Analytica Chimica Acta, 288 (1994) 57–69
Elsevier Science B.V., Amsterdam

Characterization of smokeless gunpowder by means of diphenylamine stabilizer and its nitrated derivatives

Edgard O'N. Espinoza

U.S. National Fish and Wildlife Forensics Laboratory, Ashland, OR 97520 (USA)

John I. Thornton

Forensic Science Group, Department of Biomedical and Environmental Health Sciences, University of California, Berkeley, CA 94720 (USA)

(Received 22nd June 1992; revised manuscript received 6th November 1992)

Abstract

Smokeless gunpowder frequently contains diphenylamine as a stabilizer. The diphenylamine acts as a nitrate scavenger and in turn is nitrated by complex processes. The various nitrated congeners of diphenylamine, which are numerous, may serve to characterize a sample of gunpowder because these derivatives reflect not only the production of the gunpowder, but also its storage career and thermal history following manufacture. These derivatives of diphenylamine may be isolated and identified by means of thin-layer chromatography and liquid chromatography.

Keywords: Chromatography; Diphenylamine; Forensic analysis; Gunpowder

Modern gunpowder is of the so-called "smokeless" type, consisting primarily of nitrated cellulose, but frequently with nitroglycerine as well. Gunpowder consisting of nitrocellulose alone is termed "single-base" gunpowder, and those powders that contain nitroglycerine besides nitrocellulose are called "double-base" gunpowders.

Upon standing, and particularly under hot conditions, the nitrocellulose and nitroglycerine will deteriorate with the release of nitrate. Free nitrate will in turn cause nitrous and nitric acid to be formed, which will further degrade the gunpowder. Consequently, a stabilizer of some sort is required. In the United States and in the United Kingdom, diphenylamine (DPA) is the most commonly used stabilizer, although resorcinol and ethyl centralite are also occasionally used.

A pristine gunpowder may contain diphenylamine alone, but soon the diphenylamine, acting as a nitrate scavenger, becomes nitrated itself in one of many ways. An aged cartridge (or a new one that has been exposed to a hot environment) may contain gunpowder with a witch's brew of nitrated diphenylamine compounds. The end member of this series of nitrated compounds is hexanitrodiphenylamine, in which all available sites have been nitrated.

The diversity of nitrated diphenylamine species provides an opportunity to characterize a particular gunpowder, because the nitrated diphenylamine species reflect not only the original production but also the subsequent thermal history and storage career of the gunpowder. And in

Correspondence to: E. O'N. Espinoza, U.S. National Fish and Wildlife Forensics Laboratory, 1490 E. Main St., Ashland, OR 97520 (USA).

SSDI 0003-2670(92)00469-9

fact, many color tests have been suggested for the characterization of gunpowders; these color tests rely in major part on the reaction of diphenylamine and resorcinol congeners [1,2].

Although DPA clearly acts as a nitrate scavenger and is increasingly nitrated as gunpowder ages, the available knowledge concerning the reactions of DPA nitration has been either fragmented or poorly understood. Although the sequences of events during diphenylamine nitration have been postulated (not always altogether plausibly [3,4]), these mechanisms have not been previously evaluated for forensic purposes. In the chemistry of gunpowder and propellants, and especially with respect to nitrocellulose containing powders, stabilizers are compounds that prevent the acid catalyzed decomposition of nitrocellulose or nitroglycerin. Stabilizers exert their effect by binding decomposition products such as free acid and nitrous gases; the stabilizers themselves are converted into relatively stable compounds in this process. Neither stabilizers nor their secondary products react with the parent nitroglycerin or nitrocellulose.

In recent years there has been renewed interest in DPA as an organic indicator of gunshot discharge residue [5–9]. Although the ubiquity of diphenylamine is such that the presence of DPA alone might not unequivocally suggest that a gunshot discharge has taken place, the presence of an entire group of nitrated derivatives of DPA is highly significant. Industrial and environmental uses of DPA is not normally associated with nitrating agents.

The intent of the present research was to investigate the mechanisms of nitration of DPA and the occurrence of nitrated derivatives of diphenylamine, and to evaluate the suitability of these derivatives as a test for characterizing gunpowders for determining commonality of source. Following a discussion of the possible mechanisms of diphenylamine nitration, the experimental work conducted in the present study was divided into three avenues of inquiry, namely, thermal stability of DPA, DPA nitration mechanisms, and presence and occurrence of DPA in gunpowders by thin-layer chromatography and high-pressure liquid chromatography analysis.

General reaction mechanisms

The classical explanation of the conversion of DPA into its various derivatives is the rearrangement of *N*-nitrosoamines to the *C*-nitroso compound (The Fisher–Hepp reaction) and its subsequent oxidation. Another theoretically plausible reaction would be the oxidation of a *N*-nitrosamine to the *N*-nitramine with subsequent rearrangement, although no direct evidence for this reaction has been reported. It seems unlikely that multiple simultaneous nitration, denitration, or rearrangement of a nitro group from one carbon atom to another would occur. Based on these types of reactions, several nitration schemes seem possible. The nitration scheme depicted in Fig. 1 is based upon a series of nitrosations, denitrosations and nitrations, but does not involve rearrangements. In this scheme the only nitrosation that occurs is the original *N*-nitrosation of DPA. Schroeder et al. [10] believed this to be the principal reaction of DPA, but that some direct nitration to the mononitro derivatives also occurs. According to this view, *N*-nitroso-DPA then is supposedly nitrated largely to *N*-nitroso-4-nitro-DPA, which in turn is further nitrated, although some denitrosation results at this stage. Since the accumulation of nitro groups would produce an increasing instability of the *N*-nitroso bond, Schroeder predicted that after formation of the *N*-nitroso-dinitro-DPA, essentially complete denitrosation occurs and that further reaction is by direct nitration.

