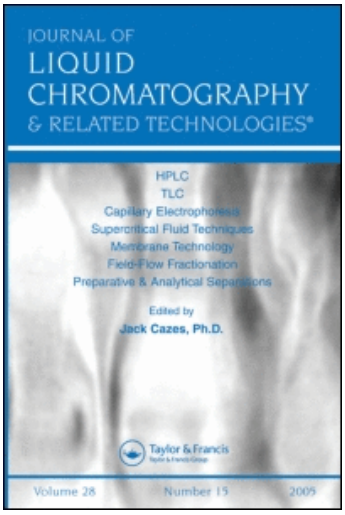

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Detection of Toxic Compounds in Polyurethane Food Bags by Liquid Chromatography/Electrochemistry

Colleen J. P. Ward[a]; D. M. Radzik[a]; P. T. Kissinger[a]
[a] Department of Chemistry, Purdue University, West Lafayette, Indiana

To cite this Article Ward, Colleen J. P. , Radzik, D. M. and Kissinger, P. T.(1985) 'Detection of Toxic Compounds in Polyurethane Food Bags by Liquid Chromatography/Electrochemistry', Journal of Liquid Chromatography & Related Technologies, 8: 4, 677 — 690
To link to this Article: DOI: 10.1080/01483918508067112
URL: http://dx.doi.org/10.1080/01483918508067112

JOURNAL OF LIQUID CHROMATOGRAPHY, 8(4), 677-690 (1985)

DETECTION OF TOXIC COMPOUNDS IN POLYURETHANE FOOD BAGS BY LIQUID CHROMATOGRAPHY/ELECTROCHEMISTRY

Colleen J. P. Ward, D. M. Radzik,
and P. T. Kissinger
Department of Chemistry
Purdue University
West Lafayette, Indiana 47907

ABSTRACT

The application of liquid chromatography/electrochemistry (LCEC) for the determination of toxic by-products of boilable cooking pouches including, 2,6-toluenediamine (2,6-TDA), 2,4-toluenediamine (2,4-TDA), aniline and phenol, in aqueous solutions is demonstrated. Separation was achieved on a 5 μm ODS reverse-phase column with a mobile phase consisting of 7.0% acetonitrile and 93.0% 0.1 M ammonium acetate buffer, pH 5.40. Detection of compounds was accomplished using a thin layer amperometric detector. Samples were directly injected without a pre-concentration or clean-up procedure. All of the potentially harmful compounds found to migrate out of the polyurethane walls of the boilable cooking pouches were routinely detectable at levels less than one picomole.

 0148-3919/85/0804-0677$3.50/0

INTRODUCTION

Recently there has been concern about the potential toxicological and cancer-inducing activity of some of the primary aromatic amines (1), and phenol families (2), therefore sufficient means of detecting and quantitating their presence is important.

Two diamines, 2,6-toluenediamine (2,6-TDA), and 2,4-toluenediamine (2,4-TDA) have previously been detected in aqueous solutions of boilable cooking bags (3). They are intermediates in the production of toluenediisocyanates used in the manufacture of polyurethanes (4). In 1982, production of the mixture of 2,4- and 2,6-TDA (80/20) by U.S. industry reached 555,994 million pounds. 2,4-Toluendiamine production alone was 162,173 million pounds (5). 2,4-Toluenediamine, previously a common ingredient used in hair dyes, was removed from such preparations in 1971 when it was reported that rats fed a diet of 0.1 or 0.06 percent 2,4-TDA developed liver cancer (6). Tests with Salmonella typhemurium have shown 2,4-TDA to cause frame-shift mutations in the mutant strain TA1538 (7). The National Cancer Institute has reported an elevated, but not statistically significant incidence of bladder and kidney tumors associated with rats treated with 2,4-TDA (8). It has been reported that there is a dose dependent relationship between the administration of 2,4-TDA and the incidence of hepatocellular tumor development in rats and female mice (9). The 2,6-TDA isomer has been suspect in causing elevated levels of kidney and liver tumors in rodents (7). Although potentially harmful in some biological systems, the toxic effects of 2,4- and 2,6-TDA in man has not been established.

