United States
Environmental Protection
Agency

Environmental Monitoring
Systems Laboratory
P.O. Box 93478
Las Vegas NV 89193-3478

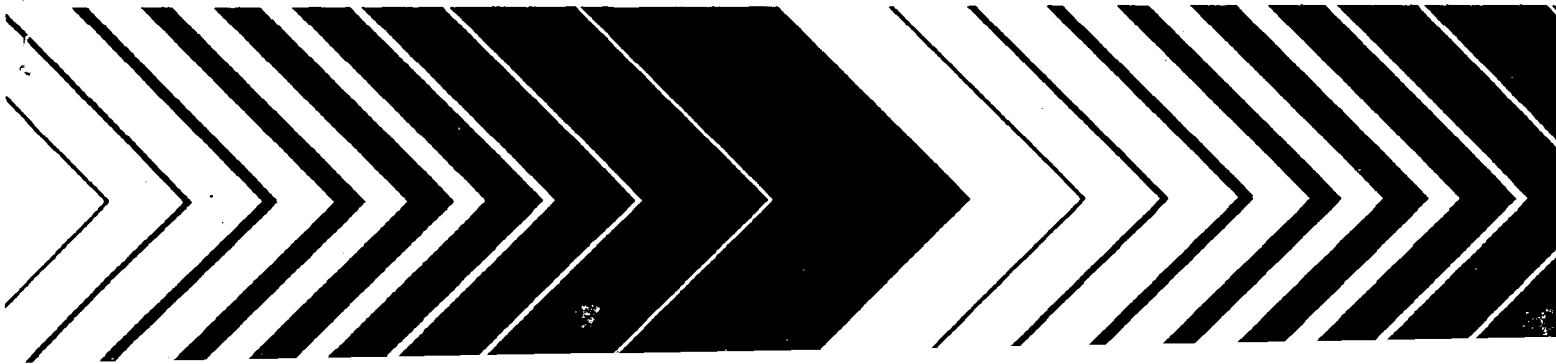
EPA/600/R-94/029
April 1994

Research and Development

EPA

Environmental Screening For Azo Dyes By Chemical Reduction And Mass Spectrometry

0182QAD94



ENVIRONMENTAL SCREENING FOR AZO DYES BY CHEMICAL REDUCTION AND MASS SPECTROMETRY

by

ROBERT D. VOYKSNER, ROLF STRAUB, and JEFFREY T. KEEVER
Analytical and Chemical Sciences
Research Triangle Institute
Research Triangle Park, North Carolina 27709

and

HAROLD S. FREEMAN and WHEI-NEEN HSU
College of Textiles
North Carolina State University
Raleigh, North Carolina 27695

CR819555

Project Officer

L. Donnelly Betowski
Methods Research Branch
Environmental Monitoring Systems Laboratory
Las Vegas, Nevada 89193

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
LAS VEGAS, NEVADA 89193

NOTICE

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development (ORD), partially funded and managed the extramural research described here. It has been peer-reviewed by the Agency and approved as an EPA publication.

The research reported in this report was adapted from two manuscripts sent to peer-reviewed journals. They are reprinted with permission from the following sources. Copyright 1993 American Chemical Society. The citations are as follows:

1. "Determination of Aromatic Amines Originating from Azo Dyes by Chemical Reduction Combined with Liquid Chromatography/Mass Spectrometry", Voyksner, R.D.; Straub, R.; Keever, J.T.; Freeman, H.S.; Hsu, W-N. *Environ. Sci.* Technol. 1993, 27, 1665-1672.

2. "Determination of Aromatic Amines Originating from Azo Dyes by Hydrogen-Palladium Reduction Combined with Gas Chromatography/Mass Spectrometry", Straub, R.F.; Voyksner, R.D.; Keever, J.T. *Anal. Chem.* 1993, 65, 2131-2136.

ABSTRACT

The U.S. Environmental Protection Agency is interested in azo dyes, which form the largest class of dyes in use and have the potential to form carcinogenic aromatic amines under reducing conditions. Therefore, a method that both affords reductive cleavage products *in-situ* and permits their characterization would also permit a better assessment of modern complex and structurally unknown textile dyes for their potential genotoxicity. A logical approach to such a method would involve the evaluation of procedures for the reductive cleavage of azo dyes and for the mass spectrometric analysis of the resulting volatile and nonvolatile aromatic amines.

Two general procedures were evaluated for the reductive cleavage of commercial azo dyes. The first procedure used solutions of sodium hydrosulfite (dithionite) and tin (II) chloride to reductively cleave 16 azo dyes. Identifications of the chemical reduction products were mainly based upon mass spectra obtained by particle beam high-performance liquid chromatography/mass spectrometry (HPLC/MS). Standards of the reduction products, when available, were used to confirm identities. The chemical reduction methods resulted in nearly complete reduction of the azo bond to form aromatic amines. Overall, tin (II) chloride was the more powerful reducing agent and yielded a greater number of products.

The second procedure was evaluated for reductive cleavage of eight commercial azo dyes using hydrogen (H2) and palladium (Pd). The reduction was accomplished directly in the heated injector of a gas chromatograph (GC); the resulting products were separated by capillary gas chromatography (GC) and characterized by mass spectrometry (MS). This *in-situ* method resulted in nearly complete reduction of the azo bond to form aromatic amines. For most of the tested (non-sulfonated) azo dyes, the in-line H2/Pd reduction/analysis procedure yielded the same or a greater number of reduced cleavage products as did reduction with tin (II) chloride in solution.

The analysis of reduced, industrial waste sludge extracts indicated the presence of identifiable aromatic amines, which originated from the reduction of unknown dye components. While the identity of the parent dyes in these sludges could not be determined, this analytical approach appears to provide the means to assess the environmental significance of a dye-manufacturing effluent based on the presence of various amines. Therefore, reductive cleavage followed by HPLC/MS permits the screening of modern, complex synthetic dyes for potentially genotoxic aromatic amines without prior knowledge of the parent dye structure.

The second procedure, the in-line reduction process, was not affected by the presence of wastewater interferences. The GC/MS analysis of reduced waste-sludge extracts indicated the presence of identifiable aromatic amines originating from the reduction of unknown dye components as well as from other reducible nitrogen-containing compounds. While the identities of the parent dyes in these sludges were unknown, this analytical approach appears to provide a means to assess the potential environmental significance of released effluent based on the detection of genotoxic aromatic amines.

This report was submitted in fulfillment of Cooperative Agreement CR819555 and contract 68-02-4544 by Research Triangle Institute under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from November 1989 to April 1993 and work was completed as of April 1993.

Contents

Figures

Tables

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

CI	—	chemical ionization
C.I.	—	Color Index
cm	—	centimeter
conc.	—	concentrated
EI	—	electron impact ionization
EtOAc	—	ethyl acetate
eV	—	electron volt
FD&C	—	Food, drug, and cosmetic
g	—	gram
GC/MS	—	gas chromatography/mass spectrometry
HPLC/MS	—	high performance liquid chromatography/mass spectrometry
HPLC/TS-MS	—	high performance liquid chromatography/thermospray mass spectrometry
i.d.	—	internal diameter
LC	—	liquid chromatography
M	—	molar
$M^{+\cdot}$	—	molecular radical cation
mM	—	millimolar
mg	—	milligram
min	—	minute
mL	—	milliliter
mm	—	millimeter
mmol	—	millimole
Mol. wt.	—	molecular weight
ms	—	millisecond
MS/MS	—	mass spectrometry/mass spectrometry
m/z	—	mass-to-charge ratio
μA	—	microampere
μL	—	microliter
μm	—	micrometer
N.D.	—	not detected
nm	—	nanometer
OAc	—	acetate
°C	—	degrees centigrade
PB	—	particle beam
PB-MS	—	particle beam-mass spectrometry
ppb	—	parts per billion
ppm	—	parts per million
ppt	—	parts per trillion
s	—	second
TIC	—	total ion current
TLC	—	thin layer chromatography
t_R	—	retention time
TS	—	thermospray
TS-MS	—	thermospray-mass spectrometry
UV	—	ultraviolet
V	—	volt
v/v	—	volume-to-volume ratio
w/v	—	weight-to-volume ratio

SYMBOLS

CH_2Cl_2	—	methylene chloride
$-CH_2OH$	—	hydroxymethyl
Cl	—	chlorine
-CN	—	cyano group
$-CO_2CH_3$	—	acetate
HCl	—	hydrochloric acid
He	—	helium
H_2	—	hydrogen (molecular)
$[M+H]^+$	—	protonated molecule
$MgSO_4$	—	magnesium sulfate
N_2	—	nitrogen
Na_2CO_3	—	sodium carbonate
$Na_2S_2O_4$	—	sodium hydrosulfite
NaOH	—	sodium hydroxide
$-NH_2$	—	amino group
-N=N-	—	azo group
$-NO_2$	—	nitro group
-OH	—	hydroxyl
Pd	—	palladium
pH	—	the negative of the log of the hydrogen ion concentration
$SnCl_2$	—	tin (II) chloride

ACKNOWLEDGMENT

We thank Dr. L. Don Betowski (EPA, EMSL-LV) for helpful discussion concerning the project, and U.S. dye manufacturers for supplying the commercial azo dyes.

SECTION 1

INTRODUCTION

Azo dyestuffs are widely used as colorants in a variety of products such as textiles, paper, leather, gasoline, and foodstuffs. These dyes are of great environmental concern to the U.S. Environmental Protection Agency due to their potential to form carcinogenic aromatic amines under reducing conditions (1). Genotoxicity among azo dyes and their synthetic intermediates has been well documented (2,3). For instance, 2-naphthylamine, 4-aminobiphenyl, 4,4'-diaminobiphenyl (benzidine) (4), and Acid Red 27 (FD&C Red 2, a food, drug, and cosmetic colorant) have been identified as actual or potential carcinogens, although evidence suggests that metabolites of some of the azo compounds are the actual genotoxic agents (5). Also, certain aromatic amines used in the past as precursors of synthetic dyes pose a potential risk to human health (6,7). This point was initially thought to be important only if workers were exposed to a genotoxic precursor during dye manufacture or when handling an impure dye containing the unreacted, genotoxic parent arylamine. It was later found, however, that even a commercially available and highly purified dye such as Congo Red (Color Index (C.I.) No. 22120, also known as Direct Red 28) can generate the known carcinogen benzidine, in the presence of mammalian enzymes (8). Also, the literature contains additional papers describing the genotoxicity of dyes or their reductive cleavage products (9-12). Therefore, it is recognized that azo dyes, as well as the amines formed from dye metabolism/reduction, must be monitored to adequately assess the potential risk to humans and the environment.

Over 140 million pounds of synthetic dyes are produced annually in the USA (13). Following the production of industrial dyes, impurities as well as a portion of the dyes themselves may be discharged in waste streams. The resulting wastewater is a complex sample matrix having variable proportions of suspended solid particles and dissolved solutes, and is often subjected to reductive or oxidative waste treatment processes to remove color prior to release. This so-called "reductive-clear" step creates a wide array of new products, and current methodology does not always provide the specificity or ability to identify them.

