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Research and Development

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Project Summary

Determination of Benzidines in Industrial and Municipal Wastewaters

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A r... to obtain accurate values for benzi... and DCB, it is believed to be (DCB... to assay the sample as soon This n... llection as possible. For perfor... ontaining chlorine, a reduc- with ... such as sodium thiosulfate selecti... added, since chlorine was compo... rapidly degrade benzidine 0.1 ppl... types c... ject Summary was devel- surface... PA's Environmental Mon- industri... d Support Laboratory, several ... OH, to announce key many ... the research project that is decrease... fully documented in a separate report ...method... of the same title (see Project Report to about 1 ppb for benzidine and DCB. ordering information at back).

Apparent levels of 10-20 ppb of benzidine were present in the dye plant effluents. For one dye plant, effluent benzidine was determined to be 9 ppb and 12 ppb using two different sets of chromatographic conditions, thus supporting the belief that benzidine is present at the level stated.

Precision and accuracy of the method were estimated from the results for five wastewater samples spiked at levels between 1 and 50 ppb. For this group of samples the recoveries were 69 ± 15% for benzidine and 76 ± 9% for DCB.

Storage of several wastewater samples for (two or seven days) at 4°C and pH 2 resulted in degradation of the compounds in several cases, probably due to oxidation or irreversible adsorption to particulate matter. Therefore,

Introduction

Under provisions of the Clean Water Act, the Environmental Protection Agency is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 "priority" pollutants and classes of pollutants to be toxic under Section 307(a). This report is one of a series that investigates the analytical behavior of selected priority pollutants and suggests a suitable test procedure of their measurement.

It presents the results obtained under Contract No. 68-03-2624, in which a method was developed for the determination of benzidine, dichlorobenzidine, and diphenylhydrazine in aqueous

effluents. This work was conducted in two phases, wherein Phase I consisted of evaluation of various analytical methodologies, and Phase II consisted of validating the most promising method using several aqueous effluent samples. Data from the Phase I study, which were presented in a report dated May 19, 1978, are also presented herein.

The objective of this study was to develop an optimized analytical method for the determination of benzidine, dichlorobenzidine (DCB) and 1,2-diphenylhydrazine (DPH) and to validate the method on a variety of aqueous effluents.

The successful completion of this program involved the fulfillment of certain directives set forth in the contract by EPA. An extensive literature review was first conducted to evaluate the previous work in the area. Subsequent work was directed toward determination and then full evaluation of an appropriate measurement technique, which best satisfied the requirements for sensitivity and selectivity, as well as the considerations of sample cost, that is, equipment, time, and training, which would be needed for the method. The stability of the benzidine compounds in water miscible solvents and their instability in chlorinated and unchlorinated buffered water at different pHs and storage temperatures were studied over the prescribed time periods. Extraction efficiency of two organic solvents was also studied for the standard compounds. The remainder of the program involved the study of the sample preparation and clean up steps which would be necessary to eliminate sample interferences. The complete method was then applied to several representative wastewater samples and an assessment was made of the precision and accuracy of the complete procedure.

Analytical Methods Development

Three analytical techniques were evaluated for potential use in the determination of benzidines: (1) direct GC/AFD, (2) GC/ECD following derivatization with a fluoroacy groupl and (3) HPLC/EC.

Direct GC/AFD

Gas chromatographic properties of the three compounds were investigated using a Hewlett Packard 5730 gas chromatograph equipped with dual FID/AFD. Columns were 6 ft x 2 mm I.D. glass, packed with a 3% loading of the liquid stationary phase on Gas Chrom Q 100/120. The stationary phases evaluated were OV-1, OV-17, SP2250DB, and OV-225, which represent a wide range of polarities. GC/FID was used to evaluate all four stationary phases and GC/AFD was evaluated for the SP2250DB stationary phase.

The four stationary phases investigated represent a wide range of polarities. Both OV-17 and SP2250DB were found to give satisfactory results. OV-1 was acceptable but some degree of tailing was noted, especially at low concentrations. OV-225 gave an unacceptable degree of column bleed.

