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HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF AROMATIC AMINES

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SUMMARY

The high-pressure liquid chromatographic conditions required for the liquid–solid separation of approximately fifty aromatic monoamines and diamines were established in this study. The separations were carried out on various silica gels using chloroform and/or cyclohexane mobile phases. The primary separation mechanism depended upon an interaction between the amino group and the slightly acidic adsorbent and in most cases is related to the basicity of the amine. The general parameters developed here should apply to the separation of other aromatic amines not considered in this study.

INTRODUCTION

Aromatic amines are valuable chemicals in many areas of industry and research. Classically important in dye chemistry, the physiological activity of these compounds make them of interest in the biomedical field. Underscoring their physiological activity is the fact that eight of the fourteen chemicals controlled by the second emergency standard issued by the Occupational Safety and Health Administration are aromatic amines[1]. Isomeric aromatic diamines have become increasingly important in studying the effects of chemical structure on the properties of polyimides[2,3], polyamides[4], epoxies[5], and urethanes[6]. As research with this class of compounds increases, their separation and identification becomes more important.

A review of the chromatography literature reveals many amine separations. However, these are primarily thin-layer, ion-exchange, or gas-chromatographic analyses of aliphatic amines. High-pressure liquid chromatography (HPLC) has been used in only a few cases to separate aromatic amines[7,8]. Since this technique offers speed, the potential for sample scale up, and a gentle environment for these reactive species, the use of HPLC in the analysis of amines is expected to increase. However,

no study has been reported on the HPLC separation of aromatic amines as a class of organic compounds*.

The present investigation concerns the HPLC separation of approximately fifty aromatic monoamines and diamines, is a continuation of work correlating amine basicity with chromatographic retention[9], and directly supports polymer research programs at the NASA–Langley Research Center. This report provides a summary of pertinent chromatographic parameters and discusses a proposed mechanism for separating aromatic amines on silica gel. An understanding of this proposed mechanism along with the parameters presented should provide a starting point for the separation of amines not considered in this study.

EXPERIMENTAL

Apparatus

A Model ALC 202/R401 liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) equipped with a Model 6000 solvent delivery system was used throughout this study. The fixed-wavelength (254 nm) ultraviolet (UV) detector was employed and the chromatograph was operated under ambient temperature conditions. A 25-μl Series "B-110" Pressure-Lok liquid syringe (Precision Sampling, Baton Rouge, La., U.S.A.) was used to inject the analytical samples.

Chromatograms were recorded on a Hewlett-Packard 7100B strip chart recorder at a 0.51 cm/min chart speed.

Columns

A brief description of each absorbent used in this study is given in Table I. Corasil I, Corasil II, Porasil A, Porasil B, and Porasil C columns were prepared by packing each adsorbent into 61 cm × 2.4 mm I.D. stainless-steel tubing using the modified tap–fill technique[10]. Approximately 3.2 g of Corasil and 1.2 g of Porasil were required to pack these columns which were fitted on the injector end with a silanized glass wool plug and on the detector end with a 5-μm stainless-steel frit. A 25 cm × 2.1 mm I.D. stainless-steel Zorbax-SIL column was obtained prepacked from a commercial source.

TABLE I

PROPERTIES OF COLUMN PACKING MATERIALS

Packing	*Particle form*	*Particle size (μm)*	*Surface area (m^2/g)*
Corasil I	Porous pellicular layer	37–50	7
Corasil II	Porous pellicular layer	37–50	14
Zorbax-SIL	Porous beads microspheres	6– 8	>200
Porasil A	Porous beads	37–75	350–500
Porasil B	Porous beads	37–75	125–250
Porasil C	Porous beads	37–75	50–100

* *Editor's note:* There are some papers, for example, H. Engelhardt *et al.*, *Anal. Chem.*, 46 (1974) 338; C. H. Chu and D. J. Pietrzyk, *Anal. Chem.*, 46 (1974) 331, 334, 335.

Materials

Common aromatic amines were obtained from commercial sources. The more complex amines were synthesized in-house. Ref. 11 gives the synthesis of many of these compounds. Amine basicity measurements were determined in acetonitrile–water (9:1) by titration with perchloric acid solution using a calibrated glass electrode[12]. The reagent-grade chloroform and cyclohexane mobile phases were filtered prior to use.

