperscript{-} ions.

\[
\begin{align*}
\text{Ag(II)} + e & = \text{Ag(I)} & E^0 &= 1.79 \, \text{V}, \, k_s = 0.10 \, \text{cm/s} & 2.4 \\
\text{NO}_3^- + e & = \text{NO}_3^- & E^0 &= 1.82 \, \text{V}, \, k_s = 1\times10^4 \, \text{cm/s} & 2.5 \\
\text{NO}_3^- + \text{Ag(I)} & = \text{NO}_3^- + \text{Ag(II)} & k_f / k_b &= 1\times10^4 / 3111 & 2.6 \\
\text{NO}_3^- & = P_3 & k_f / k_b &= 1\times10^4 / 1 & 2.7 \\
\text{Ag(II)} & = P_4 & k_f / k_b &= 1\times10^4 / 1 & 2.8
\end{align*}
\]

\textit{Scheme 2.3.} Proposed reaction mechanism of AgNO\textsubscript{3} oxidation in MeCN at a Pt electrode for CV digital simulations. In Eqs. 2.4-2.8, \(E^0\) is the standard redox potential, \(k_s\) is the standard heterogeneous rate constant, \(k_f/k_b\) is the ratio of homogeneous rate constant of the forward reaction to the backward reaction, and \(P_3\) and \(P_4\) are reduction products of NO\textsubscript{3}\textsuperscript{•} and Ag(II), respectively.

\textit{Figure 2.7.} Simulated CV of AgNO\textsubscript{3} oxidation in MeCN. In addition to parameters listed in Scheme 2.3 above, the following parameters were used: \(\alpha = 0.5\), initial [Ag(I)] = [NO\textsubscript{3}\textsuperscript{•}] = 0.007 M, area of electrode = 0.0314 cm\textsuperscript{2}, scan rate = 50 mV/s, \(T = 298.15\ \text{K}\), diffusion coefficients for all species: \(5\times10^{-6} \, \text{cm}^2/\text{s}\).

The contribution from Ag\textsuperscript{+} ions is in fact generally less than 10\% under present experimental conditions. Because 0.70 mM Ru(bpy)\textsubscript{3}Cl\textsubscript{2} was used for the ECL measurements, 1.4 mM of Ag\textsuperscript{+} ions were needed to react with Cl\textsuperscript{-} ions to form AgCl...
precipitates. Consequently, no difference in ECL enhancement was observed between AgNO$_3$ and NaNO$_3$ when their concentrations were less than 1.4 mM.

Figure 2.8. Comparison of (a) AgNO$_3$ with (b) NaNO$_3$ on ECL intensity of 1.0 mM HMTD-0.70 mM Ru(bpy)$_3$Cl$_2$-0.10 M TBAP in MeCN.

The effect of Ag$^+$ on ECL enhancement was further investigated with silver benzoate (C$_6$H$_5$COOAg) and AgBF$_4$. As shown in Figure 2.9a, in a 1.0 mM HMTD-0.70 mM Ru(bpy)$_3$(ClO$_4$)$_2$-0.10 M TBAP MeCN solution, ECL intensity is increased linearly with the addition of C$_6$H$_5$COOAg. When 0.70 mM Ru(bpy)$_3$(ClO$_4$)$_2$ was replaced with 0.70 mM Ru(bpy)$_3$Cl$_2$, a similar trend in ECL enhancement was observed (Figure 2.9b).

In this case, however, the effective [Ag$^+$] in solution is reduced by ~1.4 mM due to insoluble AgCl formation as explained earlier. Comparable ECL enhancement of AgBF$_4$ for the HMTD/Ru(bpy)$_3^{2+}$ system was also noted (not shown). Therefore, Ag$^+$ ions enhancement on ECL is essentially independent of the anions used. On the basis of data shown in Figures 2.8b and 2.9a, ~2.6 times in ECL enhancement from NO$_3^-$ as
compared to Ag\(^{+}\) can be calculated, which is consistent with the AgNO\(_3\) reaction mechanism proposed in Scheme 2.3. There would be no ECL enhancement of Ag\(^{+}\) obtained, if no direct oxidation of Ag(I) at an electrode occurred as described previously\(^{32,35}\). In this respect, ECL is a much more sensitive technique than voltammetry for verifying the insensitive oxidation of Ag(I) in MeCN.

![Graph showing ECL Peak Intensity vs. [C\(_6\)H\(_5\)COOAg] (mM)](image)

**Figure 2.9.** Effect of Ag\(^{+}\) on ECL intensity of 1.0 mM HMTD with (a) 0.70 mM Ru(bpy)\(_3\)(ClO\(_4\))\(_2\) or (b) 0.70 mM Ru(bpy)\(_3\)Cl\(_2\) using different concentrations of C\(_6\)H\(_5\)COOAg.

Since ECL production of the HMTD/Ru(bpy)\(_3\)^{2+} system initiates from the oxidation of Ru(bpy)\(_3\)^{2+} and HMTD (Scheme 2.2), in principle, any process that could increase the generation of Ru(bpy)\(_3\)^{3+} and HMTD\(^{+}\) should enhance the ECL intensity. Although electrochemical oxidations of HMTD and AgNO\(_3\) are located in the similar potential region (Figures 2.2 and 2.5), electrogenerated Ag(II) and NO\(_3\)^{−} species should be strong enough to oxidize Ru(bpy)\(_3\)^{2+} and HMTD in the diffusion layer proximity to the electrode. This is because the standard reduction potentials for Ag(II) and NO\(_3\)^{−} species
are more positive than their apparent oxidation peak as revealed by digital simulations, and the standard redox potential for HMTD$^{\ddagger}$/HMTD should be less positive than or close to its apparent oxidation peak, given that HMTD, like many other tertiary amines, involves a slow electron-transfer oxidation process (e.g., $k_s \leq 0.01$ cm/s).$^{40,43}$

\[
\text{Ru(bpy)}_3^{2+} - e \rightleftharpoons \text{Ru(bpy)}_3^{3+} \tag{2.9}
\]

\[
\text{HMTD} - e \rightarrow \text{HMTD}^{*+} \tag{2.10}
\]

\[
\text{Ag(I)} - e \rightarrow \text{Ag(II)} \tag{2.11}
\]

\[
\text{NO}_3^- - e \rightarrow \text{NO}_3^* \tag{2.12}
\]

\[
\text{NO}_3^* + \text{Ag(I)} \rightarrow \text{NO}_3^- + \text{Ag(II)} \tag{2.13}
\]

\[
\text{Ag(II)} + \text{HMTD} \rightarrow \text{Ag(I)} + \text{HMTD}^{**} \tag{2.14}
\]

\[
\text{NO}_3^* + \text{HMTD} \rightarrow \text{NO}_3^- + \text{HMTD}^{**} \tag{2.15}
\]

\[
\text{HMTD}^{**} \rightarrow \text{HMTD}^* + \text{H}^+ \tag{2.16}
\]

\[
\text{Ru(bpy)}_3^{2+} + \text{HMTD}^* \rightarrow \text{Ru(bpy)}_3^{3*} + \text{Product}_1 \tag{2.17}
\]

\[
\text{Ru(bpy)}_3^{3+} + \text{Ag(II)} \rightarrow \text{Ru(bpy)}_3^{2+*} + \text{Ag(I)} \tag{2.18}
\]

\[
\text{Ru(bpy)}_3^{3+} + \text{NO}_3^* \rightarrow \text{Ru(bpy)}_3^{2+*} + \text{NO}_3^- \tag{2.19}
\]

\[
\text{Ru(bpy)}_3^{3+} + \text{HMTD}^* \rightarrow \text{Ru(bpy)}_3^{2+*} + \text{Product}_2 \tag{2.20}
\]

\[
\text{Ru(bpy)}_3^{3+} + \text{Ru(bpy)}_3^{3+} \rightarrow \text{Ru(bpy)}_3^{2+*} + \text{Ru(bpy)}_3^{2+} \tag{2.21}
\]

\[
\text{Ru(bpy)}_3^{2+*} \rightarrow \text{Ru(bpy)}_3^{2+} + h\nu \tag{2.22}
\]

*Scheme 2.4.* Proposed ECL mechanism of the HMTD/Ru(bpy)$_3^{2+}$ system involving ECL enhancement by AgNO$_3$ in MeCN at a Pt electrode, in which Eqs. 2.9–2.12 are direct oxidations at the electrode, Eqs. 2.13–2.15 and 2.17 are chemical reactions with electron transfers in the solution proximity to the electrode surface, Eq. 2.16 is the deprotonation of an $\alpha$-C H from HMTD$^{\ddagger}$ radical cation generated electrochemically (Eq. 2.10) and chemically (Eqs. 2.14–2.15), and Eqs. 2.18–2.21 are possible pathways to form excited state Ru(bpy)$_3^{2+*}$ species that emit light via Eq. 2.22.
An ECL mechanism of the HMTD/Ru(bpy)$_3^{2+}$ system involving ECL enhancement by AgNO$_3$ in MeCN at a Pt electrode is proposed in Scheme 2.4. In this mechanism, chemical oxidations of Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{3+}$ and HMTD$\cdot\$ to [HMTD]$^{2+}$ by Ag(II) and NO$_3^{-}$ are not included, because their contributions to the overall ECL enhancement could be minor.

Limit of Detection of HMTD

![Graph showing the relationship between [HMTD] and ECL peak intensity.](image)

*Figure 2.10.* Relationship between [HMTD] and ECL peak intensity. (a) Without addition of AgNO$_3$, and (b) with addition of 7.0 mM AgNO$_3$. Other experimental conditions were the same as in Figure 2.5. Each data point represented the mean of four separate runs.

Using above described ECL strategies, the limit of detection of HMTD in MeCN was found to be 0.15 mM in the absence of AgNO$_3$ (Figure 2.10a) and 50 µM in the presence of 7.0 mM AgNO$_3$ (Figure 2.10b), which, respectively, are ~3.3 and 10 times lower than the detection limit obtained from a method based on HPLC separation and FTIR detection.$^{10}$ Because as little as 0.50 mL of a test solution was needed for running
an ECL experiment, the absolute detection limit of HMTD using AgNO₃ as the ECL enhancing agent was calculated to be 5.2 μg. This value is much higher than that obtained from the MS-based method, probably due to the relatively high ECL background caused by AgNO₃ and a relatively large volume of test solution required for ECL studies.

