The Determination of Benzidine in Wastewaters

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The Environmental Protection Agency's Proposed List of Toxic Pollutants has recently been released (Federal Register, 1973). In addition to mercury, cadmium, cyanide, and certain chlorinated hydrocarbons, it has named benzidine (4,4'-diaminobiphenyl), a toxic compound which compared to metals and pesticides has received little attention by environmental groups. Benzidine, as well as other substituted anilines, has also been included on the Occupational Safety and Health Administration's list of chemical carcinogens (C & E News, 1974). The attention has been a result of studies which have demonstrated that benzidine can have carcinogenic effects on the urinary bladder in humans (GOLDBLATT, 1947), (GOLDBLATT, 1958), (WALPOLE, *et al*, 1958) and in several tissues of a number of rodent species (CLAYSON, 1964).

In the present work we report our results and observations in developing analytical procedures for the determination of benzidine in the part-per-billion range in wastewaters (raw sewages, secondary effluents, rivers, industrial wastes).

Wastewater samples from the Los Angeles County area were routinely spiked with known amounts of benzidine/methanol standards. These spiked samples were then subjected to a sample pretreatment procedure followed by colorimetric, GLC, or TLC analysis.

Colorimetric Analysis

The colorimetric technique employed was a modification of the procedure developed by EL-DIB (1971). The chemistry of the test involves the conversion of benzidine to its diazo salt and subsequent coupling to resorcinol to produce a diazo dye which has an intense absorption band at 550 nm. The following modifications were made in EL-DIB's procedure to allow for wastewater analysis: (1) The original sample pH was adjusted to 11 with 10 N NaOH. The alkaline sample was then vacuum filtered to remove suspended solids and

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iron and magnesium ions as the hydroxides. The purpose of this procedure was to remove suspended matter which could cause light scattering during colorimetric analysis. (2) The blank or reference solution was the actual wastewater sample, except no $NaNO_2$ was added in the diazotization step. All other reagents and manipulations were the same as for the sample. (3) Using 10 cm cells and a double beam instrument, the detection limit for benzidine was improved from *ca* 100 ppb (EL-DIB, 1971) to *ca* 5 ppb.

Since primary, secondary, and tertiary alkyl amines decompose or lead to products other than diazo salts when subjected to diazotization (ROBERTS, *et al*, 1964), the colorimetric analysis is selective for aromatic amines. However, it was found that the colorimetric procedure exhibited poor selectivity for benzidine compared to other aromatic amines. The test might be of use if a gross substituted aniline count were required, but the poor selectivity for benzidine makes the test unsuitable for most wastewater determinations in the presence of other aromatic amines.

TLC Analysis

A TLC system was developed which employed Silica Gel G (0.25 mm thickness) and (95 + 5) benzene/methanol as the solid and liquid phase respectively. The spots were developed by UV-radiation or by spraying with 0.01 % ethanolic fluorescein dye. Typical R_f values were as follows: diphenylamine (85), 1-napthylamine (70), o-tolidine (51) and benzidine (37). Detection limits for the test were in the range, 0.4 - 0.5 μg, for a 5 μl spot.

As can be seen by the experimental data, the test is selective for benzidine compared to other aromatic amines, however, the overall sensitivity of the test is quite poor.

GLC Analysis (preferred)

The chromatographic system consisted of a Perkin-Elmer Model 900 gas chromatograph equipped with dual 6'x1/8" i.d. glass columns and dual flame detectors which were operated in the reference/sample mode. Instrumental parameters were as follows: injection port (275° C); detector manifold (275° C); nitrogen (40 ml/min); hydrogen (20.5 psi); and air (31.0 psi). The column packing was a mixture of OV-17 (4.7%), QF-1 (5.0%) and DC-200 (0.5%) on 80/100 mesh Gas-chrom Q. Ten μl sample volumes were injected with a 50 μl Hamilton gas-tight syringe fitted with a stainless steel needle.

Chromatograms were routinely recorded with the column temperature at 200° C or programmed from 150 - 250° C. For isothermal versus programmed operation, benzidine detection limits were 0.1 mg/l and 0.5 mg/l respectively.

Although PTGC gave a decreased sensitivity to benzidine, this temperature mode has the advantage of being able to separate a mixture of aromatic amines exhibiting a wide boiling point range. For example, when a mixed standard containing 1-napthylamine, diphenylamine, benzidine, and 3,3'-dimethoxybenzidine was temperature programmed (150-250°C @ 3°/min., holding 12 min. @ 150°), the relative retention times were 1.00, 1.46, 5.50, and 7.23 respectively (See Figure 1).

Because the GLC analysis is sensitive and selective for benzidine, it is the analytical technique of preference compared to the colorimetric or TLC analysis.