Several methods have been reported for the determination of the 2,4- and 2,6-TDA isomers, including LCUV (3,10), TLC(11), and GC (12-14). These methods are either too time consuming,

require clean-up, and pre-concentration steps, derivatizations and other reactions, or they are not sensitive enough to detect the compounds at the levels at which they exist in real samples.

Approximately 665 million pounds of aniline and 2.61 billion pounds of phenol were produced in the U.S. in 1983 (17). Despite such wide-spread use, neither compound has been adequately tested for toxic or carcinogenic effects. However, aniline (18, 19), and phenol (20) are considered acutely toxic substances. Aniline and phenol have been shown to be easily determined by LCEC(15,16).

The potentially toxic consequences of direct exposure of the public to 2,4-TDA, 2,6-TDA, aniline, and phenol is a great concern. With the availability and general acceptance of boilable cooking bags, it is necessary to determine those chemicals that can migrate from the polyurethane food bags into the contents of the bag. Liquid chromatography/electrochemistry provides a simple, direct approach with low detection limits for the determination of 2,4-TDA, 2,6-TDA, aniline and phenol in real samples. This is the first time that aniline and phenol have been directly detected in aqueous solutions of boilable cooking pouches.

MATERIALS AND METHODS

Instrumentation

A Bioanalytical Systems commercial LC-154 liquid chromatograph was used in all studies (Bioanalytical Systems, Inc., West Lafayette, IN). The system consisted of an LC-4B amperometric detector equipped with either a glassy carbon, single or a dual-electrode set in parallel configuration. The column was a Biophase 5-μm ODS column (250 x 4.6mm) from Bioanalytical Systems. A rotary injection valve with a 100-μL injection loop was used in all experiments.

Chemicals and Reagents

Chemical standards were purchased from the following sources: 2,4-diaminotoluene (2,4-toluenediamine), and 2,6-diaminotoluene (2,6-toluenediamine), Aldrich, Milwaukee, Wisconsin; aniline and phenol, Mallinckrodt Inc., St. Louis, Missouri. Buffers were prepared with distilled - deionized water and filtered through a Nylon-66 filter with a pore size of 0.2 μm. Acetonitrile was distilled in glass and filtered before use. All other compounds were of the highest available reagent grade.

Procedure for Boiling Bags Experiments

Consumer available, boilable cooking pouches (8" x 9") were used for all experiments. Bags were filled with 25 mL of distilled - deionized water or various pH buffers. Prior to boiling, inert glass beads were added to weigh down the bags. All bags were thermally sealed with a Dazey®, "Seal-a-Meal" (model 5005) and introduced into boiling distilled - deionized water. The length of boiling time is as stated in the text.

Liquid Chromatographic Conditions

For the separation and detection of standards, a mobile phase of 7.0% acetonitrile and 93.0% 0.1 M ammonium acetate buffer, pH 5.40 was utilized. The mobile phase was filtered and degassed prior to use. The flow rate was 1.5 mL/min. The detector potential was poised at +850 mV versus a Ag/AgCl reference electrode, unless otherwise specified.

Characterization and Quantitation

Chromatographic retention time offers a means of identification based on the comparison of an unknown to a

standard. Capacity factor, k', is the value most often compared, when holding mobile phase conditions constant for samples and standards. In addition, electrochemical detection using a thin-layer amperometric cell allows for the characterization of compounds based on their electrochemical properties. In order to choose an appropriate operating potential (at which to work), a normalized hydrodynamic voltammogram for each standard is prepared. A detailed discussion of the preparation of a normalized hydrodynamic voltammogram has been presented elsewhere (21). In order to verify that sample peaks eluting at the same retention time with standard peaks are the same compound, electrochemical characteristics may be compared. The current response observed for the sample and standard peaks at various potentials were compared to their current response at the limiting current plateau. These current ratios are most accurately obtained by utilizing a dual electrode detector in the parallel-adjacent configuration. The potential of one electrode is varied, while the second electrode is poised at a potential on the current limiting plateau. Current ratios for samples and standards were collected and compared. These ratios can be used as a secondary proof of peak identity.