Conclusions

Highly explosive compound HMTD was used as an ECL coreactant and determined with ECL in the presence of Ru(bpy)₃²⁺ and the ECL enhancing agent AgNO₃ in MeCN at a Pt electrode upon the anodic potential scanning from 0 to 2.0 V vs Ag/Ag⁺. This technique provided an easy and sensitive way for quantifying HMTD in μM concentration levels. ECL enhancement of the HMTD/Ru(bpy)₃²⁺ system by AgNO₃ was primarily ascribed to the chemical oxidations of HMTD by electrogenerated strong oxidizing agents NO₃⁻ and Ag(II) species, which resulted in the increase in [HMTD•⁺] and hence the ECL intensity. ECL data and CV digital simulations revealed that direct oxidation of Ag(I) to Ag(II) in MeCN at a Pt electrode certainly occurred, although the reaction appeared in the same potential region as NO₃⁻ oxidation. A standard reduction potential of 1.82 V vs Ag/Ag⁺ (or 2.01 V vs NHE) for the NO₃⁻/NO₃⁻ couple was estimated based on CV simulations, which is about 470 mV less positive than that estimated from a group of thermodynamic and kinetic measurements in aqueous media.
References

(2) Pumera, M. *Electrophoresis* **2008**, *29*, 269.


CHAPTER III

SELECTIVE DETERMINATION OF TRIACETONE TRIPEROXIDE USING ELECTROGENERATED CHEMILUMINESCENCE

Introduction

The increasing numbers of use of homemade explosives have been observed by the legal authorities. Among these, peroxide explosives, such as triacetone triperoxide (TATP) and hexamethylene triperoxide diamine (HMTD) are very popular among the terrorists since these compounds could be easily synthesized from the readily available chemicals.\(^1\) TATP, a white solid first synthesized by Wolffenstein in 1895,\(^2\) is one of the most sensitive explosives known with significant sensitivity towards friction, heat, and impact. Its impact sensitivity is equal to that of another high explosive, trinitrotoluene (TNT).\(^3\) The London bombing in 2005 and the attempt to bring down trans-Atlantic flight in 2001 by a ‘shoe bomber’ are the examples of the terrorist use of TATP.\(^4,5\)

TATP has no any commercial or military use as it has tendency to sublime\(^6\) and is sensitive to any kind of friction or impact which makes its movement difficult. As reviewed in Chapter I, a number of analytical techniques have been used to detect peroxide-based explosives,\(^7,8\) which include (a) mass spectrometry (MS), e.g., gas chromatographic separation coupled with mass spectrometric detection (GC/MS),\(^9-12\) desorption electrospray ionization MS (DESI/MS),\(^5,13,14\) and electrospray or laser ionization\(^15\) time of flight (TOF) MS (TOF/MS); (b) ion mobility spectrometry (IMS);\(^16-19\) (c) infrared absorption spectroscopy (IR), e.g., liquid chromatography separation-IR detection (LC/IR),\(^3,20\) GC/IR,\(^11\) and mid-IR laser spectroscopy with quantum cascade laser (QCL);\(^21-24\) (d) Raman spectroscopy;\(^10,25\) (e) luminescent techniques, e.g.,
fluorescence\textsuperscript{26-30} and chemiluminescence;\textsuperscript{31} (f) UV-vis spectrophotometry;\textsuperscript{32-35} and finally (g) electrochemistry.\textsuperscript{4,36-38}

Methods based on MS are very sensitive and can detect trace amounts of explosives but instruments are expensive and often inappropriate for field test. The results from IMS are affected by the matrices such as temperature and moisture, whereas fluorescence suffers from scattering light of luminophore impurities. Infrared, UV-vis detection techniques are often time consuming in sample preparation and are not appropriate for detection of trace amounts of explosives.

Electrogenerated chemiluminescence (ECL), a technique which produces light as a result of electrochemical reactions that take place at the electrode surface, has been considered as a powerful analytical technique because of its good selectivity, high sensitivity and fast sample analysis.\textsuperscript{39-42} ECL can detect target molecules down to picomolar concentration levels.\textsuperscript{43}

In this chapter, ECL detection and quantification of TATP as well as the differentiation of TATP from HMTD are explored at glassy carbon electrode in water-MeCN mixture solvents in the presence of added ECL emitter \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{2+} ions. The method is based on the fact that TATP contains peroxide functional groups, which, upon cathodic potential scanning, produce strong oxidizing species hydroxyl radicals (•OH) that could oxidize electrogenerated \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{+} cations to form \textit{Ru(bpy)}\textsubscript{3}\textsuperscript{2+*} excited states that emit photons.\textsuperscript{44}
Experimental Section

Chemicals

Acetonitrile (MeCN, 99.8%, HPLC grade), acetone (99.5+%, ACS reagent), sulfuric acid (95-98%, ACS reagent), sodium phosphate, dibasic (99.0%), tris-(2,2'-bipyridine)dichlororuthenium(II) hexahydrate (Ru(bpy)_3Cl_2•6H_2O, 99.95%), ammonium iron(II) sulfate hexahydrate ((NH_4)_2Fe(SO_4)_2 •6H_2O, ACS reagent), sodium azide (NaN_3, ≥99.0%), and 5,5'-dimethyl-pyrroline-N-oxide (DMPO, ≥97% for ESR-spectroscopy) from Sigma-Aldrich (St. Louis, MO); hydrogen peroxide (H_2O_2, 30%) from Fisher Scientific (Fair Lawn, NJ); sodium phosphate, monobasic monohydrate from J.T. Baker Chemicals Co. (Phillipsburg, NJ); silver nitrate (99.5%) and tetra-n-butylammonium perchlorate (TBAP, 99+%, electrochemical grade) from Fluka (Milwaukee, WI); catalase (filtered, 30,000 units/mL) from Worthington Biochemical Corp. (Lakewood, NJ) were used as received.

Synthesis and Characterization of TATP

The peroxide explosive compound, triacetone triperoxide (TATP), had to be synthesized as it is not commercially available. For this, we followed the standard procedure from the literature. 1.36 mL of H_2O_2 (30%, chilled for several hours) was put into a small beaker under ice-bath and to this, 1.9 mL of acetone (99.5+%, chilled) were added. 0.48 mL of concentrated sulfuric acid was added drop wise with constant stirring and monitoring the temperature (use of different acids may form different polymorphic forms). Temperature must be kept below 4 °C in entire process. After addition of acid the content was stirred for about 15 min and kept in a refrigerator for overnight. White solid compound was filtered, washed with water, and allowed to dry at room temperature.
before stored in a refrigerator for further use (Scheme 3.1). [CAUTION: TATP is very sensitive to heat, friction and impact so it has to be handled in small amount. Dry compound is volatile and more likely to explode so it must be stored as solution in MeCN at ~0 °C.]

Scheme 3.1. Synthesis of triacetone triperoxide (TATP).

The dried TATP was characterized by diamond crystal attenuated total reflection Fourier transform infrared (ATR-FTIR, Nicolet Nexus 470 FTIR spectrometer, Thermo Electron Corp., Madison, WI) and $^1$H NMR spectroscopy (Bruker 300 MHz NMR spectrometer).

Figure 3.1. (a) ATR-FTIR and (b) $^1$H NMR spectra of TATP.
FTIR spectra (Figure 3.1a) show a characteristic C-O stretch band at 1175 cm\(^{-1}\), which along with all other peaks and proton NMR spectra (Figure 3.1b) which show a characteristic singlet peak for all equivalent 18 protons at 1.48 ppm are consistent with the literature values.\(^{46}\) This chemical shift is very close to 1.41 ppm obtained theoretically by using ChemBiodraw Ultra 11 (CambridgeSoft Corp., Cambridge, MA). The reaction precursors produce \(^1\)H NMR peaks at 2 ppm (H\(_2\)O\(_2\) at 2.0 ppm, acetone at 2.09 ppm, and H\(_2\)SO\(_4\) at 2 ppm) as estimated theoretically using the above software. Small peaks seen in \(^1\)H NMR at 0 and 7.26 ppm were from the internal standard (TMS) and proton from incomplete deuteration of chloroform.\(^{47}\)

**Instruments Used for Electrochemical and ECL Studies**

Cyclic voltammetry (CV) was performed with an electrochemical workstation (Model 660A, CH Instruments, Inc., Austin, TX). The ECL and CV responses were simultaneously recorded with a homemade ECL instrument,\(^{40,48}\) where the CHI electrochemical workstation was combined with a photo-multiplier tube (PMT, Hamamatsu R928, Japan, biased at 700 V DC). A conventional three-electrode electrochemical cell system was used with a glassy carbon disk (3 mm diameter) as the working electrode, a platinum gauge as the counter electrode, and a Ag/Ag\(^+\) (with 10 mM AgNO\(_3\) and 0.10M TAPB in MeCN) as the reference electrode. The GC working electrode was polished before every run with 0.3-0.05 µM alumina slurry, washed with water and dried with Kim wipes tissue. The solution was degassed with nitrogen gas (Ultrapure, Nordan Smith, Hattiesburg, MS) at least 5 min to remove dissolved oxygen that could produce background ECL as the electrode was scanned in a negative potential region.
Electron Paramagnetic Resonance (EPR) Spectrometry

EPR spectra of hydroxyl radicals were recorded at a resonant frequency of 9.83 GHz, a modulation frequency of 100 kHz, and the microwave power of 20 mW using a Bruker EMX microX EPR spectrometer equipped with an ER 4119HS standard cylindrical resonator (Bruker BioSpin Corp.). The instrument was pre-calibrated using the manufacturer’s DPPH calibration sample. Hydroxyl radicals (\textsuperscript{\cdot}OH) were freshly produced chemically by Fenton reaction from 0.50 mM H\textsubscript{2}O\textsubscript{2} and 75 \textmu M ferrous ammonium sulfate in different H\textsubscript{2}O-MeCN compositions, and were trapped by 200 mM 5,5\textsuperscript{\prime}-dimethyl-pyrroline-N-oxide (DMPO) “spin trapping” agent to form DMPO/\textsuperscript{\cdot}OH “spin adduct” for EPR detection (Scheme 3.2). Experimentally, DMPO was mixed with H\textsubscript{2}O\textsubscript{2} before ferrous ammonium sulfate was added to the system. Note that the concentrations listed above were all final values in a total reaction volume of 400 \textmu L.

\[
\begin{align*}
\text{H}_2\text{O}_2 + \text{Fe(II)} & \xrightarrow{k_1} [\text{OH}^- + \text{Fe(III)}] \\
[\cdot\text{OH}] & \xrightarrow{k_2} \text{DMPO/\cdotOH}
\end{align*}
\]

Scheme 3.2. Formation of \textsuperscript{\cdot}OH radical by Fenton reaction and the DMPO/\textsuperscript{\cdot}OH adduct, where \(k_1\) and \(k_2\) are the rate constants of Fenton reaction and the spin trapping reaction, respectively.