Progress in the area of environmental monitoring of azo dyes will require the development of specific, sensitive, accurate, rugged, and cost-effective methods for the detection of environmental contaminants in a variety of matrices. The fact that numerous dyes are not volatile, are thermally unstable, and are active carcinogens places an enormous burden on conventional analytical technology. The classical approach for attempting to detect low levels of nonvolatile/thermally unstable pollutants has been based on mutagenicity tests, liquid chromatography, and gas chromatography/mass spectrometry (GC/MS) (14-16). However, these techniques have disadvantages stemming from nonspecific detection, lack of sensitivity, or incompatibility with nonvolatile, thermally unstable organics. On-line HPLC/MS using thermospray has overcome some of these disadvantages in the analysis of environmental wastes (17-20). Thermospray has proven to be a suitable technique for many azo dyes (15,21-32) with structural information obtained by MS/MS (15,22) or through repeller-induced fragmentation (27,30,31). Furthermore, particle beam (PB) MS has been used to generate electron impact ionization (EI) spectra from a series of commercial dyes (32-36). However, detection of azo dyes using these HPLC/MS techniques poses additional problems, including chromatographic separation, sensitivity, or specificity of detection, due to the extreme ranges in polarity, volatility, and stability of the dyes. This problem is particularly important when dealing with the thousands of dyes and their

homologs, reduction products, and precursors that can be found in dye-related wastes. Therefore, rather than developing several methods for the multitude of compounds that exhibit extremes in polarity and volatility in dye-related wastes, alternative methodology needs to be developed that can adequately screen for the potential formation of genotoxic compounds.

This paper reports the analysis by PB-HPLC/MS and by GC/MS of common products, originating from reduction of a variety of azo dyes. The ability to chemically reduce azo dyes, with concomitant formation of aromatic amines, followed by the direct analysis of these reduction products, can provide the means to qualitatively screen azo dye content in complex industrial effluents. In addition, known mutagenic amines formed from the reduction can be monitored to better assess the environmental risk of the waste.

Nitrogen-containing substituents such as nitro and diazo groups undergo exceedingly easy reduction by many reagents (1). The nitro group is readily converted to a series of functional forms through various degrees of reduction - occasionally to a nitroso group, more often to a hydroxylamino group, and most frequently to the amino group. By controlling the amount of hydrogen and the pH of the reaction, hydroxylamino, azoxy, azo, hydrazo, and amino compounds have been obtained in good yields by catalytic hydrogenation over 2% palladium-on-carbon at room temperature and atmospheric pressure (37). Complete reduction of nitro compounds to amines is accomplished by catalytic hydrogenation. Catalysts suitable for reductive conversion of aromatic nitro compounds to amines include platinum oxide, palladium, Raney nickel, copper chromite, and rhenium sulfide (1). Popular reducing agents for the conversion of aromatic diazo and nitro compounds to amines are iron, zinc, tin, tin (II) chloride ($SnCl_2$), hydrogen sulfide or its salts, and sodium hydrosulfite ($Na_2S_2O_4$, sodium dithionite) (37). However, the detection of azo dyes by HPLC/MS poses problems due to their wide range of polarities and volatilities, creating difficulties with chromatographic separations and MS responses. Rather than developing several methods for the multitude of azo dyes in waste streams, cost effective methodologies need to be developed that can adequately screen for the formation of potential genotoxic compounds in waste streams.

Gas chromatography (GC) employing MS detection (GC/MS) is relatively routine compared with HPLC/MS, but has rarely been used to analyze azo dyes due to their limited volatility. Therefore, only a few GC/MS methods have been reported for monitoring volatile dyes (38) or dyes in waste streams (13,39). The ability to chemically reduce dyes by hydrogenation in a palladium-filled GC injection port, followed by direct GC/MS analysis of these usually more volatile reduction products can provide the means to qualitatively screen industrial effluents for potential toxicity.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The reduction of industrial sludges containing azo dyes usually forms a nearly colorless effluent. Depending on the identity of the azo dyes in the waste, the aromatic reduction products may be more harmful to the environment than the original, untreated sludge components. The use of $Na_2S_2O_4$ or $SnCl_2$ to cleave the -N=N- group of azo dyes followed by HPLC/MS analysis of the reaction products is a possible way to assess the toxicity potential of complex waste sludges. Using a library containing the mass spectra of aromatic amines representing common couplers used to synthesize azo dyes can help in the identification of the parent dye. Also, components in effluents following the "reductive-clear" steps can be monitored. This procedure is less suitable, however, for characterizing wastes containing sulfonated (hydrophilic) azo dyes, due in part to the significantly higher number of standards required to build an effective library, and the limitations of PB-MS for the sensitive detection of the sulfonated aromatic amines. However, the latter problem can be addressed using electrospray negative ion-MS for the detection of the sulfonated compounds (32,40).

The reduction of azo dyes by H_2 in the presence of Pd at the elevated temperature of a gas chromatographic injector has the advantage that the entire analysis can be accomplished directly by GC/MS. In numerous cases, the same reduction products were observed for this in-line H_2/Pd reduction as were formed by reduction of azo dyes with $SnCl_2$ in solution. This in-line reduction/analysis procedure represents a cost-effective and simple way to screen waste streams for aromatic amines that can be formed during reductive waste treatments or in the environment by anaerobic processes. A knowledge of the amines that can be generated aids the assessment of the toxic potential of the particular waste.

The procedures outlined in this report were originally developed for the screening of waste materials for azo dyes. Since azo dyes do not have any uniquely characteristic feature that either mass spectrometry or infrared spectroscopy could use to identify as belonging to this particular class, the reduction-before-analysis approach that has been proposed is recommended for use to identify the more common aromatic amines. An aromatic amine then could be a possible indicator of the parent azo dye. Raman spectroscopy is able to identify the azo linkage in these compounds, but the application of Raman to trace analysis is still in the developmental stages. There are other methods (e.g., SW-846 Method 8321) to perform a more complete analysis to detect and quantify the parent dyes, which may be required for regulatory and enforcement purposes; however, these methods are more tedious and require purified dye standards, which are not readily available.

Besides being an effective screening method for the parent azo dyes, these procedures have proved successful for identifying potential environmental breakdown products of these dyes. The disposal of azo dyes in a reducing environmental will often result in the formation of aromatic amines. These methods, therefore, provide a better assessment of the potential toxicity of azo dyes in the environment than the analysis of the parent dye. Real environmental samples can be screened in this manner. However, the original non-reduced sample has to be retained for further examination following the screening procedure.

The direct on-column reduction of wastes using hydrogen and palladium provides an efficient and cost-effective procedure to assess the concentration of aromatic amines formed from these wastes and hence the potential risks associated with their environmental management.

SECTION 3

EXPERIMENTAL PROCEDURES

Materials

Sixteen commercial dyestuffs, identified by their C.I. name and number (Table 1), were evaluated in this study. The samples having low dye content were used after isolation from diluents by CH_2Cl_2 extraction. The dyes were obtained from the following sources: 1-9 and 16 (Aldrich Chemical Company Inc., Milwaukee, WI, USA); 10 (Ciba-Geigy, Dyestuffs and Chemicals Division, Greensboro, NC, USA); 11 (BASF, Charlotte, NC, USA); 12 (Eastman Chemicals, Kingsport, TN, USA); 13 (Sandoz Colours and Chemicals, Charlotte, NC, USA); 14 and 15 (Crompton & Knowles, Charlotte, NC, USA). All other chemicals were purchased from Aldrich. The solvents used for extractions, reactions, or liquid chromatography were "HPLC/GC grade" quality (Baxter Healthcare Corp., Muskegon, MI, USA). The water was distilled and passed through a Milli-Q Water System (Millipore Corp., Bedford, MA, USA) prior to use. Amine standards such as 2-amino-toluene, 2-methyl-1,4-diaminobenzene, aniline, 1-amino-2-naphthol, 2,4-dimethylaniline, 4-nitroaniline, 4-aminophenol, 8-amino-7-hydroxynaphthalene, 1,3-disulfonic acid disodium salt, 2-bromo-4,6-dinitroaniline, benzidine and 3,3'-dichlorobenzidine were from Aldrich and from EPA (Las Vegas, NV, USA). Sludges I and II were provided by EPA (Environmental Research Laboratory, Athens, GA, USA).

Thin layer chromatography (TLC) for solvent dyes was performed on normal-phase Macherey Nagel SIL G/UV_{254} plates (Alltech, Avondale, PA, USA) using toluene:ethyl acetate (EtOAc)/4:1 as the mobile phase. For all other hydrophobic dyes, TLC analyses used toluene:EtOAc/1:4; the hydrophilic dye (Acid Orange 10), however, was eluted with ethanol:EtOAc/4:1.

The palladium spun fibers (lot #20248) used for in-line hydrogenation experiments were 99.05% (Johnson Matthey Electronics, Ward Hill, MA, USA).

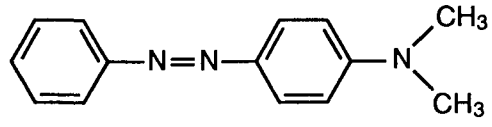
Extraction Procedures

Dyes. Dye samples having a high diluent content were separated from their commercial additives via Soxhlet extraction using methylene chloride, and then recrystallized from toluene/petroleum ether. The pure commercial dyes thus obtained were used in the subsequent analyses.

Sludges. A set of organic extracts using 60 g of sludge I and 75 g of sludge II were obtained from two 300-mL methylene chloride extractions. The organic extracts of each sludge were concentrated to give 0.2 g of purple solid from sludge I and 0.35 g of orange solid from sludge II. TLC analysis showed that both samples contained a major component along with two minor components. Each solid concentrate was divided into three equal parts, two of which were used in the reduction procedures. The third part was dissolved in acetonitrile (10 mg/mL) under a nitrogen (N_2) atmosphere and used as the unreduced control sample in the HPLC/MS and GC/MS analyses. For the blank sample, the same amount of CH_2Cl_2 as used in the extractions was evaporated to dryness and the residue dissolved in acetonitrile.

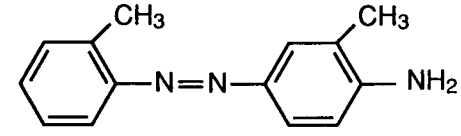
Chemical Reduction Procedures

Tin Chloride ($SnCl_2$/HCl) Reduction. The reaction was conducted in boiling methanol and required approximately 1 to 4 mmol of $SnCl_2$ (40% in conc. HCl) per mmol of dye to remove the last traces of the parent dye, as judged by TLC. The variability in the quantity of reducing agent needed can be attributed to the number and reactivity of reducible groups (e.g., N=N, NO_2) on the dye. Once the solution was decolorized (15 to 60 min-