Standard curves were prepared and used to quantitate samples.

RESULTS AND DISCUSSION

Characterization of Standards

The liquid chromatographic separation of 2,6-TDA, 2,4-TDA, aniline and phenol is shown in Figure 1. It was observed that a slight change in the pH of the mobile phase greatly altered the retention time of the amines. It has been reported (3), that phosphate buffers below pH 6.5 cause peak broadening and hence poor resolution of the toluenediamines. The

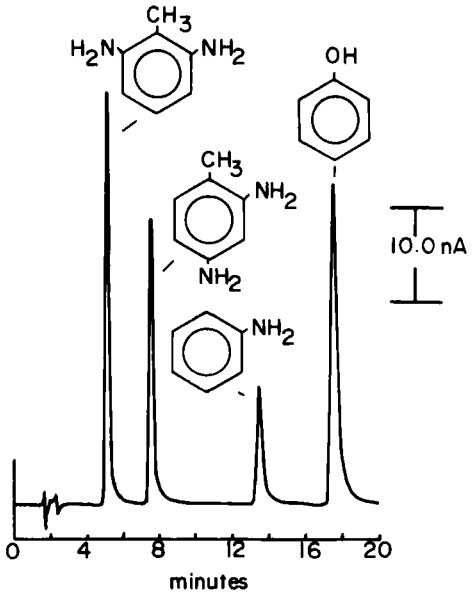

FIGURE 1. LCEC oxidative chromatogram of standards. Chromatographic conditions: BAS RP C-18 column, mobile phase of 7% acetonitrile and 93% 0.1 M $NH_4^+OAC^-$ buffer, pH 5.4, flow rate of 1.5 mL/min. 100 µL injection volume, glassy carbon working electrode at +0.85 V vs a Ag/AgCl reference electrode.

ammonium acetate buffer at pH 5.40 gave sharp peaks and adequate resolution of the toluenediamines with an injection volume of 100 µL. Hydrodynamic voltammograms for the four standards are shown in Figure 2.

Linearity of Standards

All compounds studied were detected linearly over three orders of magnitude. The detection limits for all compounds of interest were below one picomole (Table 1).

Characterization of Migrating Compounds

A chromatogram for a sample of distilled - deionized water that was boiled in a polyurethane food bag is presented in Figure

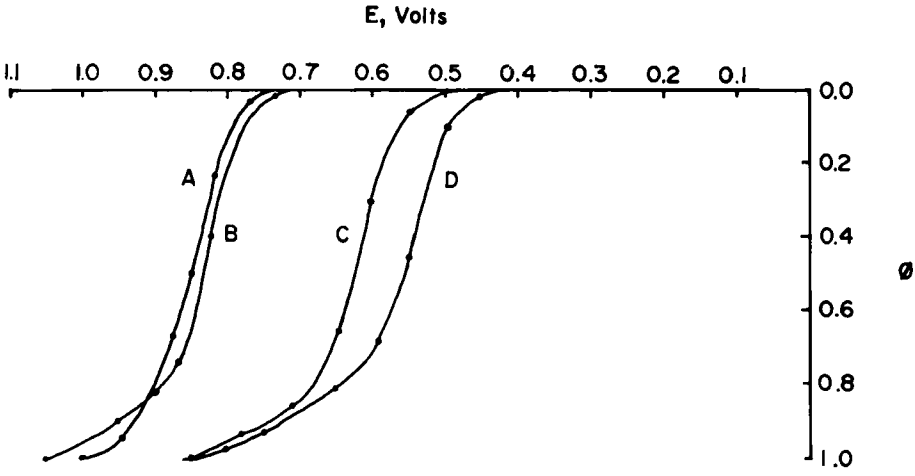

FIGURE 2. Normalized hydrodynamic voltammograms of (A) aniline, (B) phenol, (C) 2,6-TDA, and (D) 2,4-TDA.