Samples of the DMPO/\textsuperscript{\cdot}OH adduct in water-MeCN mixture were measured using a standard glass capillary (10 cm long, 2/1.2 OD/ID (mm), World Precision Instruments, Inc., Sarasota, FL) filled with the solution to a height of \(\sim\)4 cm and sealed with “Permanent Avery Glue Stic” (Office Depot, Hattiesburg, MS) at the bottom. This capillary was then placed in a normal EPR tube for spectra acquisition which took place.
exactly after 5 min of the solution preparation, unless otherwise stated. Such a “5 min time window” was necessary for solution transfer and instrument setup and tuning. The experimentally obtained EPR spectra were simulated using PEST Winsim Software (National Institute of Environmental Health Sciences, National Institute of Health, Research Triangle Park, NC) for calculating of hyperfine coupling constants.

Results and Discussion

Cyclic Voltammetry of TATP

The CV of 1.0 mM TATP (Figure 3.2a) shows that reduction of TATP is very difficult (unrecognizable reduction peak at ~ -1.0 V vs Ag/Ag+, inset in Figure 2.3). The ECL luminophore, Ru(bpy)$_3^{2+}$, however, can be readily reduced to Ru(bpy)$_3^{+}$ with a peak potential at -1.8 V vs Ag/Ag$^+$ (Figure 2.3b), suggesting that ECL could be produced only after Ru(bpy)$_3^{2+}$ reduction.

ECL Behavior of TATP

Because TATP has a poor solubility in water and Ru(bpy)$_3^{2+}$ reduction occurs at a very negative potential region (see Figure 3.2), ECL studies of TATP were first conducted in MeCN. As proposed in Scheme 3.3, up on the cathodic potential scanning, TATP could form H$_2$O$_2$ intermediate that could be reduced further to form hydroxyl radical (’OH) (Eq. 3.1). The newly formed ’OH, which is a powerful oxidant as indicated by its standard redox potential ($E^{\text{red}}_{\text{OH}^+/\text{OH}} = 1.77$~1.91 V vs NHE$^{49-51}$), could oxidize Ru(bpy)$_3^{2+}$ to Ru(bpy)$_3^{3+}$ (Eq. 3.3) and Ru(bpy)$_3^{+}$ (electrochemically reduced from Ru(bpy)$_3^{2+}$, Eq. 3.2) to Ru(bpy)$_3^{2+*}$ excited state (Eq. 3.4). Alternatively, the excited state of Ru(bpy)$_3^{2+*}$ could be generated by ion annihilation reaction (Eq. 3.5). Note that, MeCN always contains a trace amount of water (e.g., ~0.01% or ~4 mM H$_2$O for HPLC
grade MeCN) that should meet the need for H$_2$O$_2$ production from electrochemical reduction of TATP.

![CVs of (a) 1.0 mM TATP and (b) 0.60 mM Ru(bpy)$_3^{2+}$ in an electrolyte solution containing 70% volume of 0.10 M phosphate buffer (pH 7.5) and 30% volume of MeCN (i.e., 70 mM phosphate buffer in 70 (H$_2$O) : 30 (MeCN) (v/v) mixture solvent) obtained from a 3-mm diameter glassy carbon electrode at a scan rate 50 mV/s. The CV of the electrolyte solution is displayed in (c).](image)

Figure 3.2. CVs of (a) 1.0 mM TATP and (b) 0.60 mM Ru(bpy)$_3^{2+}$ in an electrolyte solution containing 70% volume of 0.10 M phosphate buffer (pH 7.5) and 30% volume of MeCN (i.e., 70 mM phosphate buffer in 70 (H$_2$O) : 30 (MeCN) (v/v) mixture solvent) obtained from a 3-mm diameter glassy carbon electrode at a scan rate 50 mV/s. The CV of the electrolyte solution is displayed in (c).

Experimentally, however, no ECL was observed from the Ru(bpy)$_3^{2+}$/TATP system in 0.10 M TBAP MeCN at bare Pt or glassy carbon electrode. To verify if the problem was really due to the poor reduction of TATP or H$_2$O$_2$ at the electrode in this solvent, several efforts were made. First, UV irradiation (254 nm UV lamp, Model ENF-40C, 0.20 AMPS from Spectronics Corp., New York) was applied to the system so that TATP can be decomposed to H$_2$O$_2$ prior to ECL detection. Second, the working electrode (Pt or glassy carbon) modified with crystalline Prussian blue (ferric ferrocyanide) which could catalytically reduce H$_2$O$_2$ was employed in the ECL testing.
Neither helped the ECL generation. Surprisingly, in MeCN even the Ru(bpy)$_3^{2+}$/H$_2$O$_2$ system did not produce any notable ECL signals. Scheme 3.4 summarizes the above observations.

\[
\text{TATP} + e \rightarrow [\text{H}_2\text{O}_2]^{+} \rightarrow \text{OH}^{*} \\
\text{Ru(bpy)}_3^{2+} + e \rightarrow \text{Ru(bpy)}_3^{+} \\
\text{Ru(bpy)}_3^{2+} + \text{OH}^{*} \rightarrow \text{Ru(bpy)}_3^{3+} + \text{OH}^{-} \\
\text{Ru(bpy)}_3^{+} + \text{OH}^{*} \rightarrow \text{Ru(bpy)}_3^{2+*} \\
\text{Ru(bpy)}_3^{+} + \text{Ru(bpy)}_3^{3+} \rightarrow \text{Ru(bpy)}_3^{2+*} + \text{Ru(bpy)}_3^{2+} \\
\text{Ru(bpy)}_3^{2+*} \rightarrow \text{Ru(bpy)}_3^{2+} + \text{hv} \\
\]

\textit{Scheme 3.3.} Proposed ECL mechanism of TATP in the presence of Ru(bpy)$_3^{2+}$ upon the cathodic potential scanning.

\textit{Scheme 3.4.} No “reductive-oxidation” type ECL was produced from TATP and H$_2$O$_2$ in MeCN in the presence of Ru(bpy)$_3^{2+}$.

When UV-irradiated TATP MeCN solution was transferred to a water-MeCN mixture medium containing Ru(bpy)$_3^{2+}$ and a suitable supporting electrolyte, strong ECL signals were observed. For example, in 70:30 (v/v) water-MeCN mixture containing 1.0
μM Ru(bpy)₃²⁺ and 70 mM phosphate buffer, 0.50 mM TATP (with UV irradiation) starts to produce ECL at ~1.5 V vs Ag/Ag⁺ and reaches the maximum at ~1.8 V vs Ag/Ag⁺ (Figure 3.3a). The ECL profile is coincident with the reduction of Ru(bpy)₃²⁺ to Ru(bpy)₃⁺ (Figure 3.3b), suggesting that TATP (or H₂O₂) must be reduced at a less negative potential region as revealed earlier in Figure 3.2. ECL generation from the Ru(bpy)₃²⁺/H₂O₂ system in water-MeCN has been reported previously.⁴⁴

![Graph](image)

*Figure 3.3.* (a) ECL and (b) CV responses of 0.50 mM TATP (with UV irradiation for 30 min) in 1.0 mM Ru(bpy)₃²⁺ water-MeCN (70:30, v/v) mixture containing 70 mM phosphate buffer at a 3-mm glassy carbon electrode with a scan rate of 50 mV/s.

Since the concentration of Ru(bpy)₃²⁺ could influence the ECL production, its optimum concentration was determined. When 0.50 mM of TATP is used (with UV irradiation), 0.60 mM Ru(bpy)₃²⁺ produces the maximum ECL response in 70:30 (v/v) water-MeCN mixture (Figure 3.4). As shown in Figure 3.5, with the exposure of TATP
MeCN solution to UV irradiation, the ECL intensity of the irradiation product (i.e., H$_2$O$_2$) increases within the first 25 min, and then remains essentially unchanged within the next 15-20 min. This suggests that under present UV irradiation conditions, i.e., with a 0.20 AMPS UV lamp at 254 nm and an experimental time scale of 45 min, TATP was

**Figure 3.4.** Effect of Ru(bpy)$_3^{2+}$ concentration on ECL generation of 0.50 mM TATP (with UV irradiation for 30 min) in 70:30 (v/v) water-MeCN containing 70 mM phosphate buffer at a 3-mm diameter glassy carbon electrode with a scan rate of 50 mV/s.

**Figure 3.5.** Effect of UV irradiation time on TATP decomposition. A 0.20 AMPS UV lamp at 254 nm was used, and the ECL of 0.50 mM TATP (with various UV irradiation intervals) was conducted in 70:30 (v/v) water-MeCN containing 0.6 mM Ru(bpy)$_3^{2+}$ and 70 mM phosphate buffer at a 3-mm diameter glassy carbon electrode with a scan rate of 50 mV/s.
gradually decomposed and H$_2$O$_2$ was relatively stable. The decomposition of H$_2$O$_2$ under UV irradiation has been reported previously.$^{33}$

Effect of Solvent Composition on ECL Intensity

Figure 3.6a shows the effect of solvent composition on the ECL peak intensity of 0.5 mM TATP (with UV irradiation for 30 min). With the addition of water to MeCN, ECL intensity increases and maximizes at a water volume of 70 % (i.e., 70:30 (v/v) water-MeCN). Because TBAP supporting electrolyte is sparely soluble in water-MeCN mixture solvent, a final concentration of 70 mM phosphate buffer was used as the supporting electrolyte.

![Figure 3.6](image_url)

*Figure 3.6. Effect of solvent composition on the ECL peak intensity of (a) 0.5 mM TATP (with UV irradiation for 30 min), and (b) 1.0 mM H$_2$O$_2$ in an electrolyte solution containing 0.6 mM Ru(bpy)$_3^{2+}$-70 mM phosphate buffer at a 3-mm diameter glassy carbon electrode with a scan rate of 50 mV/s.*

This buffer was prepared from 71 mL 0.10 M disodium phosphate and 29 mL of 0.10 M sodium phosphate, resulting in 100 mL of 0.10 M phosphate buffer solution having pH 7.5. When the irradiated 0.50 mM TATP solution is replaced with 1.0 mM H$_2$O$_2$, a very
similar profile of the ECL intensity versus solution composition was obtained (Figure 3.6b). At 70:30 (v/v) water-MeCN solutions, the ECL intensity ratio of 0.50 mM TATP to 1.0 mM H2O2 has a value of 1.4, which is consistent with the TATP UV irradiation decomposition reaction shown in Eq. 3-7.

\[
\text{TATP} \quad \{[(\text{CH}_3)_2\text{CO}_2]_3 \} + 3\text{H}_2\text{O} \rightarrow 3\text{H}_2\text{O}_2 + 3(\text{CH}_3)_2\text{O}
\]

3.7

Limit of Detection of TATP

In this section, three different modes of ECL detection of TATP, namely, UV irradiation-ECL detection, acid treatment-ECL detection, and direct ECL detection, are employed and compared.