Procedure

Sample sizes of 2–3 μl of solutions containing 1 mg/ml of solute were injected and the capacity factor k' was calculated from the equation $k' = (t_r - t_0)/t_0$, where t_r is the sample retention time and t_0 is the hold-up time. Carbon tetrachloride was injected into the mobile phase to determine the hold-up time of each column.

RESULTS AND DISCUSSION

The chromatographic conditions which provided a satisfactory separation in a reasonable time were determined by changing the polarity of the mobile phase for one of the adsorbents and then, when necessary, repeating the process using an adsorbent of higher or lower surface area as appropriate. Thus, both mobile phase polarity and adsorbent surface area were considered in optimizing a separation. Table II summarizes the chromatographic conditions and gives k' values for the separation of approximately fifty aromatic amines.

Fig. 1 shows a chromatogram obtained on a synthetic mixture of four isomeric aromatic diamines. This figure was presented to demonstrate the capability of HPLC for separating complex mixtures of amines. However, most analyses were made in order to determine monomer purity prior to polymerization studies and only one major

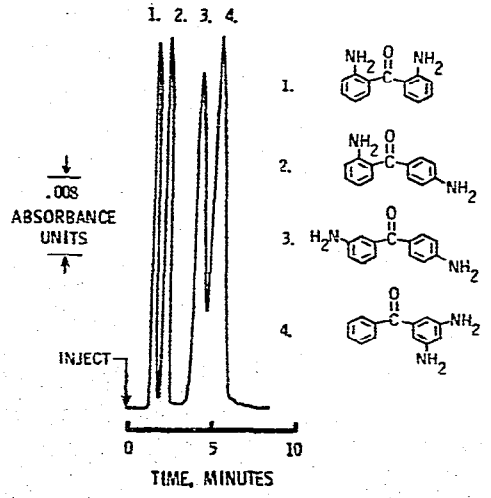


Fig. 1. Chromatogram of diaminobenzophenone isomer mixture. Conditions: column, 61 cm × 2.4 mm I.D., packed with Corasil II; solvent, chloroform; flow-rate, 1.5 ml/min; pressure, 625 p.s.i.; sample size, 3 μl (1 μg/μl); room temperature; detector wavelength, 254 nm.

TABLE II

CONDITIONS FOR SEPARATION OF AROMATIC AMINES

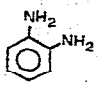
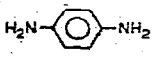
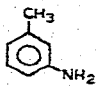
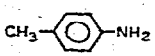
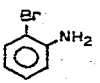
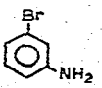
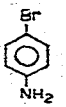
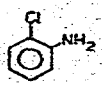
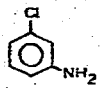
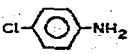
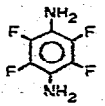
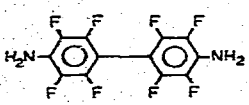
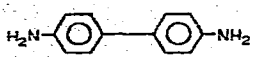
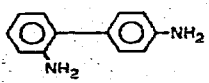
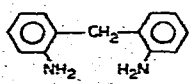
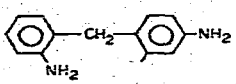
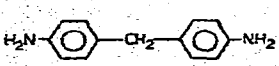
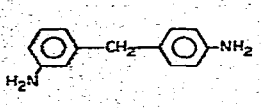
Structure	*Column*	*Mobile phase*	*Flow-rate (ml/min) [p.s.i.]*	*k'*
	Corasil II	chloroform	1.0 [500]	0.40
	Corasil I	chloroform	0.5–2.0 [325–1000]	0.82–6.92
	Corasil II	chloroform		
	Zorbax-SIL	0.7% methanol–chloroform	0.5 [2000]	5.70–15.09
	Porasil A	chloroform–cyclohexane (75:25)	0.5 [400]	0.89–1.52
	Porasil B	chloroform–cyclohexane (75:25)	0.5 [350]	0.75–2.16
	Porasil C	chloroform–cyclohexane (75:25)	0.5 [350]	0.38–0.72
	Corasil I	cyclohexane	2.0 [1300]	2.42
				7.52
				11.68