TABLE 1

Linear Regression Analysis for Some Compounds That Migrate From Polyurethane Food Bags

COMPOUND[a]	RANGE (pmol)[b]	SLOPE Y ($x10^{-2}$)	INTERCEPT	CORRELATION COEFFICIENT
Phenol	0.52-5242	1.82	1.43	0.999
2,6-Diaminotoluene	0.77-5140	5.22	4.90	0.999
2,4-Diaminotoluene	0.81-4882	3.17	3.65	0.999
Aniline	0.50-2000	4.63	-4.12	0.999

[a]y = peak current response, nanoamps; x = picomoles injected.

[b]Range is based on the responses of at least seven standards. The lower end of the range is the limit of detection.

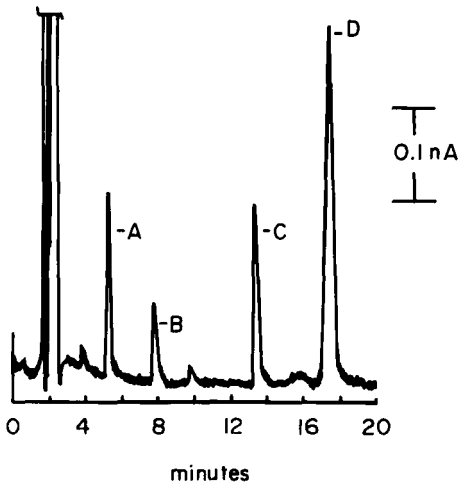

FIGURE 3. Chromatogram of a sample taken from a polyurethane bag that was boiled with 25 mL of water. (A) 2,6-TDA, (B) 2,4-TDA, (C) aniline, (D) phenol. Chromatographic conditions: BAS RP C-18 column, mobile phase of 7% acetonitrile and 93% 0.1 M $NH_4^+OAC^-$ buffer, pH 5.4, flow rate of 1.5 mL/min. 100 µL injection volume, glassy carbon working electrode at 0.85 V vs a Ag/AgCl reference electrode.

3. The chromatographic conditions were identical to those used to separate standards. Comparison of capacity factors, k', and electrochemical current ratios of standard and sample peaks allow for the positive identification of sample components. Electrochemical current ratios were obtained using the dual-parallel electrodes as explained previously under the Materials and Methods section. Table 2 and 3 list the values for the comparison of standards and sample components.

Dependence of Migration on pH

The purpose of these experiments was to determine the influence pH has on the concentration and species found to migrate from the polyurethane matrix into the contents of the

TABLE 2

Voltammetric Characterization of
2,6 and 2,4- Diaminotoluene

	k'	+650 mV	+550 mV	+500 mV	+450 mV
2,6-Diaminotoluene[a]					
Standard	2.35	0.74	0.19	0.05	0.02
Bag Boiled With Water	2.44	0.65	0.15	0.09	0.04
2,4-Diaminotoluene[a]					
Standard	3.98	0.74	0.51	0.21	0.04
Bag Boiled With Water	3.99	0.79	0.44	0.20	0.086

[a]Currents normalized to that observed at +900 mV.

TABLE 3

Voltammetric Characterization of Aniline and Phenol

	k'	+1000 mV	+950 mV	+900 mV	+800 mV	+750 mV
Aniline[a]						
Standard	8.00	—	1.00	0.84	0.22	0.04
Bag Boiled With Water	7.98	—	1.00	0.85	0.21	0.05
Phenol[b]						
Standard	10.75	0.94	0.81	0.64	—	—
Bag Boiled With Water	10.73	0.89	0.76	0.65	—	—

[a]Currents normalized to that observed at +950 mV.
[b]Currents normalized to that observed at +1.05 mV.

bag. Three buffers - pH 3.50 (ammonium acetate), 6.00 (ammonium acetate) and 8.20 (sodium phosphate) - of ionic strength 0.01 were boiled in the food bags (8" x 9"), for one hour. 100μL samples were directly injected onto the liquid chromatographic system. Compounds were quantitated based on standard calibration curves. For each buffer, three trials were run and the amount of 2,4-TDA, 2,6-TDA and phenol were quantitated as an average. (Table 4). There was no observable change in the migration pattern of the components at pH 3.50 or 6.00. The concentration of components found in the polyurethane bags boiled with the pH 8.20 buffer showed a distinct increase. The concentration of phenol detected was more than twice that determined at pH 3.50 or 6.00.