As shown in Figure 3.7, for TATP UV irradiation-ECL detection mode, as low as 2.5 µM TATP can be detected, which is 400 and more than three times lower than that from a LC/IR based detection\textsuperscript{33} and a horseradish peroxidase treatment-based UV-vis detection method,\textsuperscript{33} respectively.

\(\text{Figure 3.7.} \ (A) \ \text{ECL intensity as a function of TATP concentration for the UV-irradiation-ECL detection scheme. (B) ECL responses obtained from different concentrations of TATP (with UV irradiation for 30 min): (a) 0, (b) 2.5 µM, and (c) 0.50 mM. All experiments were conducted in 70:30 (v/v) water-MeCN with 0.6 mM Ru(bpy)\textsubscript{3}^{2+}-70 mM phosphate buffer at a 3-mm diameter glassy carbon electrode using a scan rate of 50 mV/s.}\)
ECL detection of TATP can also be achieved after TATP is treated with acid, resulting in the formation $\text{H}_2\text{O}_2$.\textsuperscript{4,38} The ECL signal produced from obtained $\text{H}_2\text{O}_2$, however, was found to be acid concentration related. In highly concentrated acidic media, ECL was greatly quenched. With 12 mM HCl (final solution medium’s “pH” ~7), TATP produces ECL responses which are comparable to the direct detection (see below) and the limit of detection is also similar, i.e., 2.5 µM (Figure 3.8). Interestingly, as shown in Figure 3.9, acid treated TATP produces two ECL peaks: the first one at -1.8 V vs Ag/Ag\textsuperscript{+} is believed to be associated with the first electron reduction of Ru(bpy)\textsubscript{3}\textsuperscript{2+} to Ru(bpy)\textsubscript{3}\textsuperscript{+}, and the second one at -2.0 V vs Ag/Ag\textsuperscript{+} remains unclear, although it might be related to the Ru(bpy)\textsubscript{3}\textsuperscript{+}/Ru(bpy)\textsubscript{3}\textsuperscript{0} electron-transfer process. Both ECL peaks show good linear relationship with TATP concentration (Figure 3.8).

![Figure 3.8](image)

*Figure 3.8.* ECL peak intensity as a function of TATP concentration. TATP was treated with 12 mM HCl, and the ECL measurements were conducted in 70:30 (v/v) water-MeCN mixture containing 0.6 mM Ru(bpy)\textsubscript{3}\textsuperscript{2+}-70 mM phosphate buffer at a 3-mm glassy carbon electrode using a scan rate of 50 mV/s. (a) first ECL peak, and (b) second ECL peak as shown in Figure 3.9.
Figure 3.9. (a) CV and (b) ECL responses from TATP (pre-treated with 12 mM HCl) using the same experimental conditions as described in Figure 3.8.

Figure 3.10. (a) ECL and (b) CV responses of 0.50 mM TATP directly detected in 0.60 mM Ru(bpy)$_3^{2+}$ water-MeCN (70:30, v/v) mixture containing 70 mM phosphate buffer at a 3-mm glassy carbon electrode with a scan rate of 50 mV/s.

In water-MeCN mixture solvents, TATP can produce ECL directly without need of any treatment, as shown in Figure 3.10. The ECL intensity, however, is relatively
weak as compared with results obtained from UV irradiation. Figure 3.11 illustrates the relationship between the ECL peak intensity and TATP concentration, where a limit of detection of 2.5 µM is evident. Although the three ECL detection modes give the same TATP detection limit of 2.5 µM, direct detection can be completed within 5 min. In contrast, two- and seven times longer time would be required to detect the same TATP sample, should the acid treatment and the UV irradiation modes be chosen, respectively.

![Graph](image)

**Figure 3.11.** Relationship between ECL peak intensity and TATP concentration when TATP was directly detected in 70:30 (v/v) water-MeCN mixture containing 0.60 mM Ru(bpy)$_3^{2+}$-70 mM phosphate buffer at a 3-mm glassy carbon electrode with a scan rate of 50 mV/s.

EPR Spectroscopy of Hydroxyl Radical (·OH)

The stability of hydroxyl radical ·OH in various compositions of water-MeCN solvent was estimated by EPR spectroscopy. The rate constants for Fenton reaction ($k_1$, Scheme 3.2) and for hydroxyl radical trapping with DMPO ($k_2$, Scheme 3.2) have a value of 76-84,52,53 and 3.4×10$^9$ M$^{-1}$ s$^{-1}$,54 respectively. The rate constants for hydroxyl radical quenching with various species such as ·OH itself, OH$, Fe(II)$, and many organic
molecules are reported to be in the range of \(10^9-10^{10} \text{ M}^{-1} \text{ s}^{-1}\),\(^{55,56}\) which is comparable to or larger than \(k_2\). As a result, the stability of hydroxyl radical in solution could be reflected on the stability of the spin trapping adduct DMPO/•OH (Scheme 3.2), assuming that the adduct is sufficiently stable and its stability is independent of the solvent composition.

Figure 3.12. Combined EPR spectra of DMPO/•OH produced from Fenton reaction (0.50 mM H\(_2\)O\(_2\), 200 mM 5,5′-dimethyl pyrroline-N-oxide (DMPO), and 75 µM Fe(NH\(_4\)\(_2\))(SO\(_4\))\(_2\)) in different water-MeCN (v/v) solution compositions. The spectra were recorded after reactions occurred for 5 min.

Figure 3.12 shows the EPR spectra of DMPO/•OH adduct produced in a series of solution composition of water-MeCN mixture. As expected, the spectra consist of a characteristic, 1:2:2:1 quartet.\(^{54}\) Very weak EPR signals are observed from solutions
containing 5-20% (v/v) of water. With the increase in water contents, the EPR signal gradually increases and maximizes at a mixture solvent containing 70% (v/v) of water (Figure 3.13). This EPR intensity of DMPO/•OH versus H₂O volume % profile coincides with the ECL intensity of TATP and H₂O₂ versus H₂O volume % profile shown in Figure 3.6. This suggests that the stability of •OH is solvent composition dependent, which is a determining factor for ECL generation from TATP or H₂O₂. In MeCN, •OH could hardly survive, so no ECL was observed from TATP or H₂O₂. On the other hand, TATP and H₂O₂ showed their highest ECL responses in 70:30 (v/v) water-MeCN solution, because in such a medium, the •OH radical possesses its highest stability.

![Figure 3.13. EPR spectra intensity of DMPO/•OH adduct (taken from Figure 3.12) as a function of solution composition.](image)

The stability of the DMPO/•OH adduct in different water-MeCN mixture solvents was also monitored over time. The adduct was relatively stable and well-defined EPR spectra could be recorded even after Fenton reaction occurred for 2 h in 100%-50% (v/v) water-MeCN solutions. Quantitative analysis of the decay in EPR intensity after 5 and 20 min Fenton reaction reveals that ~30% decrease for every solution composition is
obtained (Table 23.1), implying that the DMPO/•OH adduct had the similar stability irrespective of solvent composition, as we assumed earlier.

**Table 3.1.** Change in EPR Intensity With Change in Solvent Composition and With Change in Time

<table>
<thead>
<tr>
<th>Solvent Composition (% of H₂O in MeCN)</th>
<th>EPR Intensity (after 5 min, ×10⁶)</th>
<th>EPR Intensity (after 20 min, ×10⁶)</th>
<th>Change in EPR Intensity (±5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.15</td>
<td>0.11</td>
<td>-27</td>
</tr>
<tr>
<td>30</td>
<td>0.90</td>
<td>0.60</td>
<td>-33</td>
</tr>
<tr>
<td>50</td>
<td>1.7</td>
<td>1.2</td>
<td>-29</td>
</tr>
<tr>
<td>70</td>
<td>2.9</td>
<td>1.9</td>
<td>-34</td>
</tr>
<tr>
<td>90</td>
<td>2.5</td>
<td>1.8</td>
<td>-28</td>
</tr>
</tbody>
</table>

*Figure 3.14. EPR spectra simulation of DMPO/•OH adduct obtained from Fenton reaction in the presence of the spin trapping agent 5,5'-dimethyl pyrroline-N-oxide (DMPO) after 5 min reaction. Solvent composition: 70:30 (v/v) water/MeCN.*

Simulations of the experimentally obtained EPR spectra confirmed that the 1:2:2:1 quartet is representative of virtually identical coupling of the free electron to the nitroxide nitrogen (aN = 14.85 G) and the beta hydrogen of the pyrroline ring (aH = 14.85 G).
G) in the DMPO/•OH adduct (Scheme 3.2). Figure 3.14 shows an example of such a simulation.

Elimination of Trace Amounts of H$_2$O$_2$ from TATP

Trace amounts of H$_2$O$_2$ from the laundry detergent could lead to the false positive ECL response. This undesired H$_2$O$_2$ has to be removed before the experiment. As demonstrated in Figure 3.15, strong ECL signals from 1.0 mM H$_2$O$_2$-0.60 mM Ru(bpy)$_3^{2+}$ system are almost completely suppressed after the addition of catalase enzyme to the solution, in which unwanted H$_2$O$_2$ is decomposed to H$_2$O and O$_2$ (Eq. 3.8), thus removing false positive ECL.

$$2\text{H}_2\text{O}_2 \xrightarrow{\text{Catalase}} 2\text{H}_2\text{O} + \text{O}_2 \quad \text{3.8}$$

![Figure 3.15](image)

*Figure 3.15.* ECL responses of 1.0 mM H$_2$O$_2$-0.60 mM Ru(bpy)$_3^{2+}$ system (a) before and (b) after the addition of 5 μL of catalase. The experiments were conducted in 1.0 mL of 70:30 (v/v) water-MeCN containing 70 mM phosphate buffer at a 3-mm diameter glassy carbon electrode with a scan rate of 50 mV/s.