To corroborate this nitration mechanism, Schroeder conducted further experiments [11–13] with a series of gunpowders into which he incorporated various derivatives of DPA and then heated the mixtures to accelerate the release of nitrate. Each of these propellants was doped with one (but only one) of the following compounds: *N*-nitroso-DPA, 4-nitro-DPA, 2-nitro-DPA, 3-nitro-DPA, 4,4′-dinitro-DPA, 2,4,4′-trinitro-DPA and hexanitro-DPA. The results showed that, except 4-nitroso-DPA, the heated powders were always found to contain the derivatives to be expected based on Fig. 1. Only traces of *N*-nitroso-2-nitro-DPA were present in that powder which originally contained 2-nitro-DPA, and yet *N*-nitroso-4-nitro-DPA was an important product

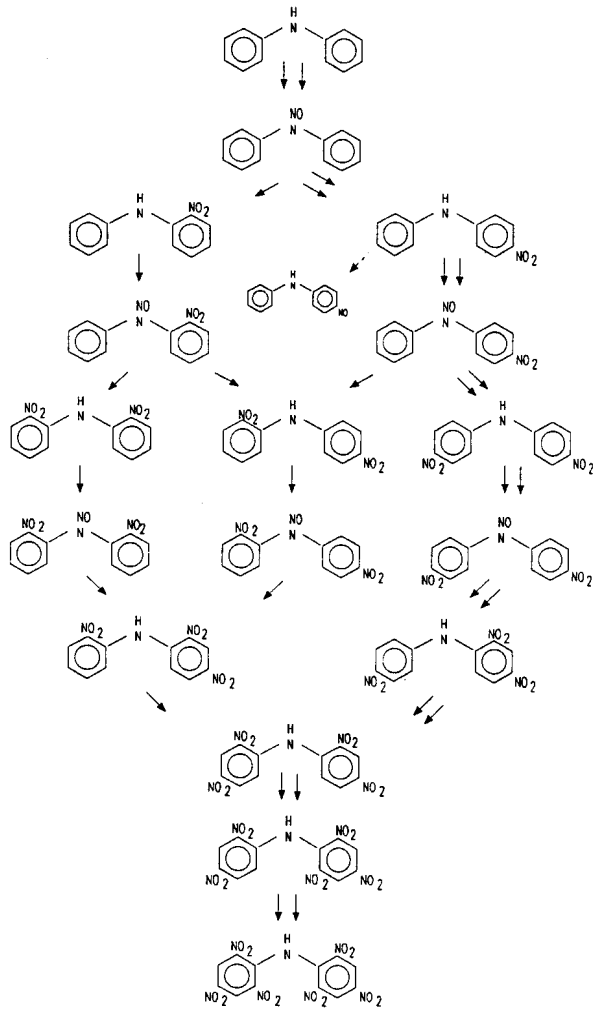

Fig. 1. Scheme of plausible nitration and nitrosation mechanisms of diphenylamine.

from 4-nitro-DPA. Denitrosation was demonstrated by the isolation of 4-nitro-DPA from the powder which originally contained *N*-nitroso-4-nitro-DPA. 4-Nitroso-DPA was found to be a very reactive compound which was depleted rapidly. Small amounts of 4-nitroso-DPA were isolated, but the reactivity of this derivative is such that it probably would not accumulate in gunpowder under any conditions.

On the basis of this it was concluded that the first reaction of DPA in smokeless powder is the nitrosation to *N*-nitroso-DPA, and that further reaction probably involves direct nitration to form *N*-nitroso-4-nitro-DPA. The probability of rearrangement of nitrated derivatives decreases as the DPA compounds become increasingly nitrated; the formation of the trinitro-DPAs and more highly nitrated compounds would probably involve direct introduction of the nitro group into the molecule.

Nitration mechanisms

The nitration of DPA in gunpowder has long been surrounded by a paradox, however. Nitration, in the classical sense, requires the presence of a strong acid to promote the formation of the nitronium cation. Although sulfuric acid is used in the manufacture of nitrocellulose, the trace amounts (typically ca. 0.5% sulfuric acid or less) found in nitrocellulose are insufficient to explain the nitration of DPA that accompanies the aging of gunpowder. On the other hand, nitrocellulose is an unstable compound in which thermal decomposition dissociates the ester bond [14–16]. This can produce nitrogen dioxide, a major decomposition product [17], which in turn can initiate a nitration reaction. This aspect of the chemistry of gunpowder may have been short shrift in previous work.

Explanations appearing in the literature concerning the nature of the nitration processes in DPA have ranged from classical to radical (the latter in the sense of electron transfer and radical pair collapse). The following discussion offers a review and summary explanation of both the classical and radical kind.

ELECTROPHILIC SUBSTITUTION: A CLASSICAL THEORY

Classically, nitration can be accomplished under the following conditions: (a) with nitric acid alone or with inorganic acids such as sulfuric acid; (b) with nitric acid in organic solvents such as nitromethane, acetic acid or acetic anhydride; (c) with dinitrogen pentaoxide in organic solvents; (d) with nitronium salts in organic solvents; (e) via nitrosation followed by oxidation. Although these conditions differ markedly from each other and the nitration rate of a given compound depends

on extant conditions, in all of the above the mechanism of nitration is essentially the same. The attacking entity is the nitronium ion, NO_2^+. Evidence of the existence of nitronium ion in nitrating media comes from Raman spectrum studies. A solution of nitric acid in sulfuric acid contains bands at 1400 and 1050 cm^{-1} that are not attributable to molecular nitric acid. Similar lines are also observed in other nitrating systems, namely dinitrogen pentaoxide in nitric acid (1400 and 1050 cm^{-1}), nitric acid in perchloric or selenic acid (1400 cm^{-1}) and crystalline nitronium perchlorate (1400 cm^{-1}). This last compound is completely ionized into nitronium ions and perchlorate ions [18], so that the 1400 cm^{-1} line is attributed to the nitronium ion. The 1050 cm^{-1} line is interpreted as the hydrogen sulphate ion in nitric acid–sulphuric acid, and to the nitrate ion in dinitrogen pentaoxide [19]. Raman spectroscopic measurements and other data are consistent with the establishment of the following equilibria:

$$H_2SO_4 + HNO_3 \rightleftharpoons HSO_4^- + [H_2O^+ - NO_2]^+ \quad (1)$$

$$[H_2O^+ - NO_2]^+ \rightleftharpoons H_2O + NO_2^+ \quad (2)$$

$$H_2SO_4 + H_2O \rightleftharpoons HSO_4^- + H_3O \quad (3)$$

The overall equilibrium may be expressed as:

$$2H_2SO_4 + HNO_3 \rightleftharpoons NO_2^+ + HSO_4^- + H_3O^+ \quad (4)$$

Evidence that nitronium ion is an active entity in diphenylamine nitration from thermally degraded nitrocellulose was shown by Yasuda [20], who effectively nitrated DPA in acetone by using dinitrogen pentaoxide. Nitration by dinitrogen pentaoxide is attributed to the ion pair $[NO_2^+NO_3^-]$, or by free nitronium ions by dissociation of this ion pair.