The results indicate that the migration of chemicals could possibly be base catalyzed. This data suggests alkaline food substances may enhance the migration of chemicals into the contents of the bag. The lack of data for the aniline component may be explained by the variations in the batches of polyurethane food bags produced. The batch used in these experiments did not yield any data that could be deemed statistically correct.

Dependence of Migration on Boiling Time

A pH 8.20 buffer of ionic strength 0.01 was selected to be boiled in the polyurethane bags. The maximum boiling time for the three bags used was three hours. A syringe was used to collect 300 μL aliquots from each of the bags at specific time intervals after the start of boiling. A 100 μl injection volume from each aliquot was then directly injected into the chromatographic system. Peaks were quantitated based on standard calibration curves. Figure 4 shows a plot of 2,4-TDA, and 2,6-TDA, detected at the various boiling times. Both

TABLE 4

Influence of pH on the Detection of Compounds[a]

pH of Buffer	2,6-TDA(pmol)	2,4-TDA(pmol)	Phenol (pmol)
3.50	0.92±0.06	0.91±0.60	2.06±0.30
6.00	0.81±0.04	0.94±0.60	2.00±0.34
8.20	1.05±0.03	1.18±0.62	5.59±0.32

[a]Three buffers of different pH and constant ionic strength (0.01), were boiled in polyurethane food bags (8"x9"), for 1 hour. Values are the means of 3 trials ± standard deviation of the mean expressed as pmol. E = +1.00 V.

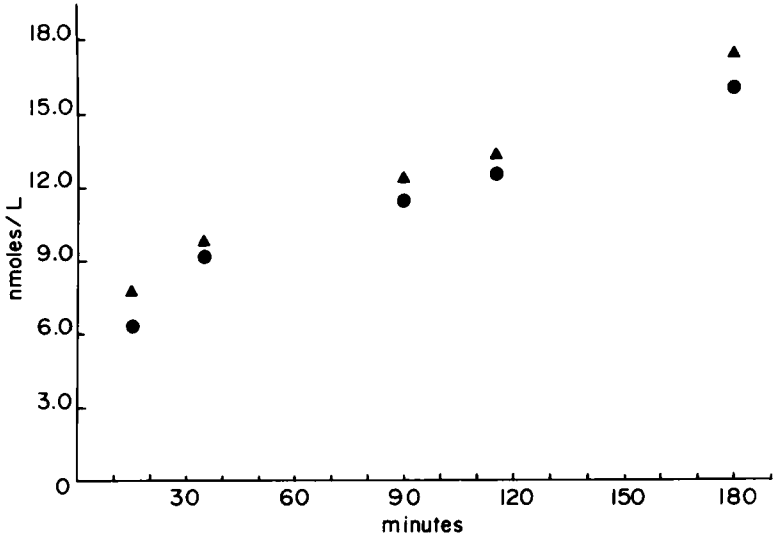

FIGURE 4. Concentration of 2,6-TDA, ● , and 2,4-TDA, ▲ , produced over time. 25 mL of pH 8.20 phosphate buffer with a ionic strength of 0.01 was boiled in polyurethane bags and sampled at various times; E = +1.00 V.

components show a steady increase in the amount migrating with time. At the end of the three hour boiling period there was no evidence that the rate of migration was slowing.

CONCLUSION

This report demonstrates the applicability of liquid chromatography/electrochemistry for the determination of 2,4-TDA, 2,6-TDA, aniline and phenol in aqueous extracts of boilable cooking bags. The selectivity of LCEC allowed for the direct determination of migration components without a clean-up or preconcentration step. Peak identities were confirmed based on retention time and voltammetric characterizations. The superior detection limits achievable with the electrochemical detector combined with the separation capabilities of liquid chromatography, easily allows the determination of trace quantities of these species.

REFERENCES

1. Radomski, J.L., The Primary Aromatic Amines: Their Biological Properties and Structure Activity Relationships, Ann. Rev. Pharamacol. Toxicol. 19, 129 (1979).