Scheme 3.5 lists how TATP can be selectively detected in the presence of H$_2$O$_2$.

The test sample is first treating with catalase enzyme so that H$_2$O$_2$ contaminant is decomposed to H$_2$O and O$_2$. The remaining catalase is then deactivated by sodium azide.
(NaN₃). In this way, the electrogenerated H₂O₂ from TATP reduction would not be decomposed by the enzyme. Finally, direct ECL detection of treated TATP can be carried out in water-MeCN mixture media with added Ru(bpy)₃²⁺ species. Figure 3.16 compares the ECL responses from four different systems, confirming that the selective detection of TATP described above is operative and the added NaN₃ has no effect on the ECL generation of TATP.

Scheme 3.5. Flow-chart of the elimination of H₂O₂ from TATP and direct ECL detection of TATP.

Figure 3.16. Comparison of ECL intensities obtained from (A) 0.50 mM TATP, (b) 0.50 mM + 0.50 mM NaN₃, (C) 0.50 TATP + 0.50 mM H₂O₂, and (D) 0.50 TATP + 0.50 mM H₂O₂ + 5 μL catalase + 0.50 mM NaN₃. The experimental conditions were the same as in Figure 3.15.
Detection of TATP in the Presence of HMTD

Hexamethylene triperoxide diamine (HMTD), another common peroxide-based explosive, also has peroxide functional groups which could produce ECL in the same fashion as TATP (Figures 3.17A(a) and (b)). Moreover, cathodic ECL responses from HMTD and TATP mixture are expected to be additive, as shown in Figure 3.17A(c).

In addition to peroxide functional groups, HMTD contains tertiary amine functional groups which are capable of producing ECL in anodic potential scanning (Figure 3.17B). In other words, TATP could produce ECL on cathodic potential scanning only, whereas HMTD could produce ECL on both cathodic and anodic potential scanning (Figure 3.17).

Figure 3.17. (A) Cathodic ECL of (a) 0.50 mM HMTD, (b) 0.50 mM TATP, and (c) 0.50 mM HMTD + 0.50 mM TATP in the presence of 0.60 mM Ru(bpy)$_3^{2+}$-70 mM phosphate buffer in 70:30 (v/v) water-MeCN mixture solvent at a 3-mm glass carbon electrode with a scan rate of 50 mV/s; (B) Anodic ECL of (a) 0.50 mM TATP, (b) 0.50 mM HMTD, and (c) 0.50 mM TATP + 0.50 mM HMTD, using the same experimental conditions as in (A).

Conclusions

The highly explosive TATP was determined with cathodic scanning ECL in the presence of Ru(bpy)$_3^{2+}$ at glassy carbon working electrode. Solvent composition has
profound effect on the ECL generation, which was found to be associated with the stability of electrogenerated hydroxyl radicals. Optimum ECL can be produced when water-MeCN mixture solvent has a volume ratio of 70:30, which corresponds to the strongest EPR signal observed from the DMPO/•OH adduct generated in the same solvent composition. Although UV irradiation, acid treatment, and direct ECL methods provide the same TATP detection limit of 2.5 μM, direct ECL approach is much fast (~5 min) and easy to operate. Elimination of contaminated H₂O₂ from TATP with catalase, and the differentiation of TATP from HMTD have also been accomplished in the studies presented in this chapter.
References


CHAPTER IV
DETECTION OF TRINITROTOLUENE BY ELECTROGENERATED CHEMILUMINESCENCE QUENCHING METHOD

Introduction

2,4,6-Trinitrotoluene (TNT) is an odorless solid that exists as colorless orthorhombic crystals or yellow monoclinic needles but does not occur naturally in the environment.\(^1\) The groundwater or seawater contamination has occurred due to the production, storage, testing, and disposal of explosives at military installations, resulting in the environmental problems because these toxic and persistent compounds can leach from soil.\(^2\text{-}^4\) At the end of World War II, an estimated 300,000 tons of explosives (mainly TNT) were disposed to the sea.\(^1\) TNT is considered as toxic as it produces toxic and mutagenic effects to life including human being.\(^5\) TNT is a flammable solid that has a melting point of 355 K and autoignites at 748 K.\(^6\) It is a prime component in a majority of munitions in use by the military and the terrorist forces around the world.\(^7\)

The methods with ability for rapid on-site detection of TNT are highly desired. Currently, several methods of detection of TNT are used, which include those based on fluorescence,\(^6\text{-}^8\text{-}^{18}\) amperometry,\(^19\text{-}^20\) surface plasmon resonance,\(^5\text{-}^21\text{-}^29\) mass spectrometry,\(^30\text{-}^{35}\) chromatography,\(^36\text{-}^{40}\) Raman spectroscopy,\(^41\text{-}^{45}\) and ion mobility spectroscopy.\(^46\text{-}^{47}\) In addition to amperometry, other electrochemical techniques have also been used to detect TNT.\(^48\text{-}^{51}\) Each individual detection method described above has its own advantages, but some drawbacks remain. For example, fluorescence techniques generally suffer from the luminescent impurities, the ion mobility spectroscopic technique suffers from the matrix effects, and chromatographic separation-mass
spectrometric detection methods require sample preparation and are not appropriate for field tests. These techniques use expensive instruments that frequently require skilled manpower to operate. The immunoassay-based electrogenerated chemiluminescence (ECL) technique has been used to detect TNT in soil and water sample,\textsuperscript{52-54} which is very sensitive and selective, but requires extra effort in sample preparation. Methods based on ECL quenching have been recently employed in the detection and quantification of phenols, hydroquinone, and catechols.\textsuperscript{55,56} Because each nitro group in TNT could undergo sequential 4e\textsuperscript{−} to 6e\textsuperscript{−} reduction to form hydroxylamine and amine within a potential range of 0 to ~-1.0 V vs. SCE,\textsuperscript{57-62} TNT could be used to quench or inhibit the ECL signals generated from a known “oxidative-reduction” type standard such as the Ru(bpy)\textsubscript{3}\textsuperscript{2+}/TPrA (TPrA = tri-\textit{n}-propylamine) system. As a result, a simple and inexpensive method for field detection of TNT may be developed.

When the Ru(bpy)\textsubscript{3}\textsuperscript{2+}/TPrA system is used, both Ru(bpy)\textsubscript{3}\textsuperscript{2+} and TPrA are oxidized upon the anodic potential scanning:

\[
\begin{align*}
\text{TPrA} - e & \rightarrow \text{TPrA}^{++} \\
\text{Ru(bpy)}_3\textsuperscript{2+} - e & \rightarrow \text{Ru(bpy)}_3\textsuperscript{3+}
\end{align*}
\]

4.1 4.2

TPrA\textsuperscript{*} free radicals are produced after the deprotonation of the newly produced TPrA\textsuperscript{++}:

\[
\text{TPrA}^{++} \xrightarrow{\text{H}^+} \text{TPrA}^* 
\]

4.3

The TPrA\textsuperscript{*} radical is a strong reducing agent with a redox potential of ~-1.7 V vs SCE,\textsuperscript{63} and is the key species of ECL generation. In the absence of TNT, the TPrA\textsuperscript{*} radical reduces Ru(bpy)\textsubscript{3}\textsuperscript{2+} to Ru(bpy)\textsubscript{3}\textsuperscript{+} that annihilates with Ru(bpy)\textsubscript{3}\textsuperscript{3+} to form the excited state Ru(bpy)\textsubscript{3}\textsuperscript{2+*} that emits light.
\[ \text{Ru(bpy)}_3^{2+} + \text{TPrA}^* \rightarrow \text{Ru(bpy)}_3^+ + \text{P1} \] 4.4

\[ \text{Ru(bpy)}_3^{3+} + \text{Ru(bpy)}_3^{3+} \rightarrow \text{Ru(bpy)}_3^{2+}^* + \text{Ru(bpy)}_3^{2+} \] 4.5

\[ \text{Ru(bpy)}_3^{2+}^* \rightarrow \text{Ru(bpy)}_3^{2+}^* + \text{hv} \] 4.6

Alternatively, the excited state \( \text{Ru(bpy)}_3^{2+}^* \) can be produced via the following process:

\[ \text{Ru(bpy)}_3^{3+} + \text{TPrA}^* \rightarrow \text{Ru(bpy)}_3^{2+}^* + \text{P1} \] 4.7

In the presence of TNT, however, part or the entire portion of the TPrA\(^*\) radicals could be consumed by TNT, resulting in significant decrease or complete quenching of the initial ECL signals of the \( \text{Ru(bpy)}_3^{2+}/\text{TPrA} \) system:

\[ n\text{TPrA}^* + \text{TNT} \rightarrow \text{TNT}^{-} + \text{P2} \] 4.8

Additionally, \( \text{Ru(bpy)}_3^{3+}^* \), another key species for ECL generation (Eq. 4.5) could be consumed by TNT:

\[ n\text{Ru(bpy)}_3^{3+} + \text{TNT} \rightarrow \text{Ru(bpy)}_3^{2+} + \text{TNT}^{-} \] 4.9

which leads to further decrease in ECL intensity.

**Experimental Section**

**Chemicals**

The chemicals used in this study were: acetonitrile (MeCN, 99.8%, HPLC grade), tris-(2,2'-bipyridine)dichlororuthenium(II) hexahydrate (Ru(bpy)\(_3\)Cl\(_2\)•6H\(_2\)O, 99.95%), and tri-\(n\)-propylamine (TPrA, 99+% (all from Sigma-Aldrich, St. Louis, MO); 2,4,6-trinitrotoluene (TNT, 99.6%, 1000 µg/mL, Supelco analytical, PA); sodium phosphate, monobasic monohydrate (J.T. Baker Chemicals Co., Phillipsburg, NJ); and silver nitrate (99.5 %) and tetra-\(n\)-butylammonium perchlorate (TBAP, 99+%,}
electrochemical grade) (both from Fluka, Milwaukee, WI). All chemicals were used as received.

**Electrochemical and ECL Studies**

The ECL and CV responses were recorded simultaneously with a homemade ECL instrument combined with an electrochemical workstation, which has been described in detail in previous chapters. Note that the test solution was used without purging with nitrogen as the electrode potential was scanned in anodic direction and oxygen from air had no effect on ECL production.

**Fluorescence Studies**

Fluorescence experiments were carried out on a PTI Qunata Master™ 40 intensity based spectrofluorometer, with a slit width of 1.00 nm, excitation wavelength at 350 nm, and an emission wavelength range from 500 to 690 nm.