Sample Pretreatment

In order for a sample to be analyzed by colorimetry, TLC, and/or GLC, it was necessary to subject the sample to a pretreatment procedure. The filtration/extraction/concentration scheme shown in Figure 2 is a generalized procedure which allows for the analysis of raw sewages, secondary effluents, rivers, or industrial wastewaters.

During the concentration of the final ether phase, an apparent photodecomposition of benzidine was observed. The problem has been avoided by adding 25% methanol to the final ether phase before concentrating. The reasoning behind the addition of methanol was based on the observation that benzidine standards were stable in methanol solution over a period of weeks.

For a typical GLC or TLC determination, the pH of a 200 ml sample was adjusted to *ca* 11 by addition of 10 N NaOH. The solution was filtered through two glass fiber filter papers and the filters washed with near boiling 3 N NaOH. The filtrate was then extracted 3 times with 50 ml of diethylether. The ether extracts were combined and extracted 3 times with 25 ml of 2N HCl. The acid extracts were combined and neutralized by addition of 10 N NaOH. The solution was allowed to cool, and then re-extracted 3 times with 50 ml of diethyl ether. 40 ml of absolute methanol was added to the ether extracts and the solution was concentrated to a final volume of 1-2 ml, using a stream of filtered air and a hot plate.

Depending on the nature of the sample and the analytical technique to be used, the pretreatment procedure can be shortened. For instance, if a secondary effluent is to be analyzed, it is usually possible to neutralize the initial filtrate and then analyze by the colorimetric procedure, or if GLC and TLC analysis is desired, the sample can be extracted directly with ether and the extract concentrated. Usually, samples which have high dissolved and/or suspended solids must be subjected to a more detailed pretreatment. In general, it was found that the extent to which the pretreatment procedure could be shortened was somewhat empirical.

The overall detection limit of the analysis was determined by the initial sample size and the volume of the final concentrate. The practical detection limit was in the range of 2-3 μg/l.

For unchlorinated wastewaters, experimental spike recoveries ranged between 70 - 95%. When chlorinated wastewaters were spiked with benzidine, low recoveries were realized. Apparently, these low recoveries were due to the reaction of benzidine with hypochlorous acid to produce a chloramine-type structure (eq.1), which did not give a positive benzidine test.

$$2\ HOCl + H_2N\text{-}\phi\text{-}\phi\text{-}NH_2 \longrightarrow Cl\overset{H}{N}\text{-}\phi\text{-}\phi\text{-}\overset{H}{N}Cl + 2H_2O \qquad (1)$$

Since the sample pretreatment scheme is based on the protonation chemistry of amines, the described analytical methods should also be suitable for the determination of other carcinogenic anilines in wastewaters.

At present, we are continuing our work with benzidine to determine its ultimate fate in chlorinated and unchlorinated wastewater systems.

Acknowledgement

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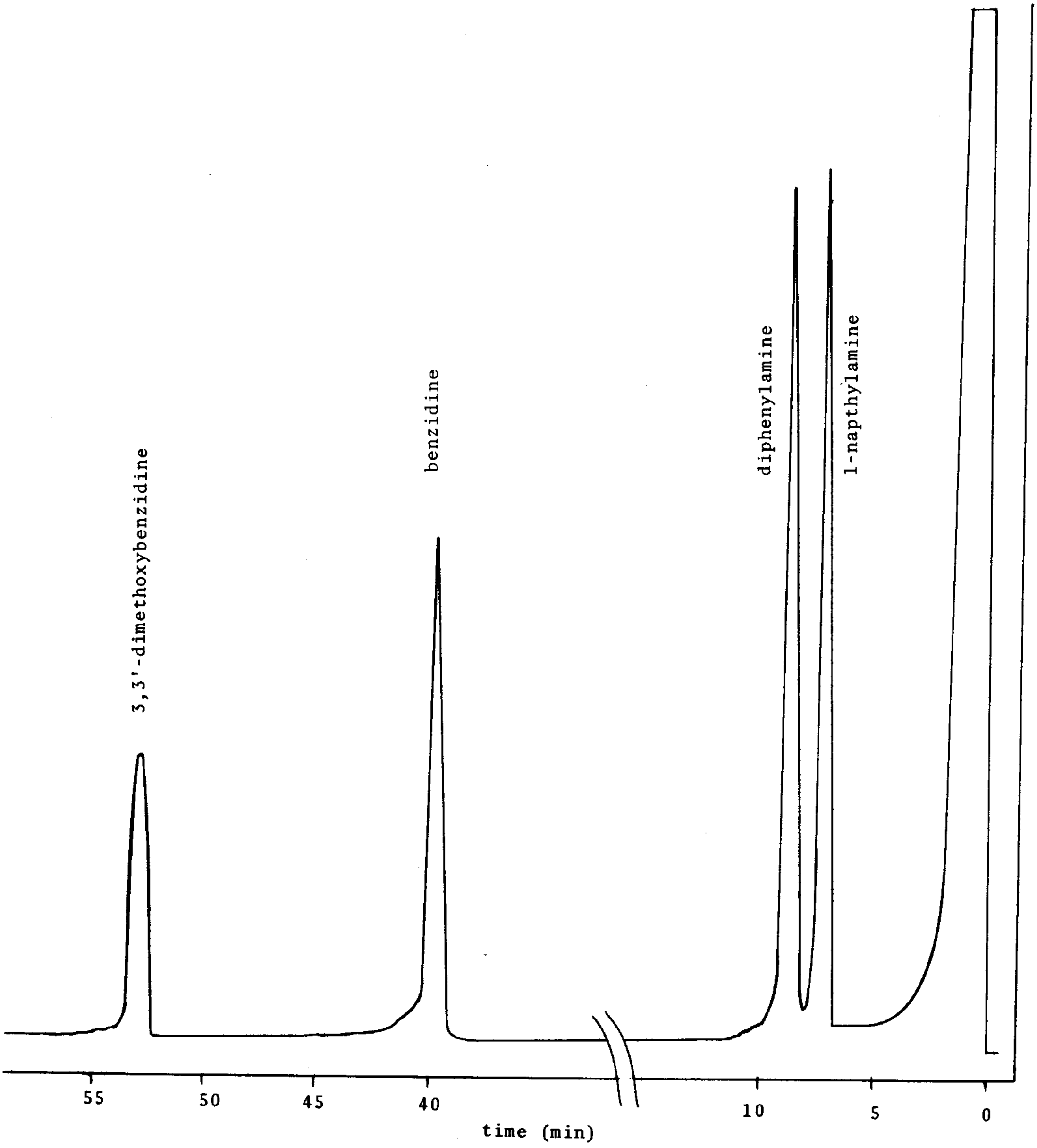