The nitrated derivatives of DPA in the experiments of Yasuda are consistent and similar to the nitrated derivatives of DPA found in gunpowder ageing studies by Schroeder et al. [10], Yasuda [21], and Volk [22] and consistent with the thermal studies of nitroglycerine conducted in the present study. Seven of the derivatives identified are nitroso compounds. Consequently, in the nitration of DPA there are either two sources of nitration (i.e., the nitrosonium ion, NO^+, and the nitronium ion, NO_2^+), or all nitration is via nitrosation with subsequent oxidation.

Nitration via nitrosation occurs in highly activated nuclei of amine derivatives, provided nitrous acid is present. The reaction appears to be in accordance, generally, with the following:

$$ArH + \underset{[slow]}{HNO_2} \rightarrow ArNO + H_2O \quad (5)$$

$$ArNO + \underset{[fast]}{HNO_3} \rightarrow ArNO_2 + HNO_2 \quad (6)$$

Since nitrous acid is continually reformed, its concentration remains constant throughout the reaction. Additionally, since the species NO^+, $H_2NO_2^+$, N_2O_4, N_2O_3 and HNO_2 are determined as nitrous acid by most conventional methods, any one of them could be acting as an effective catalyzing species to increase the rate of nitration.

In thermal degradation studies of cellulose nitrate [14], the products that could be identified by IR consisted of H_2O, HCHO, NO, CO_2, and NO_2. If nitrosation is the mechanism of DPA nitration, the following equilibrium reaction would agree with data from Yasuda [20]:

$$N_2O_4 \rightleftharpoons NO^+ + NO_3^+ \quad (7)$$

Rate limiting step in the aniline moiety of diphenylamine

Aniline is susceptible to nitronium attack because of the exposed π electrons. In the first step NO_2^+ takes two electrons of the π system to form a σ bond between it and one carbon of the phenol ring. This interrupts the cyclic system of π electrons, because in the formation of the sigma complex one carbon becomes sp^3 hybridized and no longer has an available p orbital. The four remaining π electrons of the σ complex (arenium ion) are delocalized over the remaining sp^2 carbons. In the second step the arenium ion loses a proton from the carbon that bears the nitronium. The two electrons that bonded this proton to carbon become a part of the π system. The

carbon that bears the electrophile becomes sp^2 hybridized again and an aniline derivative with fully delocalized π electrons is formed.

There exists experimental evidence that the arenium ion is a true intermediate in electrophilic substitution reactions involving benzene and its derivatives; the literature concerning DPA, however, is silent. Based on the benzene data the energy of reaction leading from DPA and the electrophile to the arenium ion is much greater than the energy of activation leading from the arenium ion to the final product. The reaction leading to the formation of the arenium ion is highly endothermic because the phenol ring loses its stabilization energy; conversely, the reaction leading from the arenium ion to the DPA derivative is highly exothermic because the phenol ring has regained its stabilization energy. The formation of the σ complex (arenium ion) is the rate limiting step in this reaction. The loss of a proton occurs rapidly compared with the arenium ion and does not affect the overall rate of reaction [19,23–25].

N-nitrosation, on the other hand, is the first nitration reaction found in ageing gunpowder studies. In the cases where *N*-nitrosation is the product of nitration, the rate limiting reaction is in the formation of the nitrosonium as shown:

$$C_6H_5NH_2 + HNO_2 \xrightarrow{[\text{slow}]} [C_6H_5NH - NO]^+ + H_2O \quad (8)$$

$$[C_6H_5NH - NO]^+ + HNO_3 \xrightarrow{[\text{fast}]} ArNO_2 + HNO_2 \quad (9)$$

Substituent effect of the amino group in the aniline moiety

The *ortho–para* directive effect of the amino group with an unshared electron pair is predominantly caused by resonance effect. This resonance operates primarily in the arenium ion and in the transition leading to it. The inductive effect of the amino group makes it slightly electron withdrawing. The difference between the electronegativities of nitrogen and carbon in aniline is not too large, however, since the carbon of the benzene ring is sp^2 hybridized it is somewhat more electronegative than it would be if it were sp^3 hybridized. Nevertheless, the resonance effect of the amino group in aniline is far more important than its inductive effect in electrophilic aromatic substitution and this resonance effect makes the amino group electron releasing and therefore *ortho–para* directing. Since the *ortho–para* directive effect is also observed in diphenylamine similar mechanisms of resonance and inductive effects are expected to be in accordance with present chemical thought. The mute literature does not support or discredit that the nitration of diphenylamine is similar to that of aniline. Since *N*-nitrosation is the first nitration reaction found in all ageing gunpowder studies, it seems plausible that in diphenylamine the inductive effect could be more important as the first step in nitrating this molecule. On the other hand, *N*-nitrosation can also be explained by the fact that secondary amines form *N*-nitroso compounds when the nitrating agent is nitrous acid.

ELECTRON TRANSFER AND RADICAL PAIR COLLAPSE, AN ALTERNATE NITRATION SCHEME

Perrin [27] proposed an alternate mechanism of electrophilic substitution reactions in aromatic molecules that are more reactive than toluene. Principally, the proposed mechanism involves an electron transfer from the aromatic to the nitronium ion followed by radical pair collapse to the σ complex intermediate:

$$NO_2^+ + ArH \rightarrow NO_2^{\cdot} + ArH^{+\cdot} \rightarrow HArNO_2^+ \quad (10)$$

For aromatics more reactive than toluene, the reaction with NO_2^+ is encounter-limited, so that all such aromatics react at the same rate. Yet although there is no intermolecular selectivity, there is intramolecular selectivity. If NO_2^+ is so reactive that it reacts at every encounter with a π system, it is reasonable to ask what distinguishes the *ortho* and *para* positions, which have only a slightly greater π electron density. It would be expected that NO_2^+ would not exhibit intramolecular selectivity, yet the selectivity is typical of

electrophilic substitution. In support of Perrin's mechanism is the fact that electron transfer to NO_2^+ is exothermic for all aromatics more reactive than toluene; additionally the estimated lifetime of an encounter pair (1×10^{-10} s) is too short to accommodate intramolecular selectivities. Therefore electron transfer mechanism provides an explanation for the encounter-limited nitration of aromatics more reactive than toluene. Electron transfer between aromatics and their radical cations is encounter-limited whenever the electron transfer is exothermic [27].