**Results and Discussion**

ECL generation from the Ru(bpy)$_3^{2+}$/TPrA system

Figure 4.1 shows the CV and ECL responses of 1.0 µM Ru(bpy)$_3^{2+}$-25 mM TPrA in MeCN containing 0.10 M TBAP at Pt electrode with a scan rate of 50 mV/s. TPrA is oxidized at ~0.6 V vs Ag/Ag$^+$ (10 mM AgNO$_3$ in 0.10 M TBAP MeCN) and the generation of ECL light starts from 0.9 V vs Ag/Ag$^+$ at which Ru(bpy)$_3^{2+}$ gets oxidized to Ru(bpy)$_3^{3+}$. The optimum concentration of ECL coreactant was determined by running an ECL experiment with 1.0 µM Ru(bpy)$_3^{2+}$ and different concentrations of TPrA with a Pt electrode. As shown in Figure 4.2, the maximum ECL response occurs with a TPrA concentration of 25 mM, which is in good agreement with the data reported previously.$^{64}$
Figure 4.1. CV and ECL of 1.0 µM Ru(bpy)$_3^{2+}$-25 mM TPrA in MeCN containing 0.10 M TBAP at a 2-mm diameter Pt disk electrode with a scan rate of 50 mV/s.

Figure 4.2. Concentration dependence of TPrA on ECL of the Ru(bpy)$_3^{2+}$/TPrA system. The experiment was conducted at a 2-mm Pt electrode with a scan rate of 50 mV/s using 1.0 µM Ru(bpy)$_3^{2+}$-0.10 M TBAP MeCN solutions.

This concentration of TPrA, therefore, was used for the entire experiment discussed below, unless otherwise indicated.
The ECL quenching experiment by TNT was carried out initially with 1.0 µM Ru(bpy)$_3^{2+}$ and 25 mM TPrA in MeCN containing 0.10 M TBAP. As shown in Figure 4.3, the ECL emission of the Ru(bpy)$_3^{2+}$/TPrA system is gradually decreased with successive addition of TNT (stock solution: 1000 µg per mL in MeCN). The ECL is reduced by ~14% (Figure 4.3b) and 68% (Figure 4.3c) with a final TNT concentration of 4.4 and 110 µM, respectively.

Figure 4.3. ECL quenching of the Ru(bpy)$_3^{2+}$ (1.0 µM)/TPrA (25 mM) by TNT at a final concentration of (a) 0, (b) 4.4, and (c) 110 µM. Working electrode: 2-mm diameter Pt, scan rate: 50 mV/s. For clarity, only forward scans of the ECL are plotted.

To examine if the ECL could be completely quenched by TNT, various concentrations of Ru(bpy)$_3^{2+}$ and TPrA were tested. When Ru(bpy)$_3^{2+}$ concentration was down to nanomolar levels, complete quenching was observed. For example, in a 5 nM Ru(bpy)$_3^{2+}$-25 mM TPrA system (Figure 4.4a), addition of 44, 88, and 132 µM of TNT results in ~76% (Figure 4.4b), 90% (Figure 4.4c), and 100% (Figure 4.4d) of the ECL quenching, respectively. Studies on the effect of lowing TPrA concentration on the ECL quenching (with 1.0 µM Ru(bpy)$_3^{2+}$) revealed that with the decrease of TPrA
concentration, TNT quenching efficiency was slightly increased. However, at low TPrA concentrations (≤10 mM), the ECL intensity of the system became weak (Figure 4.2), which made the experimental observations difficult. Furthermore, under low TPrA concentrations, no complete quenching of ECL with a high TNT concentration of 132 μM was observed. These data suggest that the quenching process shown in Eq. 4.8 is probably a relatively slow electron transfer process with respective to that of Eq. 4.9. In other words, ECL quenching by TNT via Ru(bpy)$_3^{2+}$ consumption is more favorable than direct TPrA$^-$ free radical consumption. If that is the case, under constant TPrA and TNT concentrations, ECL quenching efficiency should increase with the increase in Ru(bpy)$_3^{2+}$ concentration.

Figure 4.4. ECL quenching by TNT in the system containing 5 nM Ru(bpy)$_3^{2+}$-25 mM TPrA-0.10 M TBAP in MeCN with added TNT concentrations of (a) 0, (b) 44, (c) 88, and (d) 132 μM. A 2-mm diameter of Pt electrode and a scan rate of 50 mV/s were used for all tests.
Figure 4.5 illustrates the results of such an example, where the ECL is quenched by ~38% at 1.0 µM Ru(bpy)$_3^{2+}$, and ~45% at 10 µM Ru(bpy)$_3^{2+}$ after the system is added with 4.4 µM TNT (Figure 4.5b). Notably, increase in Ru(bpy)$_3^{2+}$ concentration linearly increases the ECL intensity with and without TNT, but the presence of constant amount of TNT shows quenching effect throughout the experiment, which confirms quenching effect is more related to the Ru(bpy)$_3^{2+}$ than to TPrA.

As shown in Figure 4.6, a linear decrease in ECL intensity of the standard Ru(bpy)$_3^{2+}$ (1.0 µM)/TPrA (25 mM) system is evident with the addition of TNT. This quenching method provides a TNT detection limit of 4.4 µM.

Fluorescence quenching of Ru(bpy)$_3^{2+}$ by TNT

*Figure 4.5. Ru(bpy)$_3^{2+}$ concentration effect on ECL (a) without, and (b) with 4.4 µM TNT addition to the MeCN solution containing 25 mM TPrA-0.10 M TBAP. A 2-mm diameter of Pt electrode and a scan rate of 50 mV/s were used for all tests.*
The fluorescence quenching study was carried out with 1.0 µM Ru(bpy)$_3^{2+}$ in MeCN with a quartz cuvette. The test solution was excited at 350 nm and Ru(bpy)$_3^{2+}$ produced the fluorescence emission peak at 620 nm. The addition of TNT results in a decrease in fluorescent emission of the test solution as shown in Figure 4.7A.

![Graph showing the relationship between ECL intensity and TNT concentration](image)

*Figure 4.6.* Relationship of ECL intensity with the TNT concentration in the system containing 1.0 µM Ru(bpy)$_3^{2+}$-25 mM TPrA-0.10 M TBAP in MeCN at a 2-mm Pt electrode at a scan rate of 50 mV/s.

The decrease in fluorescence emission with 110 µM TNT was about 42% of the emission of Ru(bpy)$_3^{2+}$ without TNT added. In order to verify the dilution effect by the addition of TNT which was in MeCN, the control experiment was carried out with the same concentration of Ru(bpy)$_3^{2+}$ and similar volumes of MeCN were added to the test solution and fluorescence emissions were recorded. The control experiment produces the results (Figure 4.7B) that suggest the effect of dilution on fluorescence emission is negligible and within the range of experimental errors.
Figure 4.7. Fluorescence quenching of 3.0 mL of 1.0 μM Ru(bpy)$_3^{2+}$ in MeCN by TNT with 1.0 cm quartz cuvette and an excitation at 350 nm. (A) TNT concentration dependence, and (B) MeCN dilution effect.

**Calculation of Quenching Constant**

The quantum yield of ECL (or fluorescence) of Ru(bpy)$_3^{2+}$ in MeCN in the absence of quenching agent ($\Phi^0$) is the rate at which the excited state decays via ECL (or fluorescence) over sum of the rates of its decay by ECL (or fluorescence) and non-radiative mechanism as:

$$\Phi^0 = \frac{K_{ECL(f)}[\text{Ru(bpy)}_{3}^{2+*}]}{K_{ECL(f)}[\text{Ru(bpy)}_{3}^{2+*}] + K_{nr}[\text{Ru(bpy)}_{3}^{2+*}]}$$

$$= \frac{K_{ECL(f)}}{K_{ECL(f)} + K_{nr}}$$

4.10

where $K_{ECL(f)}$ and $K_{nr}$ stand for the rate constants for ECL (or fluorescence) and nonradiative decay of the excited state Ru(bpy)$_3^{2+*}$. In the presence of quenching agent like TNT, the quantum yield could be expressed as:
where $K_q$ is the quenching constant of ECL (or fluorescence). By dividing Eq. 4.9 by Eq. 4.10 we get,

$$\Phi = \frac{K_{ECL(0)} [\text{Ru(bpy)}_3^{2+*}]}{K_{ECL(0)} [\text{Ru(bpy)}_3^{2+*}] + K_{nr} [\text{Ru(bpy)}_3^{2+*}] + K_q [\text{TNT}][\text{Ru(bpy)}_3^{2+*}]} \quad 4.11$$

$$= \frac{K_{ECL(0)}}{K_{ECL(0)} + K_{nr} + K_q [\text{TNT}]}$$

This is Stern-Volmer equation, in which the constant terms give the slope of a plot and is called Stern-Volmer constant ($K_{SV}$). $I$ and $I^0$ stand for the ECL (or fluorescence) intensities with and without quenching agent, TNT. The reciprocal of ($K_{ECL(0)} + K_{nr}$) gives the life time ($\tau$) of $\text{Ru(bpy)}_3^{2+*}$ excited state.

$$\tau = \frac{1}{K_{ECL(0)} + K_{nr}} \quad 4.13$$

**Figure 4.8.** Stern-Volmer plot of ECL quenching by TNT. See Figure 4.6 for experimental conditions.
On the basis of data shown in Figure 4.6 and Figure 4.7A, the Stern-Volmer plots of ECL and fluorescence quenching by TNT can be plotted as shown in Figure 4.8 and Figure 4.9, respectively. The slopes of the Stern-Volmer plots for ECL and fluorescence quenching were calculated to be $2.0 \times 10^4$ M$^{-1}$ (Figure 4.8) and $5.2 \times 10^3$ M$^{-1}$ (Figure 4.9), respectively. The quenching constant ($K_q$) calculated with Stern-Volmer constants and life time of Ru(bpy)$_3^{2+*}$ excited state in MeCN (0.562 µs)$^{67}$ came out to be $3.5 \times 10^{10}$ M$^{-1}$s$^{-1}$ for ECL quenching and $9.2 \times 10^9$ M$^{-1}$ s$^{-1}$ for fluorescent quenching. The Stern-Volmer plots suggest that the ECL quenching is about 4 times more efficient compared to the fluorescence quenching with TNT and both of these quenching processes most likely follow the dynamic process.$^{66}$ Besides the fluorescence quenching (or energy-transfer quenching, Eq. 4.14), ECL quenching of the Ru(bpy)$_3^{2+}$/TPrA system by TNT also involves processes shown in Eqs. 4.8 and 4.9. As a result, ECL quenching is more efficient than fluorescence quenching for the system reported in this chapter.
Ru(bpy)$_3^{2+*}$ + TNT $\rightarrow$ Ru(bpy)$_3^{2+}$ (TNT)$_{\text{high energy}}$