2. Deichman, W.B., Keplinger M.L., Phenols and Phenolic Compounds, in Patty FA (ed.): Industrial Hygiene and Toxicology, ed. 2 rev; Toxicology (Fassett D.W., Irish D.D., eds.). Interscience Publishers, New York, 1963, vol. 2, pp 1363.

3. Snyder, R.C., and Breder, C.V., High-Performance Liquid Chromatographic Determination of 2,4- and 2,6-Toluenediamine in Aqueous Extracts, J. Chromatogr., 236, 429 (1982).

4. Frisch K.W., and Saunders J.H., Polyurethanes: Chemistry and Technology (Flory, P.J., Mark, H., Marvel, C.S., and Melville, H.W., eds) Interscience Publishers, New York, 1962, vol. 1, pp 1.

5. U.S. International Trade Commission, Synthetic Organic Chemicals, U.S. Production and Sales, USITC publication no. 1422, (1982), pp 27.

6. Ito, N., Hiasa, Y., Konishi, Y., and Marugami, M., The Development of Carcinoma in Liver of Rats Treated with m-Toluelenediamine and the Synergistic and Antagonistic Effects with Other Chemicals, Career Res., 29, 1137 (1969).

7. Ames, B.N., Kammen, H.O., and Yamasaki, E., Hair Dyes Are Mutagenic: Identification of a Variety of Mutagenic Ingredients, Proc. Nat. Acad. Sci. U.S.A. 72, 2423 (1975).

8. Sontag, J.M., Carcinogenicity of Substituted-Benzenediamines (Phenylenediamines) in Rats and Mice, JNCI. 66, (3), 591 (1981).

9. NCI carcinogenesis technical report series No. 162, Springfield, VA,: Natl. Technical Information Service (NTIS), [PHEW publication No. (NIH) 79-1718 (NTIS) accession No. PB293593AS)], 1979.

10. Unger, P.D., and Friedman, M.A., High-Performance Liquid Chromatography of 2,6- and 2,4- Diaminotoluene, and its Application to the Determination of 2,4- Diaminotoluene in Urine and Plasma, J. Chromatogr., 174, 379 (1979).

11. Hogue Angeletti, R.A., Use of Tetracyanoethylene as a Thin-Layer Chromatographic Spray Reagent, J. Chromatogr., 36, 535 (1968).

12. Boufford, C.E., Determination of Isomeric Diaminotoluenes by Direct Gas Liquid Chromatography, J. Gas Chromatogr. 6, 438 (1968).

13. Willeboordse, F., Quick, Q., and Bishop, E.T., Direct Gas Chromatographic Analysis of Isomeric Diaminotoluenes, Anal. Chem. 40, (10), 1455 (1968).

14. Brydia, L.E., and Willeboordse, F., Gas Chromatographic Analysis of Isomeric Diaminotoluenes. 40, (1), 110 (1968).

15. Radzik, D.M., and Kissinger, P.T., Determination of Aniline and Metabolites Produced in Vitro by Liquid Chromatography/Electrochemistry, Anal. Biochem. 140, (1), 74 (1984).

16. Shoup, R.E., and Mayer, G.S., Determination of Environmental Phenols by Liquid Chromatography/ Electrochemistry, Anal. Chem. 54, 1164 (1982).

17. As reported in Chem. Eng. News, 62, (24), 32 (1984).

18. As reported in J. Chem. Ed. 62, (1), A19 (1972).

19. Jenkins, F.P., Robinson, J.A., Gellatly, J.B.M., and Salmond, G.W.A., The No-effect Dose of Aniline in Human Subjects and a Comparison of Aniline Toxicity in Man and the Rat, Fd. Cosmet. Toxicol. 10, 671 (1972).

20. Gosselin, R.E., Hodge, H.C., Smith, R.P. and Gleason, M.N., Clinical Toxicology of Commercial Products, Williams and Wilkins, 5th ed., Baltimore, (1976) pp 271.

21. Miner, D.J., Rice, J.R., Riggin, R.M. and Kissinger, P.T., Voltammetry of Acetaminophen and its Metabolites, Anal. Chem. 53, 2258 (1981).