The electron transfer mechanism resolves the paradox of intramolecular selectivity without intermolecular selectivity, since the attacking species exhibiting the intramolecular selectivity (NO_2), is different from the one exhibiting no intramolecular selectivity (NO_2^+). Radical pair collapse, on the other hand, is quite likely to exhibit selectivity, due to the nonuniform spin density in the aromatic radical cation. There is a force of attraction between the NO_2 and each carbon of the radical cation. At long distances this force is strongest toward those positions of greatest spin density. At short distances this force is strongest toward those positions where bond formation will produce the most stable σ complex. Therefore, radical pair collapse is determined by a composite of spin density and σ complex stability. An effective test for the existence of this mechanism in diphenylamine was done by Lindblom [26], who performed a controlled-potential electrolysis of a mixture of diphenylamine and NO_2. The applied half-wave potential was incapable of oxidizing NO_2, but sufficed to generate the radical cation of diphenylamine. When the two radicals diffuse together, they combine to form the σ complex. The nitrated compound 4,4′-dinitrodiphenylamine was produced without HNO_3 and H_2SO_4. The observation that the same nitrated diphenylamine product is formed both electrochemically and via NO_2^+ is strong evidence that the radical pair is involved not only in the electrochemical synthesis but also in aromatic nitration.

Electron transfer and radical pair collapse also explains the paradox of nitration in gun powders without HNO_3 and H_2SO_4, which has defied classical nitration mechanisms. Additionally, the first nitration step of DPA in gun powders is *N*-nitrosation. Electron transfer from very reactive aromatics to NO^+ has been observed [27]. These data are consistent in that if nitration in gun powders is through an electron transfer and radical pair collapse, diphenylamine has such reactivity as to support this mechanism.

EXPERIMENTAL

Materials

Reagent grade 2,4-dinitrodiphenylamine, 2,5-dinitrophenol, 4-nitrophenol, *para*-diethylaminobenzaldehyde and *N*-nitrosodiphenylamine were purchased from Aldrich; 2-nitrodiphenylamine and 2-nitroresorcinol were purchased from Eastman; 4-nitrodiphenylamine, 4-nitrosodiphenylamine, *N*-nitrosodiphenylamine and 2,4-dinitrophenol were purchased from Pfaltz and Bauer; diphenylamine and triethylamine were purchased from Baker; methanol and tetrahydrofuran were purchased from Fisher; pharmaceutical-grade nitroglycerin was obtained from Marion Merrel Dow.

Pyrolytic flask experiments

A 1000-ml Kimax round bottom flask was modified to act as an electro-pyrolytic chamber. The sample tub that closed the electrical circuit was constructed of platinum and the electrical wires were of tungsten to support high temperatures. The stoppered end of the flask was modified to receive a Kontes thin-layer chromatography (TLC) tank inlet/outlet purge valve to allow the flask to be filled with nitrogen. A variable AC autotransformer was then connected to the tungsten terminals. The incandescence of the samples tub, and therefore indirectly the temperature, was measured with an optical pyrometer. The pyrolytic derivatives were identified by TLC.

Ageing studies of DPA nitration

Pharmaceutical grade nitroglycerin was obtained from Marion Merrel Dow (Nitro-Bid, 9 mg tablets), which yielded a total of 1620 mg of nitroglycerin. This was separated into three tubes

containing 540 mg of nitroglycerin each. Each aliquot was diluted to 6 ml with methylene chloride and 3 mg of diphenylamine in methylene chloride was added (0.6% w/v, DPA in methylene chloride). The tubes were incubated at (1) room temperature, (2) 37°C, and (3) 65°C. Nitration at the three different temperatures was stopped after 27 days and the 3 aliquots were nitrated at 120°C for 70 h. The nitrated derivatives were then identified by TLC.

TLC of gunpowders and nitrated derivatives of diphenylamine

Two-dimensional chromatography was done by the method of Volk, described in Refs. 21 and 22, on 254-nm UV fluorescence 20 × 20 silica gel plates (0.25 mm) (Brinkman Instruments), and developed with benzene–carbon tetrachloride–1,2-dichloroethane (50:30:25). The plates were dried and developed in the second dimension with petroleum ether–ethyl acetate (80:20). The plates were then dried, visualized with long-wave ultraviolet radiation (356 nm) and then sprayed with *p*-diethylaminobenzaldehyde (1 g in 75 ml methanol + 25 ml concentrated sulfuric acid). One-dimensional silica paper chromatography was done on Toxi-Lab TLC Systems purchased from Baxter. Toxi-grams were developed with benzene–carbon tetrachloride–1,2-dichloroethane (50:30:25) and visualized with potassium