The use of AFD for the detection of the benzidines was investigated using SP2250DB. By operating isothermally at 230°C, a sensitivity of about 1 ng on column can be achieved for benzidine and DCB; but under these conditions, the DPH peak appears in the solvent front.

It was noted for each column that two peaks arose from the injection of DPH, and furthermore, that the larger of the peaks elutes much earlier than would be expected for a reasonably polar compound of this molecular weight. In order to confirm the identity of the eluted components, GC/MS was used, employing a 30 meter SE-30 glass capillary column.

A single sharp peak was observed eluting at 210° from a temperature programmed run. The mass spectrum revealed it to be a component with a molecular ion at 182 amu, whereas DPH has a molecular ion at 184. The component resulting from DPH injection was suspected to be azobenzene (M.W. 182) which was confirmed by the injection of pure azobenzene. It is apparent, therefore, that DPH instantaneously decomposes to azobenzene in the GC injection port.

GC/ECD

The use of various derivatization reagents to form highly electron capturing derivatives was investigated. The following derivitization reagents were evaluated: (1) trifluoroacetic anhydride (TFA); (2) pentafluoropropionyl anhydride (PFPA); (3) heptafluorobutyric anhydride (HFBA); (4) trifluoroacetyl imidazole (TFI); and (5) heptafluorobutryl imiazole (HFBI).

Numerous problems were encountered using this approach, the most serious of which was the production of multiple peaks and the incomplete reaction of DPH with all of the reagents studied. Benzidine reacted well with the HFBI but not any of the anhydrides or the TFI. DCB derivatized the best of the three amines and failed to form a derivative only with TFA. Similar results were obtained using both the anhydrides and imidazoles. The DCB-HFBA derivative was formed with good yield, whereas there was a relatively poor yield of about 10% of benzidine-HFBA derivative. No underivatized amine was present in either case, so some decomposition must have occurred in the benzidine derivatization reaction. Both benzidine and DCB were successfully derivatized using HFBI. As stated earlier, HFBI derivatization of DPH was not successful, since no derivative formation was observed.

On the basis of these results, this approach did not seem useful for the analysis of benzidines and was not further investigated, since an alternate technique, HPLC/EC, was working quite well. However, it appears that HFBI would be the best derivatizing reagent to use for benzidine and DCB, if one wished to assay these compounds by GC/ECD.

HPLC with Electrochemical Detection

This approach proved useful almost immediately, so following a short period of column evaluation, a set of parameters was selected which gave excellent resolution, sensitivity, and reproducibility. Several reversed phase and ion exchange columns were preliminarily evaluated, including: Zorbax ODS and CN, μ Bondapak C-18, Lichrosorb RP-18, RP-8, and RP-2, Spherisorb ODS, and Whatman Partisil SCX. All the ODS, CN, and RP-8 packings gave poor efficiency for the amines—less than 2000 plates in all cases—although they gave efficiencies of greater than 10,000 plates for anthracene, which is commonly used for determining column efficiency. The Whatman SCX column was very poor in that it gave badly tailing peaks. The RP-2 column was found to give excellent efficiency for the amines—greater than 6000 plates. Better resolution of the DCB and DPH was achieved using acetonitrile instead of methanol under the conditions evaluated.

Based on the results mentioned above, a set of HPLC conditions was selected which gave optimum resolution and sensitivity for the compounds of

interest. These conditions are listed below:

Column—RP-2 5 micron 25 cm x 4.6 mm I.D.
Mobile phase—50% acetonitrile - 50% sodium acetate buffer 0.1M pH 4.7
Detector—Electrochemical (glassy carbon) 3 mm diameter, Bioanalytical Systems model LC2
Detector potential—0.8V
Injection volume—25 microliters

Using the electrochemical detector, the minimum detectable quantities were 0.05 ng for benzidine, 0.1 ng for DCB, and 0.3 ng for DPH at a signal to noise ratio of five to one. Linear response for the components is obtained from 0.1 - 400 nanograms injected for DCB and benzidine (a linear dynamic range of 10^3). Above 400 nanograms, a rapid fall from linearity is observed for DCB and benzidine. Precision of peak heights for eight replicate injections of benzidine was ±2.8% at the 50 nanogram level.