Conclusions

The high explosive compound 2,4,6-trinitrotoluene (TNT) was used as an ECL quenching agent in the Ru(bpy)$_3^{2+}$/TPrA system. The TNT was found to quench both ECL and fluorescence emission of Ru(bpy)$_3^{2+*}$ in MeCN. This detection technique provides a simple and easy way of detecting TNT at lower micromolar levels. It was realized that the Ru(bpy)$_3^{2+}$/TPrA ECL was quenched by depleting Ru(bpy)$_3^{+}$ and TPrA$^-$ free radical from the system which otherwise would have produced ECL light as well as excited state energy transfer process as suggested by the fluorescence studies.
References


CHAPTER V
ELECTROCHEMICAL DETECTION OF PENTAERYTHRITOL TETRANITRATE

Introduction

The detection of explosive compounds has become very crucial because of its increasing use by the terrorist groups in the places of public importance, such as airports, train stations, power stations, governmental buildings, etc. The explosive detection plays the key role in investigating the crime scenes and counter-terrorism act of screening in the airport check points. Since the terrorist attacks of September 11, 2001, the detection of high explosives has been in the forefront of the analytical chemists. As a result, the development of analytical techniques for trace-level detection of explosives has always been in need with high sensitivity and selectivity. Pentaerythritol tetranitrate (PETN) is one of the common high explosives used in terrorist attacks because this can be used with plastic filler and molded for concealment—the infamous ‘shoe bomber’ had concealed PETN in his shoes. PETN attracts considerable interest as it can be converted to a sheet form or forms Semtex mixed with RDX, an example of plastic explosives.

There are several methods of detection of high explosives such as PETN, namely, mass spectrometry, ion mobility spectrometry, spectroscopic methods such as Raman spectroscopy, Infra red, fluorescence, terahertz, chromatographic, and electrochemical methods. The mass spectrometric method obviously is a sensitive method for detecting explosives but requires a skilled operator as well as expensive instrument which are not appropriate for field detection. The ion mobility spectrometry (IMS) is sensitive and can be used for trace detection but has been associated with the competitive ion/molecule
reaction with matrix, the response is sensitive to the instrument contamination with long
clearance time of instrument.\textsuperscript{43,44} The Raman spectroscopy suffers from the fluorescent
impurities,\textsuperscript{45} and high background response.\textsuperscript{46} There is an urgent need of an analytical
method of detection high explosives with high sensitivity and portability for field tests.

Electrochemical methods including amperometric method coupled with
electrophoresis\textsuperscript{41} and immunoassay-based electrogenerated chemiluminescence (ECL)
with Ru(bpy)\textsubscript{3}\textsuperscript{2+} as luminophore and electrochemically generated H\textsubscript{2}O\textsubscript{2} as a coreactant\textsuperscript{47}
have been used to detect nitrate esters. The immunoassay-based technique requires
tedious effort and time for sample preparation. The direct and easy-to-operate and
portable method is highly desired. In the present work, detection and quantification of
PETN by cyclic voltammetry (CV) will be reported. This method is based on the fact the
nitro group in PETN is electrochemically reduced at the surface of working electrode
when scanned cathodically from -0.3 to -1.05 V vs Ag/Ag\textsuperscript{+}. Based on the literature each
nitro group undergoes two electron process as suggested by the possible mechanism:\textsuperscript{48}

\begin{align*}
RONO_2 + 2H_2O^+ + 2e & \rightarrow RONO + 3H_2O \quad 5.1 \\
RONO_2 + 2e & \rightarrow RO^- + NO_2^- \quad 5.2 \\
RO^- + H_2O & \rightarrow ROH + OH^- \quad 5.3 \\
RONO_2 + 2e & \rightarrow R^- + NO_3^- \quad 5.4 \\
R^- + H_2O & \rightarrow RH + OH^- \quad 5.5
\end{align*}

\textit{Scheme 5.1.} Mechanism of reduction of organic ester.
Neither nitrite ester nor hydrocarbon were observed in the reduction process and only alcohol and nitrite ions were observed \(^{48}\), only the processes involving Eqs.5.2 and 5.3 become apparent.

**Experimental Section**

*Chemicals*

Sulfuric acid (95-98%, ACS reagent), nitric acid (70 %, ACS reagent), and pentaerythritol (99+%) were purchased from Sigma-Aldrich (St. Louis, MO). Tetra-\( n \)-butylammonium perchlorate (TBAP, 99+, electrochemical grade) and silver nitrate (99.5%) were from Fluka (Milwaukee, WI). Sodium carbonate and urea were purchased from J.T. Baker Inc. (Phillipsburg, NJ). All chemicals were used as received.

**Synthesis and characterization of pentaerythritol tetranitrate (PETN)**

Concentrated nitric acid (14 mL) was chilled to 0 °C in an ice bath for half an hour and 4.0 g of pentaerythritol was dissolved slowly with constant stirring. After complete dissolution of pentaerythritol, concentrated sulfuric acid was added dropwise and 0.03 g of urea was added to this mixture which could remove NO or NO\(_2\) produced. This mixture left to stir at 0 °C for 2 h.

The precipitate, as shown in Scheme 5.2, was obtained after mixing reaction mixture in ice cold water. This precipitate was filtered with excess of water and sodium carbonate solution (0.10 M) to remove excess acid. Then the precipitate was dissolved in acetone (250 mL) by heating in a water bath at 40 °C. PETN was recrystallized in a mixture of ethanol (75 mL) and water (100 mL) in 2 h. White needles of PETN were obtained and washed with ethanol.
Scheme 5.2. Synthesis of Pentaerythritol Tetranitrate (PETN).

PETN was characterized by proton NMR (\(^1\)H NMR) where all equivalent protons produced NMR signal at 4.7 ppm which was consistent with the literature\(^49\) as shown in Figure 5.1

Figure 5.1. \(^1\)H NMR of PETN taken in deuterated acetonitrile (CD\(_3\)CN).

FTIR spectra were also taken for starting material, i.e., pentaerythritol and PETN, which could conclude the formation of PETN. The characteristic peak for OH group of starting material at 3350 cm\(^{-1}\) disappeared for PETN which lacks that OH group.
Electrochemical Studies

The cyclic voltammetry (CV) was carried out with an electrochemical workstation, model 660A (CH Instruments, Austin, TX). The conventional three electrode system was used with test solution (1.0 mL) in a small glass vials. The working electrode used was a silver wire that had an exposure area of ~1 mm². Reference and counter electrodes were Ag/Ag⁺ (10 mM AgNO₃, 0.10 M TBAP in MeCN) and Pt gauze, respectively. The solution was purged with nitrogen (ultrapure, Nordan Smith, Hattiesburg, MS) since the potential scanning was done in cathodic direction.

Results and Discussion

Cyclic Voltammetric Studies of PETN

The cyclic voltammetric (CV) responses were recorded with a silver wire as working electrode scanned between -0.3 to -1.05 V vs Ag/Ag⁺. Other working electrodes including Pt, glassy carbon, and gold were tested, but none gave any meaningful electrochemical response. An irreversible reduction wave of PETN at around -0.9 V vs
Ag/Ag⁺ was observed (Figure 5.3), which may correspond to the reduction of part of the four nitro functional groups in PETN. However, the exact number of electron-transfer involved is unclear, as the blank shows a large background current (Figure 53a).

Figure 5.3. Forward scans of cyclic voltammetry: (a) 0.10 M TBAP in MeCN as blank, (b) 25 µM PETN in MeCN, and (c) 600 µM PETN in MeCN with 0.10 M TBAP as supporting electrolyte at a silver wire working electrode (exposure area: ~1 mm²). Scan rate was 50 mV/s. For clarity, the reverse scans were not included in the plot.

The CV response of PETN in MeCN, as shown in Figure 5.3, was weak and it did not increase with the increase of PETN concentration as expected. This is perhaps due to the fact that PETN is a significantly condensed material with a very high density of 1.77 g/cm³ at 20 °C, which may prevent it from the effective attack of electrons. Figure 5.4 shows a dynamic range of 25 µM to 1.0 mM, with a detection limit of PETN at ~25 µM. Among the available methods of detection of PETN this current electrochemical technique seems to be inexpensive, portable, fast, and easy to handle. The methods based on mass or ion mobility spectrometry are expensive and cumbersome in handling the experiment.
Future Work

Immunoassay based ECL detection of PETN with TPrA as coreactant and Ru(bpy)$_3^{2+}$ as ECL luminophore could give very high ECL response$^{50}$ compared to the one with electrochemically generated hydrogen peroxide from dissolved oxygen as coreactant.$^{42}$ In this method, Ru(bpy)$_3^{2+}$ and the antibody specific to PETN could be attached to the surface of functionalized polystyrene bead and the same antibody to the magnetic bead. When the PETN sample is added to the solution containing polystyrene bead with both Ru(bpy)$_3^{2+}$ and PETN antibody and magnetic bead with PETN antibody, the sandwich-type assay is formed as shown in Scheme 5.2:
Scheme 5.3. Immunoassay based ECL detection of PETN.

When Ru(bpy)$_3^{2+}$/PETN antibody attached polystyrene beads and PETN antibody attached magnetic beads are mixed in the presence of PETN analyte which acts as antigen, a sandwich type assay is formed. This mixture is then brought to the magnet where only sandwich type assay will be attracted to the magnet because of magnetic bead. The unbound Ru(bpy)$_3^{2+}$-polystyrene beads will be separated and washed from the solution with magnet in place ensuring that unbound Ru(bpy)$_3^{2+}$ is completely removed. In ECL experiment, more Ru(bpy)$_3^{2+}$ means high ECL response, which means more PETN present in the test solution, as this PETN is responsible for the sandwich type assay formation.

Conclusion

A simple electrochemical method using cyclic voltammetry proved to be a sensitive method in detecting PETN in MeCN with silver as the working electrode. Scanned in cathodic direction from -0.3 to -1.05 V vs Ag/Ag$^+$, PETN was reduced around -0.9 V vs Ag/Ag$^+$. With this method micro molar concentrations of PETN were detected, with a limit of detection of 25 µM.
References


CHAPTER VI

CONCLUDING REMARKS

The use of explosive materials by the terrorists has become a serious threat to the modern world and there is an urgent need of analytical techniques that can be used to sensitively detect and quantify them. The techniques that make use of portable and inexpensive devices are highly desirable for field test of the explosive materials that could be confiscated from the suspect especially in the transportation hubs.