TABLE 1

Possible derivatives from diphenylamine nitration

Compound	Abbreviation
Diphenylamine	DPA
N-Nitrosodiphenylamine	*N*-NO-DPA
2-Nitrosodiphenylamine	2-NO-DPA
4-Nitrosodiphenylamine	4-NO-DPA
2-Nitrodiphenylamine	2-*N*-DPA
4-Nitrodiphenylamine	4-*N*-DPA
N-4-Dinitrosodiphenylamine	*N*-4-NO-DPA
2,4-Dinitrodiphenylamine	2,4-di-DPA
2,6-Dinitrodiphenylamine	2,6-di-DPA
N-Nitroso-2-nitrodiphenylamine	*N*-NO-2-*N*-DPA
N-Nitroso-4-nitrodiphenylamine	*N*-NO-4-*N*-DPA
2,2′-Dinitrodiphenylamine	2,2′-di-DPA
2,4′-Dinitrodiphenylamine	2,5′-di-DPA
4,4′-Dinitrodiphenylamine	4,4′-di-DPA
4-Nitroso-2-nitrodiphenylamine	4-NO-2-*N*-DPA
N-Nitroso-2,4-dinitrodiphenylamine	*N*-NO-2,4-di-DPA
N-Nitroso-2,2′-dinitrodiphenylamine	*N*-NO-2,2′-di-DPA
N-Nitroso-2,4′-dinitrodiphenylamine	*N*-NO-2,4′-di-DPA
N-Nitroso-4,4-dinitrodiphenylamine	*N*-NO-4-4′-di-DPA
2,4,6-Trinitrodiphenylamine	2,4,6-tri-DPA
2,2′-4-Trinitrodiphenylamine	2,2′,4-tri-DPA
2,4,4′-Trinitrodiphenylamine	2,4,4′-tri-DPA
2,2′,6-Trinitrodiphenylamine	2,2′,6-tri-DPA
N-Nitroso-2,2′,4-trinitrodiphenylamine	*N*-NO-2,2′,4-DPA
N-Nitroso-2,4,4′-trinitrodiphenylamine	*N*-NO-2,4,4′-DPA
2,2′,4,4′-Tetranitrodiphenylamine	2,2′,4,4′-tetra-DPA
2,2′,4′,6-Tetranitrodiphenylamine	2,2′,4′6-tetra-DPA
2,2′,6,6′-Tetranitrodiphenylamine	2,2′,6,6′-tetra-DPA
2,4,4′,6-Tetranitrodiphenylamine	2,4,4′,6-tetra-DPA
2,2′,4,6-Tetranitrodiphenylamine	2,2′,4,6-tetra-DPA
2,2′,4,4′,6-Pentanitrodiphenylamine	2,2′,4,4′,6-penta-DPA
2,2′,4,6′,6-Pentanitrodiphenylamine	2,2′,4,6′,6-penta-DPA
2,2′,4,4′,6,6′-Hexanitrodiphenylamine	2,2′,4,4′,6,6′-hexa-DPA

dichromate (0.8 g in 100 ml 60% sulfuric acid). For the gunpowder experiments, 50 mg of gunpowder was extracted with 1 ml of methylene chloride in a screw top tube at room temperature for 12 h. One and three drops, respectively, of the extract were loaded onto a Toxi-gram strip. Following development, the diameter of each analyte was measured and the area calculated. Standard regression analysis confirmed a high correlation between spot area and spot amount, and TLC spot diameter was used in subsequent experimentation. To obtain a summary statistic that could characterize individual gunpowders, the areas of the analytes were summed; the resulting area reflects the total area of DPA and its derivatives detected in the sample.

LC analysis of gunpowders

A Spectra Physics 8700 high-pressure liquid chromatograph with a Beckman 254-nm UV detector was used for analysis. The analytical column was a Phenomenex Ultramex 5C18 (25 cm × 4.6 mm i.d.) reversed-phase C_{18} column. Samples were injected with an Altex 210 injector with a 20-μl loop. The Zeiss recorder was set at 1 mV and operated at 0.5 cm min^{-1}.

Separations were obtained isocratically with methanol–water–triethylamine (74:25:1). Flow-rate was maintained at 1 ml min^{-1} and column temperature was kept at 25°C.

Stock standards at a concentration of 200 μg ml^{-1} were made for diphenylamine, *N*-nitrosodiphenylamine, 2-nitrodiphenylamine and 4-nitrodiphenylamine. 4-Nitrosodiphenylamine (100 μg ml^{-1}) and 4-nitroaniline (200 μg ml^{-1}) were prepared for internal standards. All solutions were made by dissolving the solute in 20 ml of tetrahydrofuran and adding methanol to 100 ml in a volumetric flask. Working standards and internal standards were diluted to 40 μg ml^{-1}.

Sample preparation for LC was as follows: 50 mg of gunpowder was weighed and transferred to a screw top tube. 1 ml of methylene chloride was added and allowed to extract for 12 h after which the extract solution was filtered through an Acro LC13 0.2-μm filter (Gelman Sciences 4450). 50 μl of the filtered extract was then transferred to an evaporation tube and 50 μl of the internal stan-

TABLE 2

TLC R_F values for DPA and nitrated derivatives

Compound	Silica gel		Visual	*p*-DEAB	Paper silica ToxiGram
	vertical R_F	Horizontal R_F			
DPA	0.94	0.96	N/S [a]	Blue	0.93
2-*N*-DPA	0.86	0.96	Orange/yellow	Pink	0.90
N-NO-DPA	0.72	0.96	N/S	Blue	0.78
2,4-di-*N*-DPA	0.70	0.76	Yellow	Lemon yellow	0.72
N-NO-4-*N*-DPA	0.69	0.82	Orange	Purple	0.72
2,2′-di-*N*-DPA	0.64	0.53	Yellow	Yellow	0.70
2,4′-di-*N*-DPA	0.58	0.69	N/S	Yellow/orange	0.65
N-NO-4,4′-di-DPA	0.52	0.76	Yellow	Yellow	?
N-NO-2,4′-di-DPA	0.47	0.35	Yellow	Yellow	?
4-*N*-DPA	0.45	0.62	Yellow	Purple violet	0.59
N-NO-2,2′-di-DPA	0.45	0.24	Yellow	Yellow	?
2,4,4′-tri-*N*-DPA	0.31	0.60	Yellow	Yellow	0.42
2,2′-4,4′-tetra-*N*-DPA	0.20	0.45	Yellow	Yellow	0.32
4,4′-di-*N*-DPA	0.12	0.29	Yellow	Yellow	0.24
2,2′,4,4′,6,6′-hexa-*N*-DPA	0.03	0	N/S	Fluorescence	0.03

[a] N/S = not seen.

TABLE 3

Nitration derivatives from room temperature reactions

Day	0	1	3	6	8	10	13	15	17	20	22	24	27
DPA	+	+	+	+	+	+	+	+	+	+	+	+	+
N-NO-DPA	–	–	–	–	–	–	–	–	+	+	–	–	+

dard was added to each sample and allowed to evaporate to dryness at room temperature. The sediment was reconstituted with 50 μl of tetrahydrofuran, swirled, and 200 μl of methanol was added. The samples were then analyzed by LC.

RESULTS AND DISCUSSION

A list of the possible nitrated derivatives of DPA is shown in Table 1, and TLC reference values are shown in Table 2.

Thermal stability of diphenylamine

To test the hypothesis that DPA might undergo pyrolytic degradation to certain products (e.g., phenol and aniline), the sample holder in the electro-pyrolytic chamber was loaded with 50 mg of DPA and heated to incandescence, i.e., 700–800°C, by applying an AC potential of 14 V to the filament. Oxidation within the chamber was inhibited by purging with nitrogen gas. After pyrolysis the condensates of the fumes that were generated during the experiment were analyzed by TLC for diphenylamine derivatives, aniline and phenols. Only DPA was recovered. The greatest quantity of DPA was recovered from the pyrolytic condensate and not the solid residue remaining on the sample holder (an observable ten-fold difference of sample area on TLC), implying that a large portion of the sample reached the vapor state. Similar results were noted when the sample holder was loaded with 4-nitro-DPA.