The electrode potential (0.8V) was chosen based on our study of response versus electrode potential for each of the compounds. This study shows that full (diffusion controlled) response is achieved for each compound at 0.8V versus Ag/Ag Cl and above.

Solvent Stability Studies

The stability of the three amines in water miscible solvents was studied over a 90-day period. For DCB and benzidine, acetonitrile and methanol were used. Both components were completely stable over the 90-day period. DPH was found to decompose completely in three days or less in all solvents investigated, including benzene, methylene chloride, methanol, triethylamine, acetonitrile, and acetic acid. It is apparent that DPH standards must be prepared fresh daily and thus no further studies were conducted.

Extraction Studies

Extraction studies were conducted to evaluate the extraction efficiencies of methylene chloride and chloroform preserved with 2% ethanol for the benzidines in water at pHs 2, 7, and 10.

Fifty microliters of standard solution in acetone was added to 500 mL of appropriately buffered water to yield a concentration of 10 ppb for each of the compounds in a 1000 mL separatory funnel.

The benzidines were extracted with 50 and then 30 mL of the appropriate solvent. The extract was washed with 20 mL water, and the solvent was exchanged to methanol by concentrating to 5 mL on a rotating evaporator at 35°C. Finally, the extract was concentrated to 2 mL on a vortex evaporator and prepared for HPLC by dilution to 4 mL with 0.1 M sodium acetate buffer.

The extraction methodology was found to be a very delicate area since numerous unanticipated problems developed which led to low recoveries, especially for benzidine and DPH. The stability of DPH was found to be a major problem which was not completely solved.

The following are some of the problems encountered:

1. Benzidine is substantially adsorbed on Na_2SO_4 if a normal solvent drying step is employed. The use of K_2CO_3 corrected this problem. Elimination of the drying step was found to be useful when using HPLC analysis.
2. Benzidine is heat labile so that Kuderna-Danish concentration techniques gave low recoveries. The use of rotary evaporation eliminated this problem.
3. Decomposition of benzidine occurred when concentrating methylene chloride or chloroform. The addition of 15% MeOH prior to concentration stabilizes the benzidine.

Generally, chloroform at pH 7 was found to give a more efficient extraction, expecially for benzidine. As expected, no benzidine was recovered at pH 2, whereas the less basic DCB was extracted at all pH values. DPH degraded readily in both aqueous and organic media and although both chloroform and methylene chloride gave about 70% recoveries at pH 10, no set of extraction parameters was found which gave greater than about 70% recovery. At pH 2, DPH was found to degrade to benzidine to some extent. Based on these data, it was concluded that chloroform is a satisfactory solvent for the extraction of benzidines.

Storage Stability Studies

The stability of the benzidines in water under a variety of storage conditions was investigated. Two temperatures, 4°C and room temperature, three pH levels, 2, 7, and 10, and two chlorine levels, 0 and 2 ppm, were evaluated by preparing duplicate 500 mL samples spiked with 10 ppb of DCB and benzidine or DPH. DPH was done separately since it can degrade to benzidine under certain storage conditions. The solutions were stored in amber glass bottles for 7 days prior to extraction.

None of the amines were detectable in the solutions to which chlorine was added. A pH of 2 was found to give the best results for benzidine and DCB. However, at pH 2 DPH degrades to benzidine, thus creating an undesired artifact. This problem can be overcome by employing pH 4.7 acetate buffer where both benzidine and DCB are well preserved, and DPH degrades to two unidentified components, not to benzidine. At pH 4.7, two peaks were obtained which did not interfere with DCB or benzidine, neither of which was DPH (based on chromatographic retention times).

Based on these results, it was concluded that a pH between 2 and 4 is best for preservation of the benzidines. Some artifacts due to DPH decomposition to benzidine are likely at pH 2 and below if DPH is present in the sample. Addition of a reducing agent (such as Na_2SO_3) to destroy chlorine is required.

DPH was found to be very unstable in wastewater, specifically secondary sewage. DPH, spiked at the 100 ppb level, disappeared with a half life of about 15 minutes in the presence of oxygen and about 60 minutes with oxygen removed. This result indicates that DPH analysis in wastewater is virtually meaningless, since the DPH level determined cannot be directly related to the DPH in the sample at the time of collection.