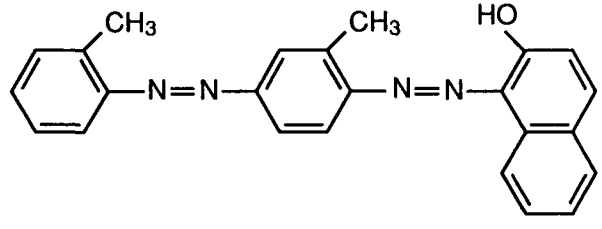
1. Solvent Yellow 2

C.I. 11020



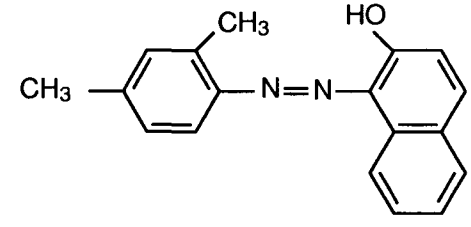
2. Solvent Yellow 3

C.I. 11160



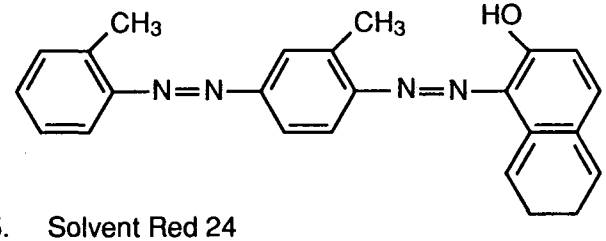
3. Solvent Yellow 14

C.I. 12055



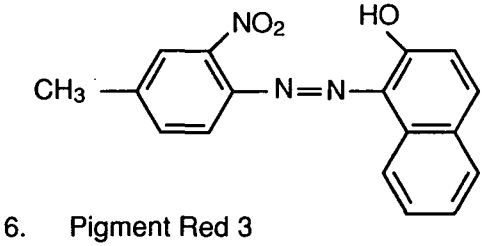
4. Solvent Orange 7

C.I. 12140



5. Solvent Red 24

C.I. 26105



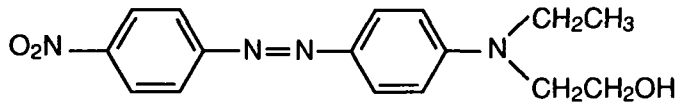
6. Pigment Red 3

C.I. 12120

0182QAD94.RPT-1

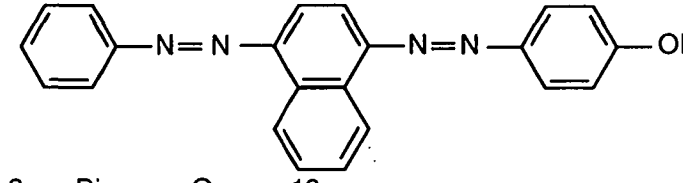
TABLE 1. AZO DYES USED IN THIS STUDY

Structure, Name, Color Index (C.I.) and Molecular Weights of Commercial Dyes



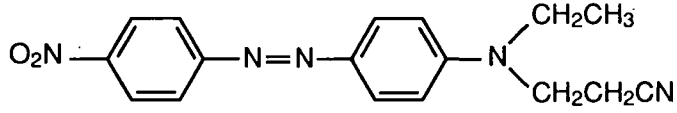
7. Disperse Red 1

C.I. 11110 (314)



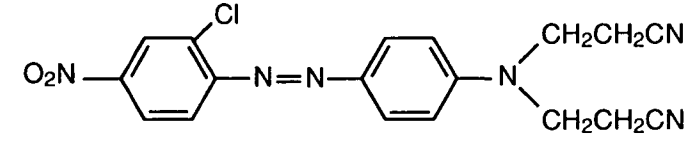
8. Disperse Orange 13

C.I. 26080 (352)



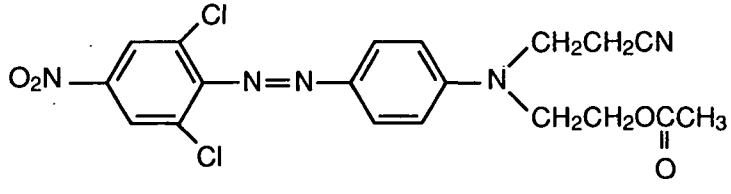
9. Disperse Orange 25

C.I. 11227 (323)



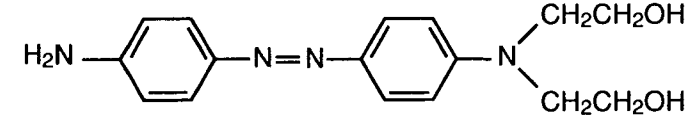
10. Disperse Orange 44

C.I. none (382)



11. Disperse Orange 30

C.I. 11119 (449)



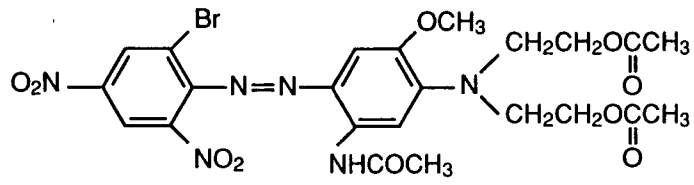
12. Disperse Black 9 (precursor)

C.I. none (300)

0182QAD94.RPT-2

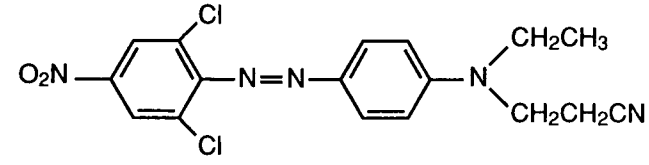
TABLE 1. AZO DYES USED IN THIS STUDY Continued

Structure, Name, Color Index (C.I.) and Molecular Weights of Commercial Dyes



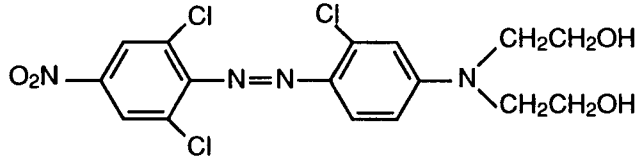
13. Disperse Blue 79

C.I. 11345 (624)



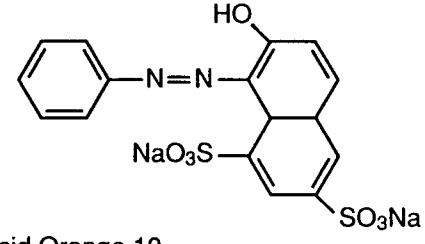
14 Disperse Orange 37

C.I. none (391)



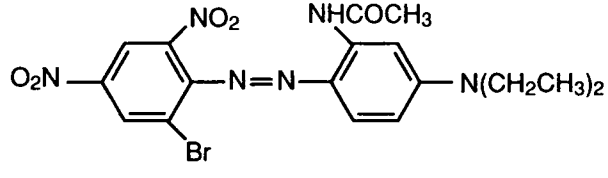
15. Disperse Brown 1

C.I. 11152 (432)

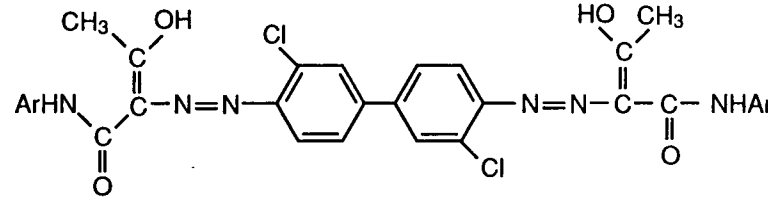


16. Acid Orange 10

C.I. 16230 (452)



17. Industrial Precursor for Disperse Blue 165 (478)



18. Generic Structure of Certain Yellow Azo Pigments

TABLE 1. AZO DYES USED IN THIS STUDY Continued

Structure, Name, Color Index (C.I.) and Molecular Weights of Commercial Dyes

utes), 5 to 10 g of solid Na_2CO_3 was added to achieve pH 7 to 8. This treatment normally produced a milky emulsion, which was concentrated to remove the methanol, diluted with EtOAc, and filtered or centrifuged to separate the white, solid precipitate. The EtOAc layer was dried over $MgSO_4$ and concentrated. TLC was then used to determine the number of products and to confirm that reductive cleavage of the dye was complete. A portion of the reaction product mixture was then dissolved in acetonitrile (10 mg/mL) and stored under N_2 for HPLC/MS analysis.

Sodium Hydrosulfite ($Na_2S_2O_4$) Reduction. A solution (or suspension) of 1 mmol dye in boiling methanol, under N_2 atmosphere, was treated with 14 to 28 mmol of $Na_2S_2O_4$ (25% w/v in water) solution until decoloration was completed. This treatment required about 5-12 min depending on the structure of the dye. However, Solvent Red 24 (5; a disazo colorant) and Pigment Red 3 (6; a sparingly soluble colorant) required about 98 mmol of the reducing agent to effect decoloration. The workup of this reaction involved the evaporation of methanol under nitrogen followed by EtOAc extraction of the remaining mixture. The EtOAc extract was concentrated, and the mixture obtained was dissolved in acetonitrile (10 mg/mL) and stored under N_2 for HPLC/MS analysis.

Chemical Reduction of Sludge Extracts

Tin Chloride ($SnCl_2$/HCl) Reduction. Solutions of the concentrate from extracts of sludges I and II were dissolved separately in methanol or methylene chloride and treated with 1 M $SnCl_2$ (in conc. HCl). This caused an immediate decolorization of the solution. The solution was made basic (pH 10 to 12) with NaOH and then extracted with methylene chloride. The extract was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was dissolved in acetonitrile (10 mg/mL) and stored under N_2 for HPLC/MS analysis.

Sodium Hydrosulfite ($Na_2S_2O_4$) Reduction. Solutions of the concentrate from extracts of sludge I and II were dissolved separately in methanol:methylene chloride (1:1) and boiled with 1 M $Na_2S_2O_4$ solution for 20 minutes. Very little color change was apparent at the end of this treatment. The resulting solutions were evaporated to dryness, and the residue dissolved in acetonitrile (10 mg/mL) and stored under N_2 for HPLC/MS analysis.

Instrumentation

Equipment for HPLC/PB-MS. The chromatography was performed using a Waters Series 600 multisolvent delivery system (Waters Assoc., Inc., Milford, MA, USA) controlled by a Waters 600-MS System Controller. The samples were injected with a Waters model U6K universal liquid chromatograph injector and separated on a Spherisorb ODS II, 5-μm particle size, 25-cm x 4.6-mm i.d. column (Regis Chemical Company, Morton Grove, IL, USA) for dye samples, and on an Alltech Solvent Miser, C_{18}, 5-μm particle size, 15-cm x 2.1-mm i.d. column (Alltech Assoc., Inc., Deerfield, IL, USA) for sludge extracts. A Waters 484 MS Tunable Absorbance Detector set at 254 nm was placed in-line before the model 59980A PB interface (Hewlett Packard, Palo Alto, CA, USA). The PB interface was connected to a Hewlett Packard quadrupole mass spectrometer, model 5988A. The equipment and conditions used for HPLC/thermospray (TS)-MS is described elsewhere (32).

Equipment for GC/MS. For the analysis of reduced azo dye standards, a GC/MS system consisting of a Finnigan MAT 4500 GC (Finnigan MAT Co., San Jose, CA, USA) and a Finnigan MAT 4500 quadrupole MS were used. A 15-m x 0.32-mm i.d., 0.25-μm DB-1 (100% methyl) fused silica column (J & W Scientific, Folsom, CA, USA) was used for the separation of the reduction products.

For the in-line hydrogenation experiments, a Hewlett-Packard GC, model 5890 (Hewlett-Packard, Palo Alto, CA, USA), with both open injection liners and liners packed with palladium filaments, was connected to a Hewlett-Packard (model 5988A) single quadrupole MS for detection and characterization of the reduction products. A 12.5-m x 0.28-mm i.d., 0.33-μm crosslinked methyl silicon fused silica column (Hewlett Packard) was used for separation.

Equipment and Conditions for High Resolution Measurements. The high resolution mass spectrometer (VG ZAB-E; Fisons Instruments, Manchester, U.K.) scanned from m/z 50 to 450 at a rate of 10 s per scan with a reset time of 2 s and a response time of 0.01 ms. The ion source was operated at 150°C. The trap current was 200 μA, and electron energy was set to 70 eV. The sample holder was filled with azo dye and Pd wires in a ratio of about 1:10, w/w. The probe temperature was ramped from 50°C to 250°C at a rate of 1°C per s.

HPLC/PB-MS Conditions

Dye Samples. The injected volume was 75 μL, and the chromatography was performed with a water-methanol linear gradient (40% to 100% methanol in 20 min with a 15-min hold) at a flow rate of 0.5 mL/min. The PB desolvation chamber temperature was 60°C, and a helium inlet pressure of 2.75 bar was maintained. The ion source was operated at 200°C. The filament emission current was 0.3 mA, and the electron energy was 70 eV. The manifold temperatures were set to 90°C (front) and 45°C (rear), and the mass range was scanned from m/z 45 to 650 at a rate of 1 s per scan. The sensitivity of the system was checked by injection of a standard solution of caffeine.