Pyrolysis of *N*-nitroso-DPA was performed until fumes were no longer generated and again the chamber and sample holder were analyzed. An unknown compound was formed during this experiment that was not identified by spectrophotometric or TLC analysis. Assays for phenol and aniline were negative. The temperature reached by the electro-pyrolytic chamber was between 700 and 800°C. This was determined with an optical pyrometer and by melting point correlations. This suggests that DPA and some of its derivatives may survive a limited exposure to high temperatures in a nitrogen atmosphere, and equally important, that DPA is not thermally degraded to aniline or phenol in these experiments. These data support the empirical findings of Dahl et al. [5–7] that DPA is relatively stable in the shooting environment.

The electro-pyrolytic chamber could not be tested with air for fear of a filament failure, but it seems certain that oxidation derivatives would form under such conditions; these compounds would not resemble diphenylamine or its nitrated derivatives.

Ageing studies of diphenylamine nitration

The nitration/nitrosation of DPA by nitroglycerine at room temperature yielded the products listed in Table 3.

TABLE 4

Nitration derivatives from 37°C reactions

Day	0	1	3	6	8	10	13	15	17	20	22	24	27
DPA	+	+	+	+	+	+	+	+	+	+	+	+	+
N-NO-DPA	–	–	–	–	–	–	–	+	+	+	+	+	+

TABLE 5

Nitration derivatives from 65°C reactions

Day	0	1	3	6	8	10	13	15	17	20	22	24	27
DPA	+	+	+	+	+	+	+	+	+	+	+	+	+
N-NO-DPA	–	–	+	+	+	+	+	+	+	+	+	+	+
4-*N*-DPA	–	–	–	–	+	+	+	+	+	+	+	+	+
2-*N*-DPA	–	–	–	–	–	–	–	+	+	+	+	+	+
4-NO-DPA	–	–	–	–	–	–	–	–	+	–	+	+	+

When the nitration was carried out at 37°C, the products consisted of the compounds listed in Table 4.

Nitration/nitrosation of DPA at 65°C yielded the products shown in Table 5.

Nitration for the three different temperatures was stopped after 27 days and all three samples were nitrated at 120°C for 70 h. The nitrated compounds yielded are listed in Table 6.

A summary of the nitrated derivatives of diphenylamine encountered in this research, as well as those described in the relevant work of others, are listed in Table 7.

The presence of nitroso derivatives in addition to nitro derivatives appears to be related to temperature. This was shown in the present work and in that of others [10,21,22]. As the temperature of incubation increases, the formation of the species of the diphenylamine derivatives varies, especially in the case of nitroso derivatives.

In the ageing of gunpowders, where the temperature is dependent upon the ambient environment, the nitration pathways are straighforward, since environmental temperatures rarely exceed 45°C. Therefore, the first step in the nitration of DPA is *N*-nitrosation, followed by simultaneous *C*-nitration and *N*-denitrosation. This is most likely due to the electron withdrawing effect of the nitro group, a process that would destabilize the *N*-nitroso bond. The *C*-nitration occurs preferentially at the para position; nitration at the ortho position was noted 7 days after nitration occurred at the para position in the 65°C incuba-

TABLE 6

Nitration derivatives of all samples after 70 h at 120°C

Compound	Room temp.	37°C	65°C
DPA	–	–	–
N-NO-DPA	–	–	–
2-*N*-DPA	–	–	–
4-*N*-DPA	–	–	+
N-NO-2-*N*-DPA	–	–	–
N-NO-4-*N*-DPA	–	–	+
2,2′-di-DPA	+	–	+
2,4′-di-DPA	+	–	+
4,4′-di-DPA	+	–	+
N-NO-2,2′-di-DPA	+	–	+
N-NO-2,4′-di-DPA	–	–	–
N-NO-4,4′-di-DPA	+	–	+
N-NO-2,2′,4-tri-DPA	+	–	+
2,4,′-tri-DPA	+	+	–
2,2′-4,4′-tetra-DPA	–	–	–
2,2′-4,4′,6-penta-DPA	–	+	–
2,2′,4,4′,6,6′-hexa-DPA	–	–	+

TABLE 7

Comparison of derivatives produced from diphenylamine nitration

Compound	Schroeder et al. [10]	Yasuda [20]	Volk [22]	Current work
DPA	+	+	+	+
N-NO-DPA	+	+	+	+
2-*N*-DPA	+	+	+	+
4-*N*-DPA	+	+	+	+
N-NO-2-*N*-DPA	–	–	–	–
N-NO-4-*N*-DPA	–	+	+	+
2,2′-di-DPA	+	+	+	+
2,4-di-DPA	–	+	+	–
2,4′-di-DPA	+	+	+	+
4,4′-di-DPA	+	+	+	+
N-NO-2,2′-di-DPA	–	+	–	+
N-NO-4,4′-di-DPA	–	+	+	+
2,4,4′-tri-DPA	+	+	+	+
2,2′,4-tri-DPA	+	+	+	–
2,2′,4,4′-tetra-DPA	+	+	+	–
2,2′-4,4′,6-penta-DPA	+	+	+	+
2,2′-4,4′,6,6′-hexane-DPA	+	+	+	+

tion experiment. Further nitration at the 4'-position on the other phenyl ring occurred more often than the 2,2'-dinitro-DPA. It therefore seems reasonable to postulate that the preferential nitration pathway would be 4-nitro-DPA, followed by 4,4'-dinitro-DPA.

Schroeder et al. [10] studied DPA by heating small samples of powder at 71°C in separate tin containers for periods up to 258 days. Rough quantitative determination of the predominant nitroso and nitro derivatives of DPA was identified by column chromatography and colorimetric techniques on eleven separate samples of the propellant. These nitrated derivatives showed an increase and decrease of each compound when plotted in terms of the quantity of DPA derivatives produced.