Wastewater Studies

Based on the above studies, the following procedure was applied to the analysis of actual wastewater samples.

- Adjust pH of 500 mL to 1 L aliquot of wastewater to pH 7 and serially extract with 100, 50 and 50 mL volumes of chloroform.
- Extract the benzidines from the solvent with three 25 mL aliquots of 1M H_2SO_4.
- Neutralize the acid solution to pH 6-7 and serially extract with 30, 20 and 20 mL volumes of chloroform.
- Wash the solvent with 20 mL distilled water and exchange the solvent to methanol while concentrating to 5 mL on a rotating evaporator.

- Concentrate the methanol extract to 1.0 mL with a gentle stream of nitrogen.
- Add 4.0 mL pH 4.7 acetate buffer and analyze by HPLC with electrochemical detector.

The following water samples were selected for analysis:

1. Surface water (Olentangy River)
2. Secondary sewage effluent (Columbus, Ohio)
3. Primary sewage (Columbus, Ohio)
4. Final effluent from plant producing various organic chemicals (such as nitrobenzene, nitrophenols, o-dichlorobenzene, and chloroanilines).
5. Final effluent from plant producing benzidine based dyes.
6. Oxidation process stream from a plant producing benzidine based dyes.

Each wastewater was analyzed, unspiked, in triplicate. Six 500 mL aliquots of each sample were spiked with benzidine and DCB at a level at least five times background. Three aliquots were analyzed immediately and three were analyzed after storage, at pH 2 and 4°C, for a period of time—either two or seven days. The pH 2 level was attained by addition of 1M H_2SO_4. One gram per liter of sodium thiosulfate was added to each sample prior to spiking. The analytical results for all samples are tabulated in Table 1.

Sample 2 was a secondary sewage effluent spiked at the 4 ppb level. Results obtained were comparable to those for the surface water sample. Storage of this effluent, spiked at the 4 ppb, for 48 hours at 4°C, and pH 2, resulted in essentially no loss in recovery. The shorter storage period was used, since it was felt that the stability of benzidine in dilute aqueous solution was so unpredictable that samples should be assayed as soon as possible. Forty-eight hours appeared to be the shortest time period which could reasonably be expected for the completion of sample collection and transport to the analytical laboratory.

Sample 3 was a primary sewage sample and was spiked at the 1 ppb level. This spike level was chosen in order to validate the procedure at levels approaching the detection limit, although as a general practice higher spiking levels were used so that accuracy could be more easily determined and poor recoveries would still result in a measurable peak. As shown in Table 1, recoveries were quite good and precision was about ±10-20%. However, after storage for seven days, low recovery and poor reproducibility were observed for DCB, but not for benzidine. This result appears to indicate that DCB may be irreversibly bound to particulate matter, since this sample had a large amount of suspended matter.

Sample 4 was a final effluent sample from a plant producing various organic chemicals such as nitrophenols, o-dichlorobenzene, chloroanilines, and nitrobenzenes. Samples 1-3 were selected for this study because they represent matrices which receive inputs from a diversity of industrial processes, and thus benzidine determination might be applied to such samples in the future. Sample 4, on the other hand, was selected because it represents a matrix suspected of containing various aromatic amines (anilines) and phenols which would be likely interferences for the HPLC/EC method (since they are readily oxidized). Sample 4 was spiked at the 1 ppb level. Recovery and particularly precision for benzidine were poor both before and after a storage time of 48 hours. Recovery and reproducibility for DCB were better than for benzidine, both before and after storage. The reason for this lack of reproducibility for this sample is not clear, although it may be due to chemical oxidation, since benzidine is much more easily oxidized than DCB.