Electrogenerated Chemiluminescence (ECL) is normally a nondestructive method of detection and quantification of analytes and very sensitive especially used as the Ru(bpy)$_3^{2+}$/TPrA system (Ru(bpy)$_3^{2+}$ = tris-(2,2′-bipyridine)ruthenium (II) cation and TPrA = tri-$n$-propylamine). Peroxide-based explosives such as hexamethylene triperoxide diamine (HMTD) and triacetone triperoxide (TATP), which are very popular among terrorists because of their simple synthesis from commercially available chemicals, have proved to be sensitively and selectively detectable with ECL technique similar to the Ru(bpy)$_3^{2+}$/TPrA system.

HMTD has three tertiary amine as well as three peroxide functional groups. These functional groups can be exploited as coreactant for ECL as common coreactants such as TPrA and hydrogen peroxide (H$_2$O$_2$). HMTD produces ECL response with Ru(bpy)$_3^{2+}$ on anodic potential scanning which leads to its detection by this method. The ECL response was enhanced by the addition of AgNO$_3$ which on oxidation produces Ag(II) ions and NO$_3^-$ radicals, both of which are strong oxidizing agents and contribute toward chemical oxidation of HMTD. This chemical oxidation of HMTD will add upon the amount of HMTD$^{+}$ cation produced by direct electrochemical oxidation at the electrode surface,
which will deprotonate to HMTD’ free radical and react with Ru(bpy)$_3^{2+/3+}$ and produce higher ECL response.

Another peroxide-based high explosive, TATP, was detected by ECL technique. This contains three peroxide functional groups so it was used as coreactant similar to H$_2$O$_2$ that on cathodic potential scanning produced hydroxyl radical ‘OH as does H$_2$O$_2$. When negative potential was applied, both Ru(bpy)$_3^{2+}$ and TATP were reduced to Ru(bpy)$_3^+$ and ‘OH radical in mixture of solvents, i.e., water and MeCN. In pure MeCN, TATP did not produce any ECL response, which could be due to lack of formation of stable ‘OH radicals as verified by EPR spectroscopy. Since the explosive compound was insoluble in water so the use of mixed solvents was needed.

In contrast to direct ECL detection method, the ECL technique can also be used to detect the high explosive materials by ECL quenching, which is a kind of indirect method. The explosive material, which can quench ECL response of a well known and very efficient Ru(bpy)$_3^{2+}$/TPrA system, could be determined based on the extent of quenching. This quenching can be due to excited state energy transfer, depletion of the species (i.e., TPrA’ free radical and Ru(bpy)$_3^+$) which otherwise would produce excited state responsible for ECL light production. High explosive, 2,4,6-trinitrotoluene (TNT) was detected by ECL quenching method. TNT quenched ECL by depletion of chemically produced Ru(bpy)$_3^+$ (from Ru(bpy)$_3^{2+}$ reduction by TPrA’ free radical) and TPrA’ free radical that are responsible of formation of excited state Ru(bpy)$_3^{2+*}$ for light emission. TNT was also able to quench excited state Ru(bpy)$_3^{2+*}$ by energy transfer, which was verified by the fluorescence experiment. The quenching constant calculated using Stern-
Volmer equation was comparable to the literature value and the limit of detection of TNT was 4.4 µM.

Electrochemical method could also be used to detect another stable explosive compound, pentaerythritol tetranitrate (PETN), which is used in making plastic explosives. Cyclic voltammetry can detect PETN by measuring reduction current with silver wire as working electrode in MeCN. When negative potential was applied, PETN was reduced probably to nitrite ions. The limit of detection of PETN by electrochemical method using CV was as low as 25 µM.
APPENDIX
RESPONSE TO DR. ALVIN A. HOLDER’S COMMENTS

Q1: Page 58: The yields of all products should be given if the products are all novel in nature. Please note this, here and elsewhere. In general, can the candidate identify the peroxo stretching frequency in the FTIR spectrum? It is a common feature for such compounds.

A1: These compounds are not novel in nature so percentage yields are not reported. In FTIR spectrum, there are distinct peaks for peroxo stretching at 800 and 900 cm\(^{-1}\) for C-O and O-O (in R\(_2\)COO) for both the peroxide explosives, namely, hexamethylene triperoxide diamine (HMTD) and triacetone triperoxide (TATP). We compared the C-O stretch at ~1200 cm\(^{-1}\) with the literature value.

Q2: Figure 2.3. The candidate should acquire a square wave voltammogram to account for the shoulder around +1.5 V for [Ru(bpy)\(_3\)]Cl\(_2\) in MeCN. Is it possible to form a Ru(IV) species? Can the candidate please clarify? Based on this note, the candidate should explain how many oxidation states there are for ruthenium. What are the common oxidation states of ruthenium?

A2: Further oxidation of Ru(bpy)\(_3\)\(^{3+}\) to Ru(bpy)\(_3\)\(^{4+}\) at ~1.5 V has never occurred either in aqueous or in acetonitrile, although Ru(bpy)\(_3\)\(^{2+}\) can be reduced with three one-electron processes. However, in the latter case, electrons are added to the bpy ligand rather than ruthenium. (Tokel-Takovyan, N. E.; Hemingway, R. E.; Bard, A. J. J. Am. Chem. Soc. 1973, 95, 6582-6589; Kanoufi, F.; Zu, Y.; Bard, A. J. J. Phys. Chem. B 2001, 105, 210-216).
Barnard and Bennett (http://www.platinummetalsreview.com/dynamic/article/view/48-4-157-158) have suggested two types of oxidation states for ruthenium: low oxidation states and high oxidation states.

Low oxidation states go up to +III and high oxidation states range from +IV to +VIII, which all depend on what ruthenium binds with.

(http://www.webelements.com/ruthenium/compounds.html)

**Q3**: The candidate should give a thorough explanation for the non-linearity in the Stern-Volmer plot. Is the quenching constant a second-order rate constant?

**A3**: The Stern-Volmer quenching can be of two types: dynamic and static quenching. In dynamic quenching, the quencher molecule has to reach to the excited molecule where excited state energy transfer occurs, so this is diffusion controlled process. In static quenching, the quencher molecule forms a complex with the emitter which does not emit. The Stern-Volmer plot follows the linearity in both cases. When both dynamic and static quenching operate, the plot deviates toward the positive direction.

There is a way to determine whether the process is dynamic or static. When the temperature of the system increases, diffusion is favored where dynamic quenching is favorable and the slope of Stern-Volmer plot increases whereas the static process decreases since the complex dissociates at this temperature.

We did not verify the mechanism of quenching and mentioned that it could be dynamic as we assumed it was due to excited state energy transfer rather than complex formation (static).
Since the emitter (excited state molecule) and the quencher molecule are involved in the quenching process, it is bimolecular and follows the second order kinetics (concentration of both affect the quenching). (Keizer, J. J. Am. Chem. Soc. 1983, 105, 1494-1498; Keizer, J. J. Am. Chem. Soc. 1985, 107, 5319-5319.)

Q4: Note that the candidate is using EPR spectroscopy to detect the •OH radical, but he should give an explanation why DMPO is used in preference to other radical traps. What advantage does it have over other radical traps?

A4: There are several spin traps to trap radicals such as DMPO (5,5-dimethyl-1-pyrroline-N-Oxide), BMPO (5-tert-Butoxycarbonyl-5-methyl-1-pyrroline-N-Oxide), EMPO (2-ethoxycarbonyl-2-methyl-2,3-dihydro-2H-pyrrole-1-Oxide). The spin trap such as BMPO and EMPO can trap superoxides and form distinguishable adduct with the half life of 23 and 8.6 min., respectively, and produce different spectra from that of hydroxyl radicals. DMPO, however, does not distinguish superoxide radical since this adduct has half life of only 45 s.

Our goal was to detect •OH radicals and we were not interested in superoxide, DMPO was good for us. The DMPO superoxide adduct (with $t_{1/2} = 45$ s) would form DMPO-OH adduct eventually. (Zhao, H., et al. Free Radic. Biol. Med. 2001, 31, 599; Stolze, K., et al. Biol. Chem. 2002, 383, 813-820.)

Q5: In summary, the candidate should include a table comparing values arising from his studies with those acquired by other techniques; then give a detailed account of which technique is superior.
A5: We have mentioned the detection limit from other techniques used in detecting these explosives such as mass spectrometry (MS), MS coupled with chromatographic techniques. These techniques are very sensitive (more than ours) and detect nano- to pico-grams (page 56). Our technique detects 50 µM and we use 1.0 mL of test solution, which requires 10 µg of HMTD. We have compared with LC/FTIR technique on page 71 and mentioned that our technique is 10 times more sensitive than LC/IR.

Our technique does not require very expensive instruments as in MS and it is portable for field detection. This is advantage over MS based technique not in sensitivity.

Likewise we have mentioned the limit of detection of TATP as 2.5 µM on page 87 which is about 400 times more sensitive than LC/IR technique. This means we require 0.5 µg/mL for detection.

For second (HMTD) and third (TATP) chapters, we have clearly compared with other techniques in use for these compounds. The fourth chapter deals with the quick detection of TNT. Obviously, detection limit would be way less (0.99 µg/mL) than what can be achieved with MS (ng to pg).

Comparison of Limit of Detection of Triacetone Triperoxide (TATP)

<table>
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<tr>
<th>Techniques</th>
<th>Limit of Detection</th>
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</thead>
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<tr>
<td>FTIR, GC-FTIR(^1)</td>
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</tr>
<tr>
<td>IMS(^2)</td>
<td>1.9 µg</td>
</tr>
<tr>
<td>ESI-MS(^3)</td>
<td>62.5 ng</td>
</tr>
<tr>
<td>HPLC-IR(^4)</td>
<td>222 mg (1 mM)</td>
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<tr>
<td><strong>ECL (current method)</strong></td>
<td><strong>0.55 µg</strong></td>
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Comparison of Limit of Detection of Hexamethylene Triperoxide Diamine (HMTD)

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<tr>
<th>Techniques</th>
<th>Limit of Detection</th>
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<td>LC-MS$^5$</td>
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<td>GC, GC-FTIR$^1$</td>
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<tr>
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<tr>
<td>ECL (current method)</td>
<td>50 µM</td>
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</table>

References