Nitrates may decompose into such nitration agents as nitrous acid, nitric acid, nitrogen dioxide or nitrogen tetraoxide. These agents may react independently, or may be in an essential equilibrium with each other so that there is effectively a "single" nitrating agent that is responsible for the reactions.

Based on the current research, as well as the data of Schroeder et al. [10], Yasuda [21] and Volk [22], it is apparent that direct *N*-nitrosation occurs, since no other process could be expected to yield *N*-nitroso-DPA. It is also certain that denitrosation occurs. Direct nitrations of the phenol rings will involve (a) direct nitration of amines (including partially nitrated derivatives), and (b) direct nitration of nitrosamines (which may be accompanied or followed by denitrosation).

Presence and occurrence of DPA in gunpowders by TLC and LC analysis

The sensitivity of the Toxi-Lab TLC plates is unquestionably less than that of conventional silica gel TLC plates; extracts analyzed on both systems showed that two dimensional chromatography resolved compounds not adequately resolved or separated by one dimensional chromatography or by the Toxi-Lab system. However, because of the convenience of the Toxi-Lab system, this system was used for the narrow purpose of characterizing a large number of gunpowders and cartridges.

It was found that by using TLC, gunpowders may be successfully characterized and compared in a semi-quantitative manner to establish their provenance. It is accepted, nevertheless, that this comparison is at least in part a feature of time and that the nitration derivatives found at any moment are a reflection of the history and environment to which the propellants have been submitted. The characterization of 118 gunpowders based on the presence of DPA and its nitrated derivatives showed this approach to be straightforward and unequivocal with respect to interpretation. As in the work of Archer [28], the most frequently detected stabilizers in gunpowders was DPA and three of its derivatives: *N*-nitroso-DPA, 2-nitro-DPA and 4-nitro-DPA. In the older manufactured gunpowders, polynitrodiphenylamines were detected, inferring advanced stages of nitrocellulose degradation.

When TLC techniques were used, only 5 propellants from 118 samples could not be differentiated. The similarities in these gunpowders could be attributed to samples having experienced similar environmental conditions, or alternatively, they could be propellants that originated from a common source and were distributed to various cartridge loader manufacturers.

Assay time by TLC is about 45 min, and because the equipment needs are minimal, these analyses can be accomplished in virtually any laboratory. Increased sensitivity and quantitation could be obtained by using a scanning densitometer.

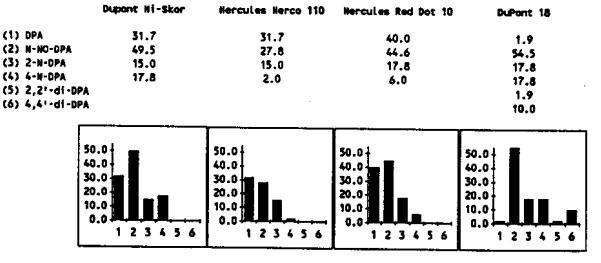

Fig. 2. Bar graph of selected gunpowders analyzed by TLC to illustrate the relative content of diphenylamine and its nitrated derivatives. Values represent area of TLC spots.

TABLE 8

Chemicals and samples tested for assay interference

p-Nitroaniline	3-Nitrophenol
m-Nitroaniline	4-Nitrophenol
Matches	*p*-Phenylphenol
Urine	Nitrobenzene
K_2NO_2	1,2-Dinitrobenzene
Phenol	1,3-Dinitrobenzene
2-Nitrophenol	2,4-Dinitrophenol

Figure 2 exhibits bar graphs of selected gunpowders and their relative content of DPA and its nitrated derivatives.

To evaluate the possibility of false positive reactions, the chemicals listed in Table 8 were tested by TLC and it was established that differentiation was possible based on color and/or R_F. The chemicals listed in Table 8 were chosen because of their chemistry, however implausible, or because of their historical significance in connection with the testing of gunshot discharge residues.

A typical chromatogram of DPA and nitrated derivatives reference compounds is shown in Fig. 3; the peaks are well-resolved, symmetrical, and the retention times are reproducible.

Gunpowders and cartridges were analyzed for DPA, *N*-nitroso-DPA, 2-nitro-DPA and 4-nitro-DPA. Gunpowder characterization using LC techniques produced a highly stylized profile. Figure 4 illustrates the DPA derivatives from the

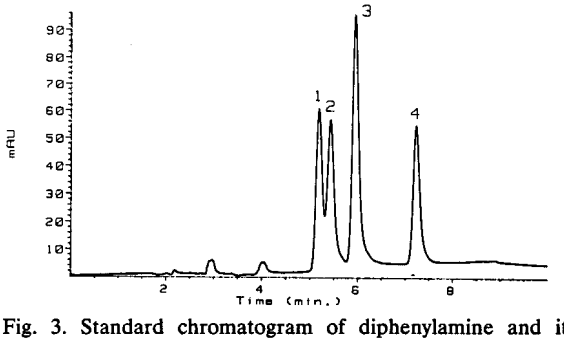

Fig. 3. Standard chromatogram of diphenylamine and its nitrated derivatives by LC. Column: Phenomenex RP C18; isocratic mobile phase of methanol–water–triethylamine (74:25:1); flow-rate 1.0 ml min^{-1}; injection volume 20 μl. Peaks: (1) *N*-nitrosodiphenylamine, (2) 4-nitrodiphenylamine, (3) diphenylamine, and (4) 2-nitrodiphenylamine.

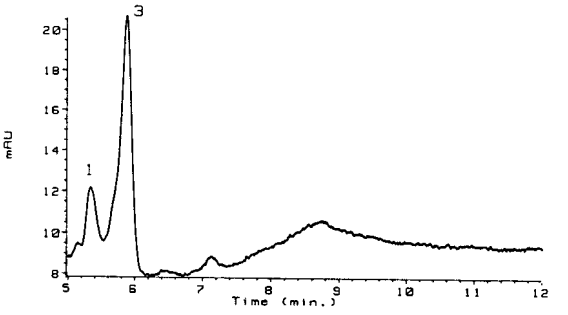

Fig. 4. LC of diphenylamine and its nitrated derivatives of gunpowder from a 7-mm Remington Magnum cartridge. Peaks: (1) *N*-nitrosodiphenylamine, and (3) diphenylamine.

gunpowder of a 7-mm Remington Magnum cartridge. Those gunpowders that contained ethyl centralite as the main source of stabilizer could easily be distinguished, since the elution of ethyl centralite did not interfere with any of the DPA derivatives of interest. Assay time by LC is about 45 min and produces a quantitative number that revealed some information regarding the current extent of nitration for a given sample.