Sludges. A 7-μL volume of extract was injected and eluted with a water-acetonitrile gradient (40% to 100% acetonitrile in 20 min, with a 25-min hold) at a flow rate of 0.2 mL/min. The changes from the HPLC conditions for the dye samples provide better separation for the broader range of components that could be found in the complex sludges. A lower flow rate was used to provide better PB sensitivity and stability, especially when operating with high aqueous-content solutions. The PB interface was operated as described above for dyes, except the ion source was set at 250°C, and the mass range was scanned from m/z 45 to 550 at a rate of 1 s per scan.

GC/MS Conditions

Reduced-Dye Standards. The injection volume was 2 μL of each standard. A temperature gradient of 50 to 325°C at a ramp rate of 10°C/min was applied. The helium (He) carrier gas rate was 1 mL/min and the split ratio 20:1 was implemented after 0.7 min. The injector and the separator temperature were maintained at a temperature of 290°C. The MS operated in the electron ionization (EI) mode and scanned from m/z 40 to 500 with a scan time of 1 s. The source temperature was 190°C.

In-Line Hydrogenation Experiment. The injection volume was 1 μL of a 1mM dye solution or sludge extract in acetonitrile. Both empty injection liners and similar glass liners packed with 11 mg of 0.08-mm palladium wire were used in the GC separation. A temperature gradient of 40°C to 300°C at a ramp rate of 10°C/min with hold at the final temperature for 4 min was applied. For the in-line hydrogenation experiments, the carrier gas consisted of He doped with 5% (v/v) hydrogen (H2) (Linde Specialty Gases, Somerset, N.J.). The carrier gas flow rate was 0.8 mL/min and the split ratio 10:1 was implemented after 0.5 min. The injector was maintained at a temperature of 300°C. The MS operated in the EI mode and scanned from m/z 30 to 600 with a scan time of 1 s. The source temperature was 200°C.

SECTION 4

RESULTS AND DISCUSSION

Chemical Reduction of Commercial Azo Dyes

The first step in achieving the principal goal of this work, to screen unknown industrial waste for the formation of potentially hazardous products after a reductive treatment, involved comparing $SnCl_2$ and $Na_2S_2O_4$ as reducing agents for the dyes shown in Table 1. For each dye, an aqueous medium in a nitrogen atmosphere was used during the reduction, since some of the anticipated reduction products might have been air sensitive. It was later determined, however, that reductive treatment of the azo dyes in the presence of oxygen yielded the same reduction products as the reaction under a N_2 atmosphere. The reduction time was optimized to maximize the yield of reduction products detected, while minimizing the amount of the parent dye remaining. Disperse dyes containing 2,6-dichloro groups (such as Disperse Orange 37 (14) and Disperse Orange 30 (11)) were generally quite resistant to chemical reduction, requiring longer reaction times to achieve a complete reductive-cleavage of the parent dyes.

The PB HPLC/MS analysis of the reduced azo dyes (Table 2) indicated that the major degradation products formed were aromatic amines generated by splitting the azo linkage. These aromatic amines were not detected in the intact dye prior to reduction. Standards, when available, were used to confirm the identity of the reduction products as well as provide an estimate for reduction efficiency. The yield of aromatic amines formed (Table 2) was based upon UV (254 nm) chromatographic peak areas. The yield ranged from 61-110% of the peak signal of the parent dye prior to reduction. These yields were consistent with the yields measured by MS when appropriate standards existed. While estimation of reduction-product yields suffers from variability due to difference in extinction coefficients, this method is probably as accurate as an estimation based on MS response in the absence of a complete set of standards.

The extraction recovery was verified gravimetrically at the 10-mg/mL level for selected, non-volatile reduction products. Recoveries were greater than 90% for disperse and solvent dyes and were about 70% for sulfonated dyes. A more thorough investigation of reduction-product recovery and yields will be the subject of future investigation and will be necessary if semi-quantitative determinations are to be performed.

Structurally simple solvent dyes (1-5) in Table 1 formed the anticipated aromatic amines after reduction (Scheme 1).

Scheme 1: $R_1\text{-}N{=}N\text{-}R_2 \xrightarrow[\text{or } Na_2S_2O_4]{SnCl_2/HCl} R_1\text{-}NH_2 + R_2\text{-}NH_2$

$Na_2S_2O_4$ reduction of Solvent Yellow 3 (2) and Yellow 14 (3) did not go to completion, as unchanged dye was detected in the extracts. Figure 1 shows the HPLC/PB-MS total ion current (TIC) chromatograms of Solvent Yellow 3 (2) following reduction with either $Na_2S_2O_4$ (B) or $SnCl_2$ (C).

TABLE 2. IDENTIFICATION OF CHEMICAL REDUCTION PRODUCTS OF COLORANTS **1-16** BY HPLC/MS

No.	Dye	Identified Reduction Products	Mol. Wt.	t_R[a]	Particle Beam m/z (Relative Intensity)[b]	UV (%)[c]
1	Solvent Yellow 2	• aniline[d]	93	12.9	93(100); 66(39)	>1
		• N,N-dimethyl-1,4-di-aminobenzene	136	28.4	136(100); 120(85); 93(37); 81(41)	88
2	Solvent Yellow 3	• 2-aminotoluene[d]	107	18.2	107(74); 106(100); 77(26); 51(15)	90
		• 2-methyl-1,4-di-amino-benzene[d]	122	30.0	122(100); 94(33); 78(26); 58(19)	3
		• unchanged dye[e]	225	25.8	225(58); 134(17); 106(100); 91(28); 77(23); 75(13); 51(4)	3
3	Solvent Yellow 14	• aniline[d]	93	13.1	93(100); 66(37)	7
		• 1-amino-2-naphthol[d]	159	19.3	159(100); 130(89); 103(26); 77(22); 51(15)	49
		• unchanged dye[e]	248	28.3	248(90); 219(7); 171(15); 143(100); 115(97); 89(10); 77(41); 51(10)	20
4	Solvent Orange 7	• 2,4-dimethylaniline[d]	121	22.1	121(100); 106(78); 77(17)	30
		• 1-amino-2-naphthol[d]	159	20.2	159(100); 130(63); 103(13); 51(11)	31
5	Solvent Red 24	• 2-aminotoluene[d]	107	22.4	107(100); 91(55); 77(49); 51(25)	18
		• 2-methyl-1,4-diamino-benzene[f]	122	40.4	122(33); 104(46); 71(41); 55(100)	16
		• 1-amino-2-naphthol[d]	159	19.1	159(100); 130(70); 103(20); 77(20)	42
6	Pigment Red 3	• 1-amino-2-naphthol[d]	159	19.5	159(100); 130(62); 103(15); 77(19)	70
7	Disperse Red 1	• 4-nitroaniline[d]	138	13.0	138(100); 108(83); 92(50); 65(75)	70

TABLE 2. IDENTIFICATION OF CHEMICAL REDUCTION PRODUCTS OF COLORANTS **1-16** BY HPLC/MS Continued

No.	Dye	Identified Reduction Products	Mol. Wt.	t_R[a]	Particle Beam m/z (Relative Intensity)[b]	UV (%)[c]
8	Disperse Orange 13	• aniline[d]	93	13.1	93(100); 66(40)	>1
		• 4-aminophenol[d,g]	109	6.2	109(10); 108(100); 80(48); 64(7)	10
		• 1,4-diamino-naphthalene	158	10.7	158(100); 109(50); 80(37)	78
9	Disperse Orange 25	• 4-nitroaniline[d]	138	12.3	138(100); 108(83); 92(50); 65(75)	18
		• N-(2-cyanoethyl)-N-(ethyl)-1,4-diamino-benzene	189	14.8	189(25); 149(100); 120(34); 92(4)	75
10	Disperse Orange 44	• N,N-bis(2-cyanoethyl)-1,4-diaminobenzene[d]	214	8.3	214(40); 174(100); 120(37); 106(5)	70
11	Disperse Orange 30	• 1,4-diamino-2,6-dichlorobenzene	176	13.8	76(27); 149(73); 124(40); 98(100); 81(56); 78(63)	10
		• N-(2-cyanoethyl)-N-(2-hydroxethyl)-1,4-diaminobenzene[f]	205	6.1	205(45); 174(85); 165(80); 120(100); 92(30); 65(20)	35
		• 2,6-dichloro-4-nitro-aniline	206	25.5	208(40); 206(60); 178(50); 176(84); 162(20); 160(30) 135(15); 133(22); 126(30); 124(100); 92(28); 90(31)	20 45
		• N-(2-cyanoethyl)-N-(2-acetoxyethyl)-1,4-diaminobenzene	247	10.0	247(1); 205(32); 174(91); 165(54); 120(100); 92(20); 65(20)	
12	Disperse Black 9	• 1,4-diaminobenzene[d]	108	8.3	108(100); 92(44); 80(66); 67(25); 52(64)	30
		• N,N-bis-(2-hydroxyethyl)-1,4-diaminobenzene	196	21.7	196(25); 165(100); 120(20); 93(14)	60
13	Disperse Blue 79	• 2-bromo-1,4,6-triaminobenzene[f]	201	10.4	203(15); 202(5); 201(10); 88(23); 70(83); 61(100)	25
		• 3-acetamido-4-[N,N-bis-(2-acetoxyethyl)amino]-1-amino-5-methoxybenzene	367	15.1 87(100)	367(10); 294(15); 208(9);	60

TABLE 2. IDENTIFICATION OF CHEMICAL REDUCTION PRODUCTS OF COLORANTS **1-16** BY HPLC/MS Continued

No.	Dye	Identified Reduction Products	Mol. Wt.	t_R[a]	Particle Beam m/z (Relative Intensity)[b]	UV (%)[c]
14	Disperse Orange-37	• 1,4-diamino-2,6-dichlorobenzene	176	23.0	176(27); 149(73); 124(40); 98(100); 84(56); 74(63)	40
		• N-(2-cyanoethyl)-N-ethyl-1,4-diaminobenzene	189	26.1	189(19); 149(100); 120(73); 106(8); 92(21)	40
		• 4-amino-2,6-dichloro-4' [N-(2-cyanoethyl)-amino] azobenzene		333	334(15); 333(27); 293(100); 265(21); 229(7); 201(33);149(49); 120(41); 100(15); 92(9)	10
15	Disperse Brown 1	• 1,4-diamino-2,6-dichlorobenzene	176	22.3	176(56); 149(28); 134(59); 98(100); 84(53)	40
		• 3-chloro-N,N-bis(2-hydroxyethyl)-1,4-diaminobenzene	230	17.4	230(21); 199(100); 155(37); 127(13)	40
16	Acid Orange 10	• aniline[d]	93	13.5	93(100); 66(40)	40

[a]t_R = retention time in TIC chromatogram (min). [b]m/z (Relative intensity) reports the major peaks (>5%) of each product down to m/z 50; a maximum of 12 ions are reported in descending m/z. [c]UV(%) = peak area of identified reduction product/Σ of the peak areas in the chromatogram of the reduced sample at a wavelength of 254 nm; the identified reduction products were <0.5% of the total peak area in the unreduced HPLC/UV analysis of the parent dye. [d]Identity confirmed with standard. [e]observed only after $Na_2S_2O_4$ reduction. [f]observed only after $SnCl_2$ reduction. [g]only identified by HPLC/PB-MS in sample reduced with $SnCl_2$.