Sample 5 was obtained from a plant producing benzidine based dyes. This sample was about one year old upon receipt and had been stored at pH 2 and 4°C during that period of time. The sample was diluted 10:1 with distilled water prior to analysis. Recoveries were very good before storage but decreased after storage. The drop in benzidine level was particularly disturbing, since there was a detectable level of benzidine in the unspiked sample, which had been stored for over a year. It seemed possible therefore that the benzidine

Table 1. *Data for Wastewater Analyses*

Sample No.	*Sample Description*		*Background (ppb)*	*Spike (ppb)*	*Recovery*	
					0 days	*7 days*
	Distilled water	*Benzidine*	*<.1*	*4*	*70 ± 7**	—
		DCB	*<.1*	*4*	*50 ± 5*	—
1	*Surface water*	*Benzidine*	*<.1*	*4*	*63 ± 6*	—
		DCB	*<.1*	*4*	*48 ± 5*	—
2	*Secondary sewage***	*Benzidine*	*<.1*	*4*	*73 ± 8*	*60 ± 12*
		DCB	*<.1*	*4*	*63 ± 7*	*58 ± 6*
3	*Primary sewage*	*Benzidine*	*.1*	*1.0*	*64 ± 5*	*71 ± 21*
		DCB	*.1*	*1.0*	*55 ± 10*	*19 ± 10*
4	*Final effluent** for organic chemicals plant*	*Benzidine*	*<.1*	*1.0*	*47 ± 46*	*52 ± 17*
		DCB	*<.1*	*1.0*	*89 ± 8*	*60 ± 21*
5	*Final effluent** from plant producing benzidine based dyes*	*Benzidine*	*12 ± 3*	*50*	*79 ± 14*	*47 ± 8*
		DCB	*<1*	*50*	*78 ± 7*	*23 ± 7*
6	*Process effluent** from oxidation process in plant producing benzidine based dyes*	*Benzidine*	*<1*	*10*	*82 ± 3*	*75 ± 7*
		DCB	*<1*	*10*	*98 ± 5*	*95 ± 3*

**Mean ± standard deviation.*

***Stored for 48 hours instead of seven days.*

background was due to matrix interferences, rather than benzidine. The extract from Sample 5 was injected at various electrode potentials to see if a lower electrode potential would result in a lower background for benzidine. The apparent level of benzidine in the unspiked sample decreased in going from 0.8 to 0.6 volts. This indicates that an interfering component, with an oxidation potential greater than benzidine, is present. However, it was still not certain whether the benzidine level at 0.6 volts was accurate. In order to check this, a second set of HPLC conditions was used. The value obtained for benzidine using the original chromatographic conditions (12 ppb) correlated closely with the value obtained using the alternate colum (9 ppb), thus indicating that the peak probably is benzidine.

The final sample, sample 6, run through the analytical scheme, was an oxidation process effluent from the benzidine based dye plant from which sample 5 was taken. This sample was also diluted 10:1 with distilled water before analysis. Due to a high background at 0.8 volts, benzidine was determined at 0.6 volts. Recoveries for benzidine and DCB spiked at the 10 ppb level were excellent both before and after storage for 48 hours at 4°C and pH 2.

An estimate of the precision and accuracy of the method can be obtained using the results for all five waste samples. For these samples, the average recovery was 69 ± 15% for benzidine and 76 ± 9% for DCB.

Summary and Recommendations

The conclusions drawn from this study are as follows:

- DPH is too unstable in both organic solvents and aqueous solutions to permit meaningful analytical data to be obtained.
- Benzidine and DCB are completely stable for at least 90 days in both methanol and acetonitrile when stored in the dark in sealed ampules.
- Benzidine and DCB can be efficiently extracted from water with chloroform at pH 7 and pH 10. At pH 2, DCB is readily extracted but benzidine is not.
- All three compounds are destroyed when stored in aqueous solutions containing 2 ppm of chlorine. In the absence of chloroform, benzidine and DCB are stable at pH 2 and pH 4.7. However, at elevated pH benzidine is more readily degraded, due to oxidative reactions.
- Analysis of several wastewater samples showed that the analytical methods developed herein work well for the analysis of benzidine and DCB down to the 1 ppb level for most samples. Dye plant samples are a special case since those utilized in this program all showed apparently detectable 10-20 ppb levels of benzidine. Confirmed measurements on two HPLC columns indicate that benzidine is present at the levels stated, although more definitive measurements should be made.

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***James E. Longbottom** is the EPA Project Officer (see below).*
The complete report, entitled "Determination of Benzidines in Industrial and Municipal Wastewaters," (Order No. PB 82-196 320; Cost: $10.50, subject to change) will be available only from:
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