The sensitivity of the LC assay was 0.25 μg ml^{-1} or 5 ng total detected when a 20-μl sample loop was used. The LC response for DPA, *N*-NO-DPA, 2-nitro-DPA and 4-nitro-DPA was linear from 50 to 4000 ng.

It is of interest to note that none of the Federal brand cartridges and some other cartridges analyzed had DPA or its derivatives as their main source of stabilizer.

A profile of DPA and its various derivatives found in 4 representative gunpowders is shown in Fig. 5. An experiment to determine the feasibility of recovery of DPA and its derivatives on the

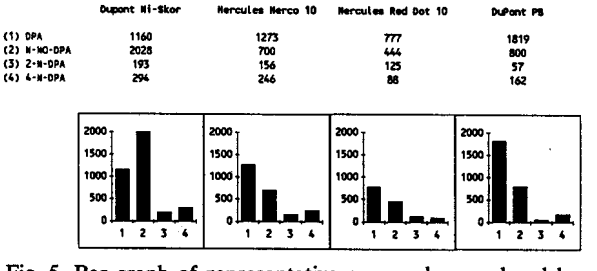

Fig. 5. Bar graph of representative gunpowders analyzed by LC showing total content of diphenylamine and its nitrated derivatives (μg ml^{-1}).

hands of a person after firing a handgun was conducted. As performed in the manner discussed above, the LC or TLC techniques were inadequate with respect to sensitivity to conclude that this type of analysis would be applicable to all shooting incidents and to all types of ammunition or firearms. Attempts to recover DPA and its derivatives from the hands of a shooter were unsuccessful in 0.22 and 0.25 caliber handguns. Successful recovery of DPA derivatives was ordinarily observed, however, with calibers equal to or greater than 0.38 Special. Increased sensitivity could be obtained, however, by measuring DPA derivatives by coupling liquid chromatography with an electrochemical detector, or the use of liquid chromatography–mass spectrometry (LC–MS); this would achieve an order of magnitude greater sensitivity and would permit the LC technique to be applied to calibers less than 0.38. In conclusion, the TLC approach to characterizing gunpowders was found useful for the purpose of distinguishing between samples of different provenance and different age. LC was found to provide a more suitable means for the quantitation of minor diphenylamine derivatives. The scheme in Fig. 1 illustrates the most likely nitration events; a large group of derivative compounds may be formed, which in turn will give gunpowders a highly stylized profile of nitrated derivatives. The mechanism of DPA nitration is most likely that of electron transfer followed by radical pair collapse. This mechanism explains the paradox of nitration in gunpowders without HNO_3 and H_2SO_4, an aspect of the chemistry of diphenylamine that has defied classical notions of nitration mechanisms.

REFERENCES

1 J.L. Booker, J. Forensic Sci. Soc., 13 (1973) 199.
2 R.S. Maloney and J.I. Thornton, J. Forensic Sci., 27 (1982) 318.
3 T.L. Davis and A.A. Ashdown, J. Am. Chem. Soc., 46 (1924) 1051.
4 T.L. Davis and A.A. Ashdown, Ind. Eng. Chem., 17 (1925) 674.
5 D.D. Dahl, S.C. Slahck and P.F. Lott, Microchem. J., 31 (1985) 145.
6 D.D. Dahl, J.C. Cayton and P.F. Lott, Microchem. J., 35 (1987) 360.
7 D.D. Dahl and P.F. Lott, Microchem. J., 35 (1987) 347.
8 J.B.F. Lloyd, Anal. Chem., 59 (1987) 1401.
9 L.S. Leggett and P.F. Lott, Microchem. J., 39 (1989) 76.
10 W.A. Schroeder, E.W. Malmberg, L. Fong, K.N. Trueblood, J.D. Landerl and E. Hoerger, Ind. Eng. Chem., 41 (1949) 2818.
11 W.A. Schroeder, M.K. Wilson, C. Green, P.E. Wilcox, R.S. Mills and K.N. Trueblood, Ind. Eng. Chem., 42 (1950) 539.
12 W.A. Schroeder, P.E. Wilcox, K.N. Trueblood and A.O. Dekker, Anal. Chem., 23 (1951) 1740.
13 W.A. Schroeder, B. Keilin and R.M. Lemmon, Ind. Eng. Chem., 43 (1951) 939.
14 J. Isler and D. Fleiger, in J.F. Kennedy, G.O. Phillips, D.J. Wedlock and P.A. Williams (Eds.), Cellulose and its Derivatives, Wiley, New York, 1985.
15 R.W. Phillips, C.A. Orlick and R. Steinberger, J. Phys. Chem. 59 (1955) 1034.
16 G. Gelernter, L.C. Browning, S.R. Harris and C.M. Mason, J. Phys. Chem., 60 (1956) 1260.
17 M.L. Wolfrom, J.H. Frazer, L.P. Kuhn, E.E. Dickey, S.M. Olin, D.O. Hoffman, R.S. Bower, A. Chaney, E. Carpenter and P. McWain, J. Am. Chem. Soc., 77 (1955) 6573.
18 D.R. Goddard, E.D. Hughes and C.K. Ingold, J. Chem. Soc., (1950) 2559.
19 R.O.C. Norman and R. Taylor, Electrophilic Substitution in Benzenoid Compounds, Elsevier, Amsterdam, New York, 1965.
20 S.K. Yasuda, J. Chromatogr., 13 (1964) 78.
21 S.K. Yasuda, J. Chromatogr., 14 (1964) 65.
22 F. Volk, Propellants and Explosives, 1 (1976) 59.
23 F. Volk, Propellants and Explosives, 1 (1976) 90.
24 T.W.G. Salomons, Organic Chemistry, Wiley, New York, 1984.
25 R. Breslow, Organic Reaction Mechanisms, Benjamin, New York, 1969.
26 T. Lindblom, in J. Hansson (Ed.), Proc. 4th Symposium Chem. Problems Connected with Stabilizers and Explosives, Sekt. Detonik Foerbraenning, Joenkoeping, Sweden, 1979.
27 C.L. Perrin, J. Am. Chem. Soc., 99 (1977) 5516.
28 A.W. Archer, J. Chromatogr., 108 (1975) 401.