The response from the reduction products is lower than that of the unreduced dye because of losses in the particle beam momentum separator due to sample volatility. The peaks for 2 appear at the end of the HPLC run since a low-pressure solvent mixer on the HPLC pump and a 4.6-mm i.d. column operating at 0.5 mL/min resulted in a 7-8 min delay in solvent changes at the detector. TIC chromatograms B and C show peaks attributable to the formation of the two reduction products (Peaks No. 1 and 3), while the parent dye (Peak No. 2) is nearly absent. PB-MS response for volatile amines (e.g., aniline) is 2-10% of the response by GC/MS (fused silica column fed directly into the MS source). Reduction products that are volatile or of low molecular weight, such as aniline from either Solvent Yellow 2 (1) or Disperse Orange 13 (8), were difficult to detect by PB-MS, due to losses in the momentum separator.

The presence of numerous substituent groups (e.g. $-NO_2$, $-CO_2CH_3$, and -CN) in addition to the azo linkage in dyes 9-11, 13, and 15, increased the number of components among the reductive-cleavage products (Scheme 2). For example, the reduction of Disperse Orange 30 (11) with $SnCl_2$/HCl also resulted in hydrolysis of the acetate to the hydroxyethyl group, giving a product with a hydroxyethyl group and a molecular weight of 205. Also, one of the major products (2,6-dichloro-4-nitroaniline) formed from cleavage of the azo bond was further reduced to give the corresponding diamine (2,6-dichloro-1,4-diaminobenzene).

Scheme 2:

for $X = NO_2$

$$R_1\text{-}N{=}N\text{-}R_2\text{-}X \xrightarrow[\text{or } Na_2S_2O_4]{SnCl_2/HCl} R_1\text{-}NH_2 + H_2N\text{-}R_2\text{-}NH_2$$

for $X = C_2H_4OAc$

$$R1\text{-}N{=}N\text{-}R_2\text{-}X \xrightarrow[\text{or } Na_2S_2O_4]{SnCl_2/HCl} R1\text{-}NH_2 + HOC_2H_4\text{-}R_2\text{-}NH_2$$

Disperse Blue 79 (13) also showed further reduction of substituents. For example, the expected reduction product, 2-bromo-4,6-dinitroaniline, was not detected. Instead, 2-bromo-1,4,6-triaminobenzene was found.

Clearly, substituent groups, such as -OAc and $-NO_2$, in azo dyes are often affected by the use of a powerful reducing agent such as $SnCl_2$. Under the right conditions, NO_2 and azo functional groups yield common diamino or triamino products, thereby simplifying the analysis. In addition, the formation of multiple reduction products reinforces the point that this approach is best suited for qualitative screening purposes and that the reduction products must be identified if a particular target azo dye is to be monitored by this methodology. Sulfonated azo dyes also pose an environmental concern. These dyes are usually precluded from PB MS analysis due to nonvolatility and lack of MS response. One acid dye (Acid Orange 10 (16)) was included in this evaluation to determine if the reduction methodology could aid in developing MS screening procedures for this type of dye. PB-MS analysis of the reduced dye only detected the volatile reductive-cleavage product, aniline. A sulfonated reduction product (8-amino-7-hydroxynaphthalene-1,3-disulfonic acid), was confirmed by thermospray mass spectrometry based on the retention time of a standard.

Both chemical reduction methods resulted in reductive cleavage for most of the commercial azo colorants studied. However, $SnCl_2$/HCl was a more powerful reducing agent, yielding a greater number of products as well as thorough conversion of the starting dye. Therefore, it is preferable to use $SnCl_2$/HCl for further reduction studies of commercial azo dyes and for industrial sludges. Treatment of the azo dyes listed in Table 2 forms some common reduction products. For example, aniline, methylaniline, or dimethylaniline are common reductive cleavage products for seven dyes (Nos. 1-5, 8 and 16); aminonaphthol is a common reductive cleavage product for four dyes (Nos. 3-6); diaminobenzenes (including monochloro and dichlorodiaminobenzenes) are common reductive cleavage products for four dyes (Nos. 11, 12, 14, and 15); and 4-nitroaniline is a reduction product for two dyes (Nos. 7 and 9). As a result, the detection of certain amines can give useful information regarding the possible structures of the parent azo dye compounds used in a manufacturing procedure.

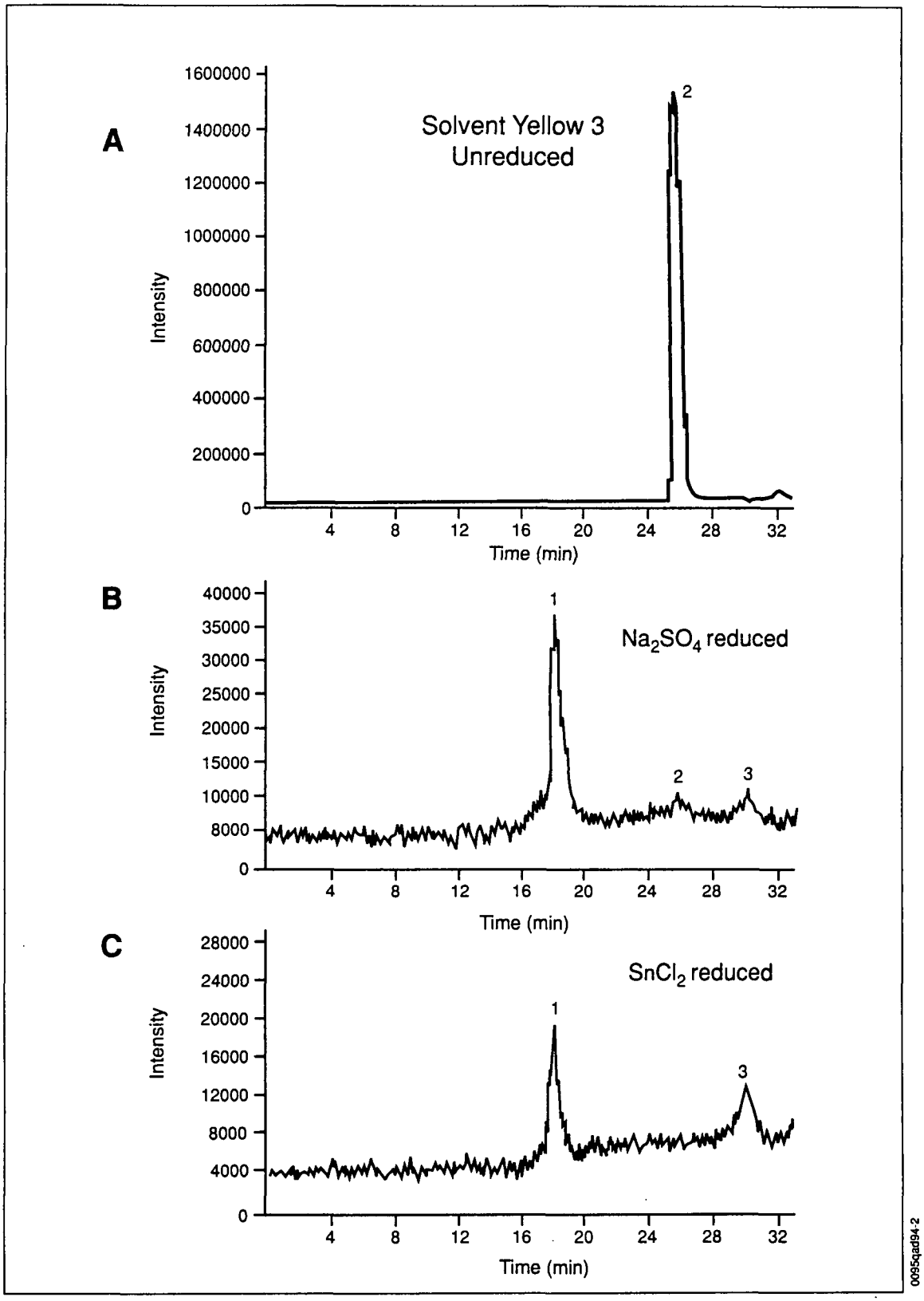

A
Solvent Yellow 3
Unreduced
Intensity
Time (min)
B
Na_2SO_4 reduced
Intensity
Time (min)
C
$SnCl_2$ reduced
Intensity
Time (min)
0095qad94-2

Figure 1

Detection of Aromatic Amines in Reduced Sludge Extracts

Extracts of sludges (I and II) from two different waste treatment sites were reduced with $SnCl_2$/HCl and $Na_2S_2O_4$, and then analyzed by HPLC/MS to screen for potentially hazardous reduction products, and for azo dyes based upon the detection of aromatic amines. The HPLC conditions were changed for the analysis of the sludges compared with the dye standards to provide a better and faster separation for a broader range of components that might be found in a sludge. This was accomplished by using a stronger elution solvent, such as acetonitrile instead of methanol, and a 150-mm x 2.1-mm column operated at a flow rate of 0.2 mL/min. A 2.1-mm i.d. HPLC column offered two advantages. First the column's operating flow rate is closer to the optimal flow rate of the PB-MS interface (0.2-0.4 mL/min) resulting in better sensitivity (especially for an aqueous mobile phase) than with the 0.5 mL/min for the 4.6-mm i.d. column. Secondly, the chromatographic peak width is narrower compared with the 4.6-mm i.d. column, resulting in a higher concentration introduced into the MS and better sensitivity. To ensure the validity of the method, all amine standards were analyzed using these new conditions to verify elution order. While the determination of the dye standards would have benefited from better resolution, it was decided no additional information would have been gained about their reduction, and they were not reanalyzed using these new conditions.

Sludge I. The HPLC/PB-MS chromatograms (A and B) of the components in both chemically reduced extracts of the wastewater sludge are presented in Figure 2. The $Na_2S_2O_4$ treatment formed four new products, while $SnCl_2$ afforded at least six new products, based upon the total ion current (TIC) chromatogram. The retention times and m/z values of the reduction products are found in Table 3. Identification of products was based mainly on mass spectral library searches (41), spectral interpretation, and available aromatic amine standards analyzed under the same HPLC/MS conditions. Thermospray (TS) ionization was used to confirm the molecular weights of the reduction products. The HPLC/TS-MS analysis also indicated that the principal blue colorant present in sludge I was the monoazo dye 17 (Table 1), molecular weight 478, an important precursor in the synthesis of Disperse Blue 165 (TS mass spectrum not shown). Analysis of the reduction products indicated that dye 17 was present at the 3-5 μg level in the sludge extract based upon the relative intensity of similar reduction products generated from Disperse Blue 79 (13). This corresponds to about 143-238-ppm level in the actual sludge sample. The TIC chromatogram C of the untreated sludge sample shows no fully identifiable mass spectrum.

Sludge II. The analysis of the extract from the $Na_2S_2O_4$ treatment closely resembled that obtained from the untreated sample. This indicates that $Na_2S_2O_4$ was not a strong enough reducing agent for the colorant(s) present. However, $SnCl_2$ treatment led to several new products, the principal of which are shown in Figure 3. Table 3 lists the retention time and m/z values of the two products (3,3'-dichlorobenzidine and N,N'-dicyclohexylurea) generated from sludge II by $SnCl_2$ treatment. Although both compounds were found in the untreated and treated extract, $SnCl_2$ treatment significantly increased the amount of the amine to near 1 μg (based on the relative response to benzidine) in the sludge extract, verifying that the parent dye contained a benzidine moiety. This corresponded to about 84 ppm of dichlorobenzidine in the sludge sample. These results suggest that the principal components in this sludge were colorants of type 18 (Table 1), a common skeleton of commercial yellow-pigment azo dyes. They are the primary type colorants for which dichlorobenzidine is still used today.