TABLE II *(continued)*

Structure	*Column*	*Mobile phase*	*Flow-rate (ml/min) [p.s.i.]*	*k'*
	Corasil I	cyclohexane	2.0 [1300]	2.77
				7.70
				12.35
	Corasil II	chloroform	1.0 [700]	0.44
				0.38
	Zorbax-SIL	chloroform–cyclohexane–methanol (90:9.7:0.7)	0.5 [2000]	7.26
				6.50
	Corasil II	chloroform	0.5 [350]	1.61
				2.10
				2.66
				2.92

(Continued on p. 574)

TABLE II *(continued)*

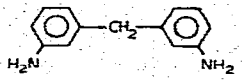
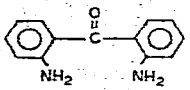
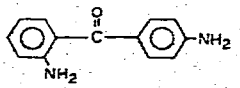
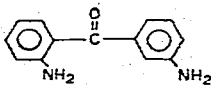
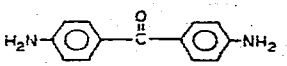
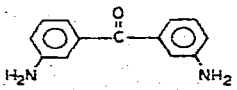
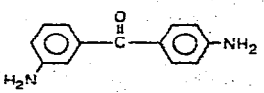
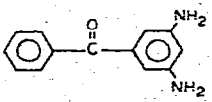
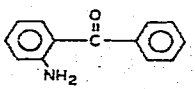
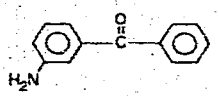
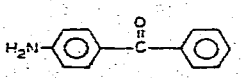
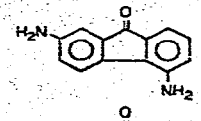
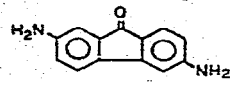
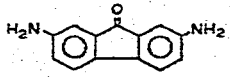
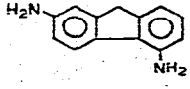
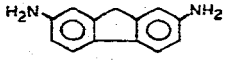
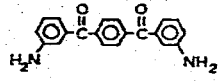
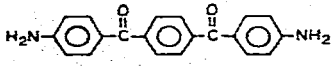
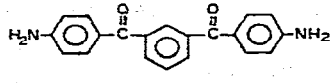
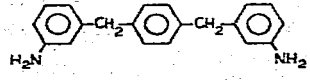
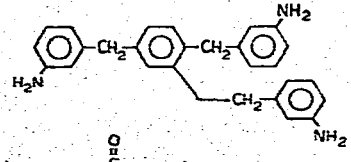
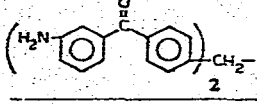
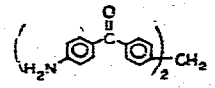
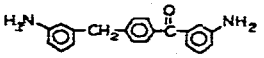
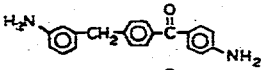
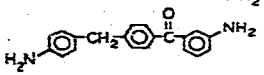
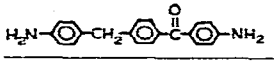
Structure	*Column*	*Mobile phase*	*Flow-rate (ml/min) [p.s i.]*	*k'*
	Corasil II	chloroform	0.5 [350]	3.23
	Corasil II	chloroform	1.0 [600]	0.34
				1.13
				1.14
				3.14
				3.44
				3.63
				5.04
	Corasil II	chloroform	0.5 [350]	0.13
				0.54
				0.54

TABLE II *(continued)*

Structure	*Column*	*Mobile phase*	*Flow-rate (ml/min) [p.s.i.]*	*k'*
	Zorbax-SIL	chloroform–cyclohexane–methanol (90:9.3:0.7)	0.5 [2000]	9.80
				12.07
				9.80
	Corasil II	chloroform	0.5 [800]	2.02
				1.54
	Zorbax-SIL	chloroform–cyclohexane–methanol (95:4.3:0.7)	0.5 [1500]	1.92
				2.64
				3.41
	Zorbax-SIL	chloroform–cyclohexane–methanol (90:9.3:0.7)	0.5 [2000]	4.33
				22.96
	Zorbax-SIL	chloroform–cyclohexane–methanol (95:4.3:0.7)	0.5 [1500]	2.23