Figure 1. Resolution of 4 aromatic amines by the column mixture OV-17(4.7%), QF-1 (5.0%) and DC-200 (0.5%) on 80/100 mesh Gas-Chrom Q, using PTGC (150-250° C @ 3°/min., holding 12 min. @ 150° C).

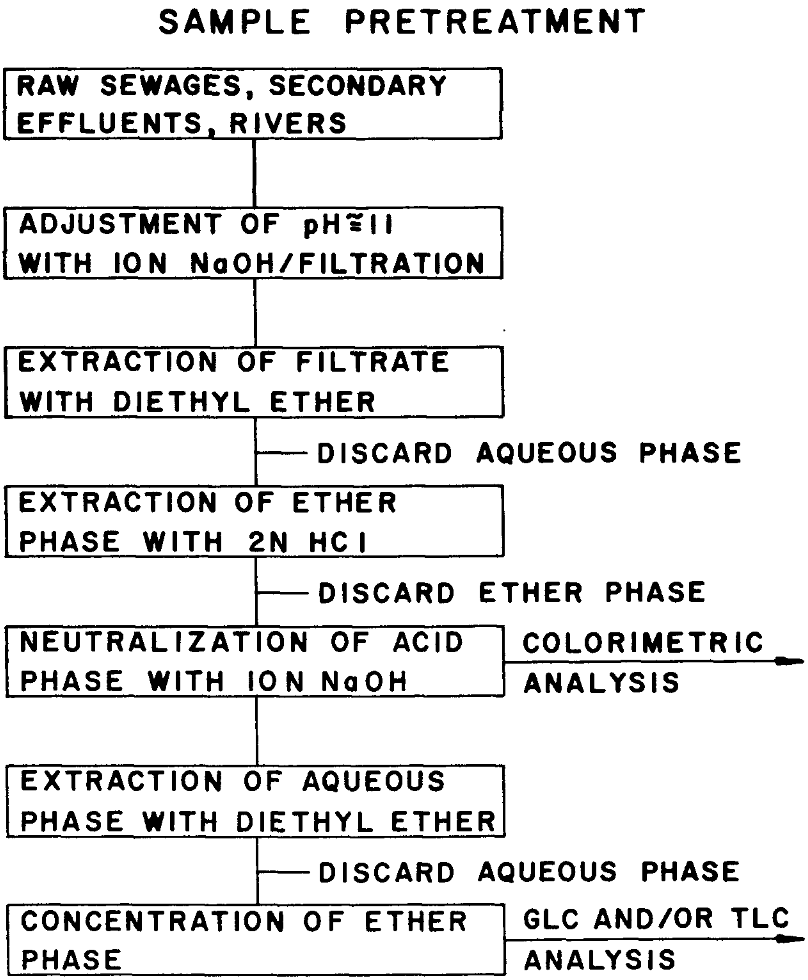

Figure 2. Procedure for pretreatment of sample prior to analysis by GLC, TLC, or colorimetry.

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