In-Situ Reduction of Commercial Azo Dyes

The ability to reduce azo dyes in a H_2-Pd injection port liner, in-line with GC/MS analysis, was tested for some of the compounds in Table 1. Initially, each dye was analyzed with an empty injection port to demonstrate the absence of aromatic amines. The GC/MS analysis for eight dyes did not detect aromatic amines in the dye formulation. Also, most dyes (except Solvent Yellow 2) failed to show intact molecular ions. Usually the ions detected in the mass spectrum are a result of thermal degradation of the parent dye in the heated injection port.

In-line reduction using the GC injection port required the presence of Pd and hydrogen and the reaction required high temperatures (> 250°C) to reduce relatively simple azo dyes such as Solvent Yellow 2. Failure to achieve one of these criteria significantly affected the reduction yield of the azo dyes. With 5% hydrogen in helium reduction yields were similar to those with pure hydrogen; this removes the risk associated with using pure hydrogen in the experiment.

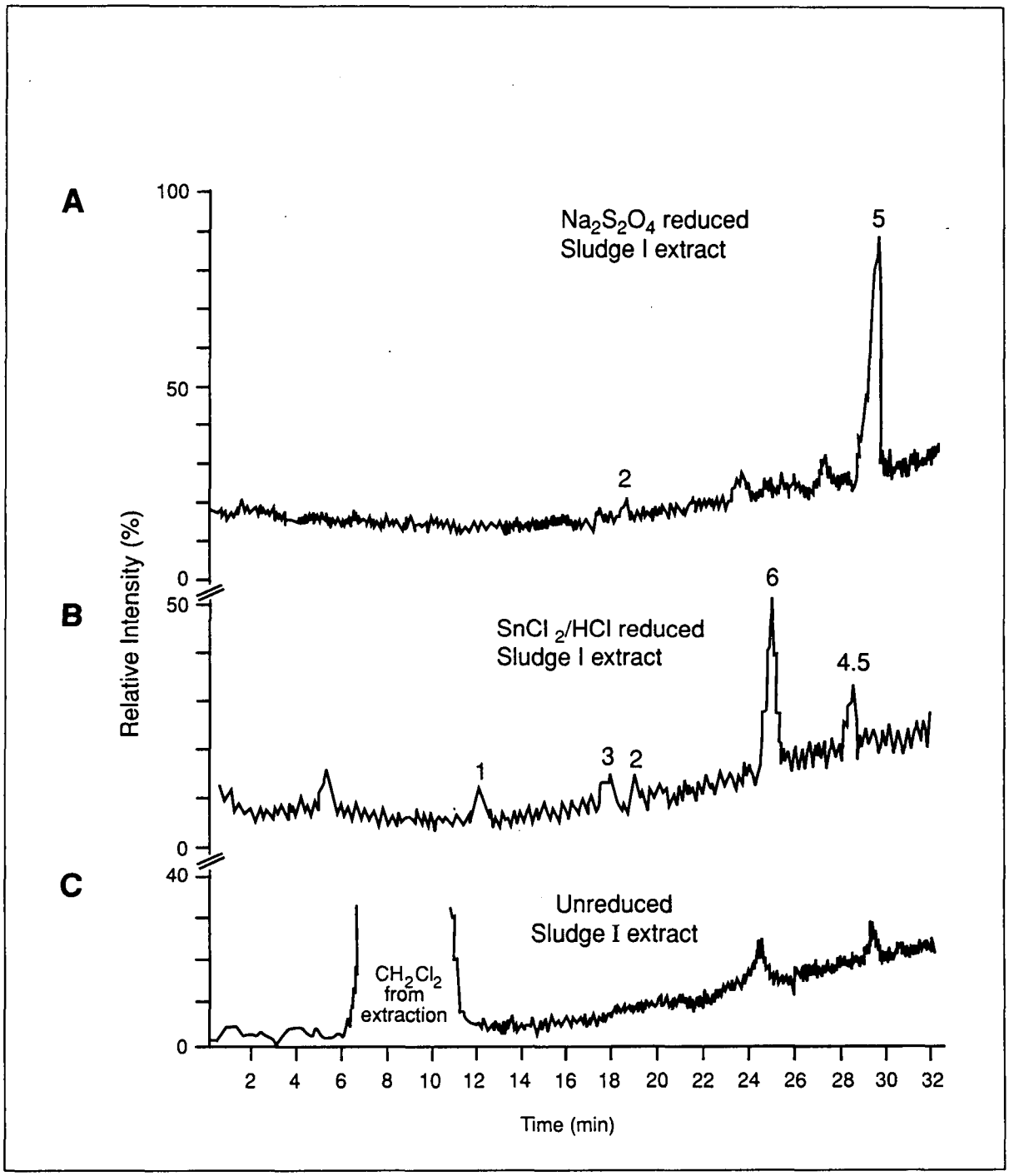

A
B
C
100
50
0
50
0
40
0
Relative Intensity (%)
$Na_2S_2O_4$ reduced
Sludge I extract
5
2
6
$SnCl_2$/HCl reduced
Sludge I extract
4.5
1
3
2
Unreduced
Sludge I extract
CH_2Cl_2
from
extraction
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Time (min)
0095qad94-3

Figure 2

TABLE 3. IDENTIFIED COMPONENTS IN REDUCED SLUDGE SAMPLES I AND II

Sludge Extract No. and (Reducing Agent Applied)	Peak No.	Identified Reduction Products	Mol Wt.	t_R[a]	Thermospray (m/z Relative Intensity) Positive[b]	Thermospray (m/z Relative Intensity) Negative[c]	Particle Beam (m/z Relative Intensity)	UV %[d]
I ($Na_2S_2O_4$)	2	2-bromo-4,6-dinitro-aniline[e]	263	18.7		322(<1); 262(100)	N.D.[f]	3
	5	4-(2-bromo-4,6-dinitro-phenylazo)-3-acetamido-N,N-di-ethyl-aniline	478	28.9	496(1); 479(100)	537(100); 477(40)	478(10); 403(52); 348(20), 253(10); 221(19); 205(100); 161(63); 79(49)	70
I ($SnCl_2$)	1	N,N-bis-(2-cyanoethyl) aniline	199	11.9	217(100); 200(36)[e]		199(7); 159(100); 106(46); 104(52); 91(42); 77(70)	7
	2	2-bromo-4,6-dinitro-aniline[e]	263	19.0		322(1); 262(100)	N.D.	1
	3	N,N-bis-(2-cyanoethyl) bromoaniline	277	17.9	295(100); 278(10)		277(1); 239(96); 237(100); 191(20); 184(46); 155(37); 125(13); 76(64)	8
	4	4-(2-chloro-4-nitro-phenylazo)-N,N-bis-(cyanoethyl)aniline	382	28.9	383(100)	441(22); 381(100)	N.D	20
	5	4-(2-bromo-4,6-dinitro-phenylazo)-3-acetamido-N,N-di-ethylaniline	478	28.9		537(100); 477(45)	N.D.	10
	6	unknown	416	24.7	417(100)		418(32); 416(30); 403(92); 401(100); 333(15); 323(12); 120(25); 105(29); 77(37)	
II ($SnCl_2$)	1	N,N'-dicyclo-hexylurea	225	17.6	226(100)		225(8); 143(12); 99(21); 61(23); 56(100)	5
	2	3,3'-dichloro -benzidine	252	20.0	270(2) 253(100)		254(68); 252(100); 223(21); 182(13); 154(27);126(31); 102(16); 86(33); 54(19)	45

[a]t_R = Retention time in TIC chromatogram (min); [b]positive ions detected were $(M+NH_4)^+$ and $(M+H)^+$; [c]negative ions detected were $(M+CH_3CO_2)^-$ and $(M-H)^-$; [d]UV % = relative UV peak area in UV_{254} chromatogram; [e]positively identified also by HPLC/UV and standard analysis; [f]negative ion detection; [g]N.D. = not detected or spectrum from more than one component.

Injection port temperatures over the region of 50 to 400°C were evaluated. Higher temperatures were more effective for most of the dyes. The intact dye or thermal degradation products of the parent dye were vaporized, then subjected to reduction in the injection port. A temperature of 300°C showed an acceptable combination of higher reduction yields and few thermal decomposition products.

The in-line H_2/Pd reduction, GC/MS approach was used to study eight dyes listed in Table 1. A summary of the reduction products identified for the dyes, based upon the EI mass spectra, are presented in Table 4 and are discussed below. Likewise, off-line solution-phase reductions of the same azo dyes using $SnCl_2$/HCl, followed by GC/MS analysis (no reduction in the GC port) provided a means to compare the number and identity of the products formed and their yields.

The H_2/Pd reduction of the solvent dyes (1 and 5) primarily resulted in the cleavage of the azo bond to form aromatic amines. The H_2/Pd reduction of Solvent Yellow 2 yielded aniline and an unexpected product (t_R = 10.21 min, $M^{+\bullet}$ = 148) together with a trace of unreduced dye. High resolution mass measurements indicated the empirical formula of $C_8H_{10}N_3$ (mass error 0.5 ppm), which is consistent with the diazonium product, 4-diazonium-N,N-dimethylaniline. The $SnCl_2$ reduction exhibited the two expected amines, aniline and N,N-dimethyl-4-aminobenzene. The H_2/Pd reduction of Solvent Red 24 primarily produced the expected amines (2-aminotoluene, 2,4-diaminotoluene, and 1-amino-2-naphthol) from cleavage of the azo bonds . The $SnCl_2$ reduction produced the same amines, except 2,4-diaminotoluene was not detected. Clearly, there is a similarity in reduction processes for the in-line H_2/Pd and solution $SnCl_2$ reduction of these solvent dyes.