(Continued on p. 576)

TABLE II (*continued*)

Structure	*Column*	*Mobile phase*	*Flow-rate (ml/min) [p.s.i.]*	*k'*
				2.82
	Zorbax-SIL	chloroform–cyclohexane–methanol (95:4.3:0.7)	0.4 [1100]	1.99
				2.18
				2.19
				2.54

component was observed. A 0.1% impurity could easily be detected if a constant UV detector response for all peaks was assumed. This assumption is reasonable since, due to the method of synthesizing these compounds, the impurity in a particular amine was often an isomer of that amine.

The adsorption of many basic organic compounds on silica gel is generally accepted to be the interaction of a base with an acidic surface[15]. One way this interaction may occur for an amine is illustrated in Fig. 2. The unshared pair of electrons associated with the amine nitrogen atom are proposed to interact with the slightly acidic hydroxyl proton. The strength of this interaction determines how long the amine is retained on the column. Thus, the separation mechanism must be related to the base strength of the amine. The stronger the base, the stronger the interaction with the acidic adsorbent, and the longer the amine takes to elute.

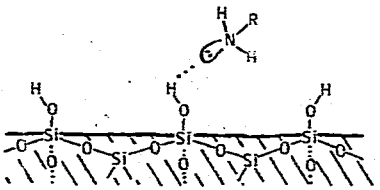


Fig. 2. Proposed acid–base interaction for separating aromatic amines on silica gel.

Fig. 3 shows two chromatograms obtained in this research which supported this concept. Tetrafluoro-*p*-phenylenediamine is expected to be less basic than *p*-phenylenediamine because the electronegative fluorine atoms tend to delocalize the two amine electrons. Thus, the fluorinated diamine should interact less with the active sites on the silica gel and elute before the unfluorinated compound. This behavior is observed in Fig. 3. Similarly, octafluorobenzidine elutes before its unfluorinated counterpart.

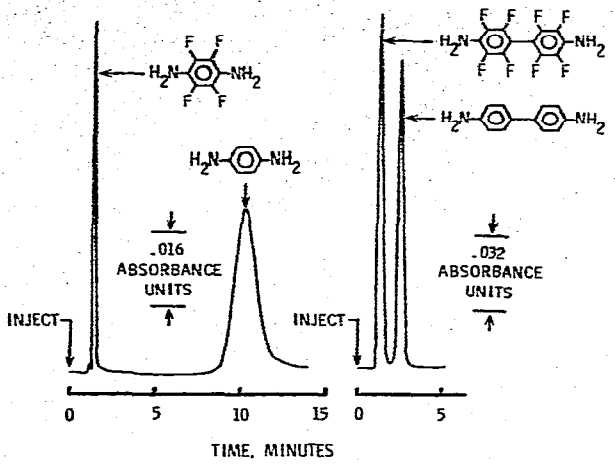
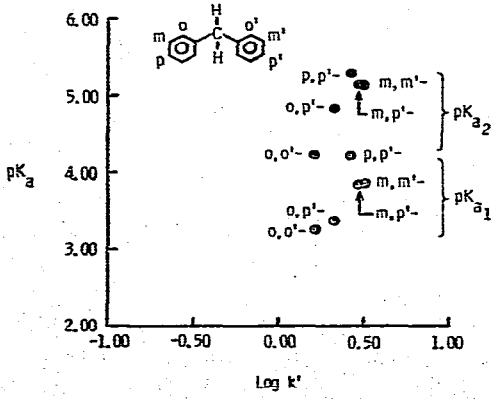


Fig. 3. Chromatogram of fluorinated and unfluorinated aromatic diamines. Conditions: same as in Fig. 1 except for the flow-rate being 1.0 ml/min (500 p.s.i.).

In an earlier phase of this research, a linear correlation between pK_b and log k' was established for four series of simple aromatic amine isomers[9]. These amines were the *o*-, *m*-, and *p*-isomers of bromoaniline, chloroaniline, toluidine, and phenylenediamine and were chromatographed on various silica gels using chloroform and/or cyclohexane mobile phases. For these four series of simple amines, the amino portion of the molecule apparently dominated the adsorption process and, thus, the proposed mechanism was in operation. However, this relationship may not be so straightforward for complex amines. Fig. 4 gives a plot of pK_a *versus* log k' for a series of methylenedianiline isomers. A single straight line connecting all points would indicate separation only by the proposed mechanism. Such a line could not be drawn with any confidence. Fig. 5 shows similar data for a series of diaminobenzophenone isomers.



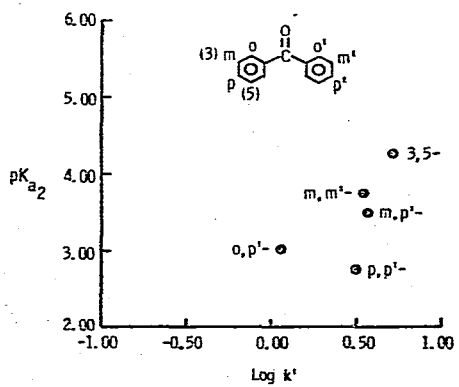


Fig. 4. Plot of pK_a *versus* log k' for methylenedianiline isomers. Conditions: same as in Fig. 1 except for the flow-rate being 0.5 ml/min (325 p.s.i.).

Fig. 5. Plot of pK_a versus log k' for diaminobenzophenone isomers. Conditions: same as in Fig. 1 except for the flow-rate being 1.0 ml/min (500 p.s.i.).

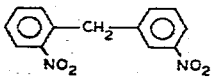
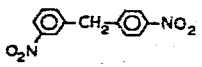
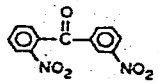
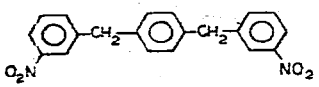
No meaningful correlation could be made between these points. Perhaps this behavior is an indication that other parts of the molecule, such as the methylene and carbonyl groups, are entering into the adsorption process.

Although linear correlations between basicity and chromatographic retention will not always exist for more complex amines, the separation of most aromatic amines is expected to reflect acid–base interactions to some extent. Thus, relative retention times are expected to correspond to relative basicities for many series of similar amines. This concept is proving especially useful in predicting the reactivities of isomeric diamines in Table II to form various polymer systems.

The conditions for the separation of four relevant aromatic dinitro compounds are given in Table III. An analysis at this stage of diamine synthesis was considered to be vital since any impurity present in the dinitro compound would be carried over with its reduced product. Thus, amine purification could often be simplified by determining the purity of the precursor dinitro species. Satisfactory separations were usually obtained by dissolving these compounds in chloroform and then injecting the solution into low-polarity mobile phases.

TABLE III

CONDITIONS FOR SEPARATION OF AROMATIC DINITRO COMPOUNDS

Structure	*Column*	*Mobile phase*	*Flow-rate (ml/min) [p.s.i.]*	*k'*
	Zorbax-SIL	cyclohexane–chloroform–methanol (97.8:1.5:0.7)	0.7 [3800]	2.03
				3.63
	Zorbax-SIL	cyclohexane–chloroform–methanol (82:17.3:0.7)	0.6 [2500]	2.84
	Zorbax-SIL	cyclohexane–chloroform–methanol (90:9.3:0.7)	0.5 [2000]	13.78

CONCLUSIONS

This study established the chromatographic conditions for separating approximately fifty aromatic monoamines and diamines. These compounds were separated on various silica gels using chloroform and/or cyclohexane mobile phases. Adsorbent surface area and mobile phase polarity were varied to optimize each separation. The primary separation mechanism for the amines depended upon an interaction between the amino group and the slightly acidic adsorbent and in most cases related to the basicity of the amines. The general conditions developed here should apply to the separation of a wide variety of aromatic amines as long as the amino group plays a major role in the adsorption process.

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