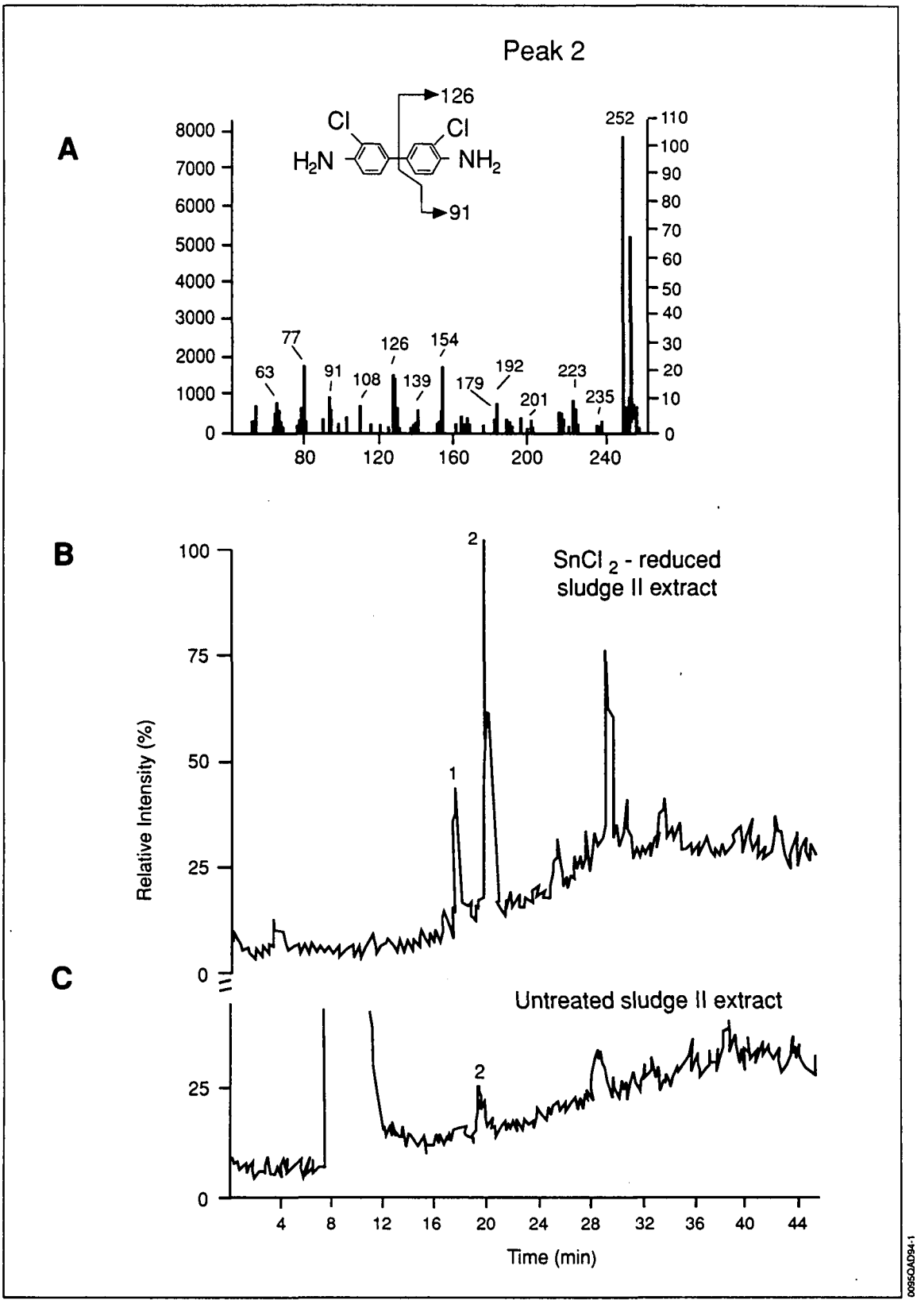

A
Peak 2
126
Cl
Cl
H_2N
NH_2
91
252
77
63
91
108
126
139
154
179
192
201
223
235
B
$SnCl_2$ - reduced sludge II extract
Relative Intensity (%)
C
Untreated sludge II extract
Time (min)
0095OA094-1

Figure 3

TABLE 4. IDENTIFICATION OF MAJOR H_2/Pd-REDUCTION PRODUCTS OF COLORANTS BY GC/MS

No.	Dye	Identified Reduction Products	Mol. Wt.	Solution-Reduction t_R (area %)[a,b,c]	Pd/H_2 Reduction t_R(area %)[a,b,d]
1	Solvent Yellow 2	• aniline[e]	93	1.26(11)	4.15(30)
		• N,N-dimethyl-1,4-diaminobenzene	136	5.00(88)	N.D.[f]
		• 4-diazonium-N,N-dimethylaniline	148	N.D.	10.24(45)
5	Solvent Red 24	• 2-aminotoluene[e]	107	2.19(93)	5.43(84)
		• 2,4-diaminotoluene[e]	122	N.D.	9.38(3)
		• 1-amino-2-naphthol[e]	159	12.35(1)	14.29(9)
		• 1-isocyanatonaphthalene	169	7.30(6)	11.94(1)
7	Disperse Red 1	• aniline[e]	93	1.39(7)	N.D.
		• 1,4-diaminobenzene[e]	108	4.13(42)	8.03(16)
		• 4-nitroaniline	138	8.17(11)	12.35(16)
		• N-ethyl-N-(2-hydroxyethyl)-1,4-diaminobenzene	180	9.38(40)[e]	13.80(62)
9	Disperse Orange 25	•1,4-diaminobenzene[e]	108	4.17(18)	8.02(11)
		• 4-nitroaniline[e]	138	8.18(3)	12.31(3)
		• N-(2-cyanoethyl)-N-ethyl-1,4-diaminobenzene	189	12.77(76)	14.92(49)
		• unknown[g]	191	N.D.	16.45(2)
		• 4-diazonium-N-(2-cyanoethyl)-N-ethylaniline	201	N.D.	15.47(17)
		• 4-hydrazine-N-2-cyanoethyl-N-ethylaniline	204	N.D.	15.71(18)
12	Disperse Black 9 (precursor)	• 1,4-diaminobenzene[e]	108	4.15(35)	8.28(53)
		• N,N-dimethyl-1,4-diaminobenzene-	136	N.D.	9.49(13)
		• N,N-bis-(2-hydroxyethyl)-1,4-diaminobenzene	196	13.88(60)	16.72(32)
14	Disperse Orange 37	• 1,4-diaminobenzene[e]	108	N.D.	8.14(7)
		• unknown	136	N.D.	10.14(9)
		• unknown	142	N.D.	10.51(20)
		• 2-chloro-4-nitroaniline	172	N.D.	13.59(7)
		• 1,4-diamino-2,6-dichlorobenzene	176	8.13(45)	12.41(17)
		• N-(2-cyanoethyl)-N-ethyl-1,4-diamino-benzene	189	11.23(52)	15.20(32)
		• 2,6-dichloro-4-nitroaniline[e]	206	N.D.	14.11(8)
15	Disperse Brown 1	• aniline[e]	93	N.D.	4.07(2)
		• 2,3-dichloroaniline	161	N.D	9.99(2)
		• N,N-diethyl-1,4-diaminobenzene	164	N.D.	10.98(1)
		• 2-chloro-4-nitroaniline[e]	172	N.D.	13.52(3)
		• 1,4-diamino-2,6-dichlorobenzene	176	8.23(100)	12.53(53)
		• unknown	189	N.D.	15.49(28)
		• 2,3,4-trichloroaniline	195	N.D.	13.05(1)
		• 2,6-dichloro-4-nitroaniline[e]	206	N.D.	14.01(10)
16	Acid Orange 10	• aniline[e]	93	1.36(2)	N.D.
		• Acid Orange 10 - 2Na - SO_3	326	17.56(98)	N.D.

[a]t_R = Average retention time in TIC chromatogram (min); [b](area %) = percentage of the total peak area in the TIC chromatogram; [c]Reaction product from aqueous $SnCl_2$ reduction; [d]Reaction product from in-line H_2/Pd reduction; [e]identity confirmed with standard; [f]N.D. = Not detected in TIC chromatogram; [g]unknown = structure of formed reduction product unknown or only partially resolved.

The disperse dyes (Nos. 7-15) tend to exhibit a greater variety of reduction products due to other reducible groups on the molecule (e.g., NO_2) and functional groups (e.g., Cl, OH) that can react under the imposed conditions. The H_2/Pd reduction of Disperse Red 1 resulted in the formation of near equal amounts of the partially reduced product (4-nitroaniline) and the completely reduced product (1,4-diaminobenzene), together with N-ethyl-N-(2-hydroxyethyl)-1,4-diaminobenzene. The $SnCl_2$ reduction produced the same products as did H_2/Pd reduction, plus a trace of aniline, possibly from loss of NO_2 and reduction of the azo linkage. The H_2/Pd reduction of Disperse Orange 25 produced six products. Three of the products (comprising 63% of the ion current) could be accounted for by reduction of the azo and nitro groups for the dye. The other three products were not as obvious. High resolution MS aided in determining the empirical formulas for the ions at m/z 201 and 204 which correspond to the respective diazonium ($C_{11}H_{13}N_4$) and hydrazine ($C_{11}H_{15}N_4$) products in Table 4 (mass error of 3.4 and 4 ppm, respectively). However, the peak of lowest relative intensity, at m/z 191, was not identified.

The $SnCl_2$ solution-reduction of this dye exhibited three products that were observed for the H_2/Pd reduction, originating from reduction of the azo and/or nitro groups. The diazonium or hydrazine products were not detected for the $SnCl_2$ reduction. The H_2/Pd reduction of Disperse Black 9 resulted in the formation of two products that formed by reduction of the azo linkage (1,4-diaminobenzene and N,N-bis(2-hydroxyethyl)-1,4-diaminobenzene). Also, losses of methanol were observed to form an N,N-dimethyl-1,4-diaminobenzene. However, this product only accounted for 13% of the ion current. The $SnCl_2$ solution-reduction only resulted in the reduction of the azo linkage to form the same two products (1,4-diaminobenzene and N,N-bis(2-hydroxyethyl)-1,4-diaminobenzene) detected by H_2/Pd reduction.

The H_2/Pd reduction of Disperse Orange 37 formed seven products. The major product (32%) was N-(2-cyanoethyl)-N-ethyl-1,4-diaminobenzene formed from reduction of the azo linkage. Four products were identified to form by combinations of the azo linkage or nitro group reductions or losses of chlorine. These products included 2,6-dichloro-4-nitroaniline, 1,4-diamino-2,6-dichlorobenzene, 2-chloro-4-nitroaniline, and 1,4-diaminobenzene and accounted for 39% of the ion current. Two products (Mol. wt. 136 and 142) were not identified. The $SnCl_2$ solution-reduction only formed two products, 1,4-diamino-2,6-dichlorobenzene and N-(2-cyanoethyl)-N-ethyl-1,4-diaminobenzene. These two products were the two main products observed by H_2/Pd reduction.

The H_2/Pd reduction of Disperse Brown 1 was similar to that of Disperse Orange 37. There were eight reduction products detected, and the main product (53%) was 1,4-diamino-2,6-dichlorobenzene. However, other products were detected that involved the reduction of a nitro group, as well as losses of nitro groups and losses or addition of Cl (e.g., aniline, 2,3-dichloroaniline, 2-chloro-4-nitroaniline, 2,6-dichloro-4-nitroaniline and 2,3,4-trichloroaniline). Also, a reduction product, N,N-diethyl-1,4-diaminobenzene was detected instead of the expected product, 3-chloro-N,N-(2-hydroxyethyl)-1,4-diaminobenzene. The $SnCl_2$ reduction only produced one product (1,4-diamino-2,6-dichlorobenzene), the same product observed in H_2/Pd reduction. While a variety of products can be formed by H_2/Pd reduction of disperse dyes, the major products correspond to the reduction products observed from $SnCl_2$ solution reductions.

The most difficult dyes to study by mass spectrometry are the acid dyes. The salts of sulfonic acid dyes cannot be volatilized without thermal degradation. Even the free acids are extremely nonvolatile (39). This may explain why H_2/Pd reduction of Acid Orange 10 failed to produce any significant peaks that could be correlated with the reduction of this dye. The $SnCl_2$ solution-reduction of the acid dye resulted in two products; the main reduction product (98%) was a partially desulfonated parent dye (M^+ = 326), and the other was aniline.

Several general reaction schemes for the H_2-Pd reduction in the GC injection port can be formulated based on the products identified in Table 4. Structurally simple dyes (Solvent Yellow 2 and Solvent Red 24) formed the anticipated aromatic amines as shown in Scheme 3.

Scheme 3: $R_1\text{-}N{=}N\text{-}R_2 \xrightarrow[\Delta]{H_2/Pd} R_1NH_2 + R_2NH_2$

The presence of other functional groups (e.g., NO_2, OH, CN or halogens) increased the number of products formed. The presence of a nitro group in the dye usually resulted in the formation of a diamine (Scheme 4).

Scheme 4:

$$R_1N{=}N\text{-}R_2\text{-}NO_2 \xrightarrow[\Delta]{H_2/Pd} R_1NH_2 + O_2NR_2NH_2 + H_2NR_2NH_2$$

Reaction products resulting in the loss of a halogen (e.g., Cl), OH, or CH_2OH from the dye were more difficult to predict, but accounted for less than 10% of the ion current for the reduced dye.

The H_2/Pd reduction yield for the simple azo dyes (e.g., Solvent Yellow 2) was above 60%. The yield was based on the molar response of the amine standard (e.g., aniline) relative to the molar quantity of the parent dye. More complex azo dyes that contained additional functional groups resulted in a wide variety of reduction products. Lower yields were often observed since the same azo cleavage products could be further reduced to form other products (e.g., Disperse Red 1 can be reduced to form 4-nitroaniline, which then can be reduced to form 1,4-diaminobenzene). However, if the molar response for the two possible reduction products are summed, the reduction yield is about 70%. Also, it was not possible to calculate the yields of the more complex dyes in this manner due to a lack of reference standards. While the reduction was not 100%, the relative intensities of the reduction products for Solvent Yellow 2 and Solvent Red 24 were consistent (standard deviation <25%) for their daily analysis over the course of the study.

The H_2/Pd reduction of azo dyes in Table 1 indicates that several common cleavage products can arise. Aniline is a common cleavage product for dyes 1, 7, and 15; 1,4-diaminobenzene for dyes Nos. 7, 9, and 12; 4-nitroaniline for dyes 7 and 9; and 1,4-diamino-2,6-dichlorobenzene for dyes 14 and 15. As a result, detection of a limited number of amines can aid in determining dye types used in manufacturing.

Detection of Aromatic Amines in Sludge Extracts after In-Situ Reduction

Extracts of sludges (I and II) from two different waste treatment sites were reduced with H_2/Pd in the injector and then analyzed by GC/MS to qualitatively screen for azo dyes based upon the detection of aromatic amines or other related reduction products. The GC/MS conditions were the same as for the dye standards.

Sludge I. The in-line reduction treatment of a wastewater sludge formed seven new products. The retention times and postulated molecular weights of these reduction products are listed in Table 5. Identification of products was based mainly on mass spectral library searches (41), manual spectral interpretation, and the analysis of available reference standards. In both the unreduced and the reduced wastewater sludge extract, numerous fairly simple aromatics have been tentatively identified. The major components present in the reduced and unreduced extracts were aniline (t_R = 4.10, $M^{+\cdot}$ = 93), 3-aminophenyl propanenitrile (t_R = 10.98 min, $M^{+\cdot}$ = 146), 2,4-dinitrobromobenzene (t_R = 12.56, $M^{+\cdot}$ = 246), 2-chloro-4-nitroaniline (t_R = 13.52 min, $M^{+\cdot}$ = 172), and (1,1'-biphenyl)-4-ol (t_R = 13.96 min, $M^{+\cdot}$ = 170). Three of the seven reduction products listed in Table 5 were tentatively identified as 4-nitroaniline, 4-nitro-1,2-diaminobenzene, and 2-nitro-1,4-diaminobenzene. These products are consistent with the principal blue colorant in sludge I that was identified by thermospray LC/MS to be the monoazo dye 17 (Table 1). However, it is evident that sludge I contains additional azo dyes or other compounds that can be reduced to form aromatic amines.

Sludge II. The H_2/Pd treatment of the wastewater sludge produced four new reduction products listed in Table 5. Most of the components identified in the unreduced extract were present after the in-line reduction. Both samples contained polysubstituted aromatic amines and polychlorinated aromatics. The major components identified in both the unreduced and the reduced wastewater sludge were aniline (t_R = 4.10 min, $M^{+\cdot}$ = 93), 1,3- or 1,4-dichlorobenzene (t_R = 4.71 min, $M^{+\cdot}$ = 146), 4-ethoxyaniline (t_R = 7.97 min, $M^{+\cdot}$ = 137), methylaniline (t_R = 5.48 min, $M^{+\cdot}$ = 107), 2-methoxyaniline (t_R = 7.01 min, $M^{+\cdot}$ = 123), 1,2,4-trichlorobenzene (t_R = 7.97 min, $M^{+\cdot}$ = 180), 2,3-dichloroaniline (t_R = 9.18 min, $M^{+\cdot}$ = 161), 2,3,4-trichloroaniline (t_R = 11.98 min, $M^{+\cdot}$ = 195), 4-chloro-2,5-dimethoxyaniline (t_R = 13.00 min, $M^{+\cdot}$ = 187), 4,4'-dichlorobiphenyl (t_R = 14.62 min, $M^{+\cdot}$ = 222), and 3,3'-dichlorobenzidine (t_R = 21.01 min, $M^{+\cdot}$ = 252). Although substituted benzidine derivatives were found in the untreated and reduced extract, H_2/Pd reduction produced higher levels of dichlorobenzidine. Comparative results, obtained from thermospray LC/MS, confirmed the occurrence of dichlorobenzidine in this sample. These results suggested that the principal component in the

sludge was a colorant of type 18 (Table 1). This is a common skeleton of commercial yellow azo pigments, which were present in the sludge II sample. These yellow azo pigments are the primary type of colorants for which dichlorobenzidine is currently used.

TABLE 5. MAJOR COMPONENTS FORMED AFTER IN-SITU REDUCTION OF SLUDGE I AND II

Sludge Extract No.	Peak No.	Identified Products	Mol. Wt.	t_R[a]
I	1	• 2-chloro-1,4-diaminobenzene[b]	142	10.73
	2	• 4-nitroaniline[b]	138	12.33
	3	• 4-nitro-1,2-diaminobenzene[b]	153	15.27
	4	• 2-nitro-1,4-diaminobenzene	153	16.01
	5	• unknown[c]	203	17.26
	6	• unknown	247	18.52
	7	• unknown	294	19.29
II	1	• 2-methylphenol[b]	108	5.62
	2	• 4-chloroaniline[b]	127	6.26
	3	• 2,5-dimethoxyaniline	153	10.43
	4	• benzidine[b]	184	17.85

[a]t_R = Average retention time in TIC chromatogram (min); [b]Identity confirmed with standard; [c]Unknown = Structure of formed reduction product unknown or only partially resolved.

REFERENCES

1. Hudlicky, M. *Reductions in Organic Chemistry,* J. Wiley & Sons, New York, 1986, p. 69-76.
2. Searle, C.E. (Editor) *Chemical Carcinogenesis,* ACS Monograph, American Chemical Society, Washington, DC, 1976.
3. Helmes, C.T.; Sigman, C.C.; Fung, Z.A.; Thompson, M.K.; Voeltz, M.K.; Mackie, M.; Klein, P.E.; Lent, B. *J. Environ. Sci. Health,* 1984, A19, 97-113.
4. Boeninger, M. *Carcinogenicity and Metabolism of Azo Dyes, Especially Those Derived from Benzidine,* DHHS (NIOSH), Publication No. 80-119, July 1980.
5. Hueper, W.C. *Occupational and Environmental Cancers of the Urinary System,* Yale University Press, New Haven, 1969.
6. *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man,* Vol. 8, Lyon, IARC 1975.
7. Freeman, H.S.; Esancy, J.F.; Esancy, M.K.; Mills, K.P.; Whaley, W.M. *Dyes Pigments,* 1987, 8, 417-422.
8. Prival, M.J.; Mitchell, V.D. *Mutation Res.,* 1982, 97, 103-116.
9. Roxon, J.J.; Ryan, A.J.; Wright, S.E. *Food Cosmet. Toxicol.,* 1967, 5, 367-369.
10. Chung, K.T. *Mutation Res.,* 1983, 14, 269-281.
11. Nesnow, S.; Bergman, H.; Bryant, B.J.; Helton, S.; Richard, A. *J. Toxicol. Environ. Health,* 1988, 24, 499-513.
12. Krishna, G.; Xu, J.; Nath, J. *J. Toxicol. Environ. Health,* 1986, 18, 119-129.
13. Reisch, M.S. *Chem. Eng. News,* 1988, 66, 7-14.
14. Venkataraman, K. (Editor) *The Analytical Chemistry of Synthetic Dyes,* J. Wiley & Sons, New York, 1977.
15. Ballard, J.M.; Betowski, L.D. *Org. Mass Spectrom.,* 1986, 21, 575-588; references cited therein.
16. Games, L.M.; Hites, R.A. *Anal. Chem.,* 1977, 49, 1433-1440.
17. Yergey, A.L.; Edmonds, C.G.; Lewis, I.A.S.; Vestal, M.L. *Liquid Chromatography/Mass Spectrometry, Techniques and Applications,* Plenum Press, New York, 1990.
18. Brown, M.A. (Editor) *Liquid Chromatography/Mass Spectrometry, Applications in Agricultural, Pharmaceutical, and Environmental Chemistry,* ACS Symposium Series 420, American Chemical Society, Washington, DC, 1990.
19. MacCarthy, P.; Klusman, R.W.; Cowling, S.W.; Rice, J.A. *Anal. Chem.,* 1991, 63, 301R-342R.
20. MacCarthy, P.; Klusman, R.W.; Rice, J.A. *Anal. Chem.,* 1989, 62, 269R-304R.
21. Covey, T.; Henion, J. *Anal. Chem.,* 1983, 55, 2275-2280.
22. Betowski, L.D.; Ballard, J.M. *Anal. Chem.,* 1984, 56, 2604-2607.
23. Voyksner, R.D. *Anal. Chem.,* 1985, 57, 2600-2605.
24. Betowski, L.D.; Pyle, S.M.; Ballard, J.M.; Shaul, G.M. *Biomed. Environ. Mass Spectrom.,* 1987, 14, 343-354.
25. Yinon, J.; Jones, T.L.; Betowski, L.D. *Biomed. Environ. Mass Spectrom.,* 1989, 18, 445-449.
26. Voyksner, R.D.; Pack, T.W.; Haney, C.A.; Freeman, H.S.; Hsu, W.-N. *Biomed. Environ. Mass Spectrom.,* 1989, 18, 1079-1086.

27. Yinon, J.; Jones, T.L.; Betowski, L.D. *Rapid Commun. Mass Spectrom.*, 1990, 4, 245-250.

28. Groepplin, A.; Linder, M.W.; Schellenberg, K.; Moser, H. *Rapid Commun. Mass Spectrom.*, 1991, 5, 203-205.

29. Lindberg, C.; Paulsen, J. *J. Chromatogr.*, 1987, 394, 117-122.

30. McFadden, W.H.; Lammert, S.A. *J. Chromatogr.*, 1987, 385, 201-211.

31. McFadden, W.H.; Garteiz, D.A.; Siegmund, E.G. *J. Chromatogr.*, 1987, 394, 101-108.

32. Straub, R.; Voyksner, R.D.; Keever, J.T. *J. Chromatogr.*, 1992, 627, 173-186.

33. Willoughby, R.C.; Browner, R.F. *Anal. Chem., 1984,* 56, 2626-2631.

34. Willoughby, R.C.; Poeppel, F. *Presented at the 35th Ann. Conf. on Mass Spectrometry and Allied Topics,* Denver, CO, USA, May 24-29, 1987, p. 289-290.

35. Vekey, K.; Edwards, D.; Zerilli, L.F. *J. Chromatogr.*, 1989, 488, 73-85.

36. Yinon, J.; Jones, T.L.; Betowski, L.D. *J. Chromatogr.*, 1989, 482, 75-85.

37. Brand, K.; Steiner, *J. Chem. Ber.*, 1922, 55, 875, 886.

38. Brumley, W.C.; Brilis, G.M.; Calvey, R.J.; Sphon, J.A. *Biomed. Environ. Mass Spectrom.*, 1989, 18, 394-400.

39. Beukelman, T.E. In *The Analytical Chemistry of Synthetic Dyes;* Venkataraman, K., Ed.; J. Wiley & Sons: New York, 1977, pp 255-267.

40. Lee, E.D.; Henion, J.D. *Rapid Commun. Mass Spectrom.* 1992, 6, 727-733.

41. *NIH/EPA/MSDC Mass Spectral Data Base (NBS Library) and Registry of Mass Spectra Data (Wiley Library).*