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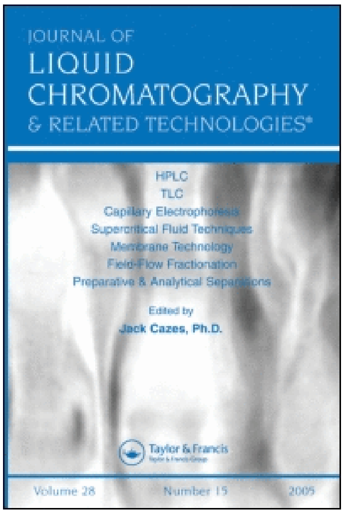

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SIMULTANEOUS DETERMINATION OF AROMATIC ISOCYANATES AND SOME CARCINOGENIC AMINES IN THE WORK ATMOSPHERE BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A reversed-phase high-performance liquid chromatographic method with UV-detection is described for the simultaneous determination of aromatic isocyanates and some carcinogenic aromatic amines which may be present together in the work atmosphere of the polyurethane industry. The air is sampled through ethanol which is made alkaline by potassium hydroxide (KOH). The isocyanates react instantaneously to the corresponding ethyl urethanes, while the amines remain in nonionized state. KOH, which has been added to catalyze the ethyl urethane reaction and eliminate the side reactions, is precipitated out with hydrochloric acid and the sample solution is evaporated to dryness. The residue is dissolved in 1 ml ethanol and water (1:1). A 50-ul aliquot of the resulting solution is chromatographed on a Rad Pak C_{18} column and eluted isocratically with a mixture of tetrahydrofuran, acetonitrile and water buffered with acetate to some exact value in the pH range 5.5-7.0. The use of this pH range is favored both by the retention behaviour and UV-detectability of the aromatic amines.

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INTRODUCTION

Isocyanates are extensively used in the manufacture of polyurethane products. Airborne isocyanates are apt to cause chronic impairment of pulmonary functions and occupational asthma (1). Other hazardous chemicals may also be present in workplace air that contains isocyanates. In the manufacture of certain cross-linked polyurethane elastomers toluene diisocyanate (TDI) is mixed with 3,3′-dichloro-4,4′-diaminodiphenylmethane (MOCA), which is a carcinogenic agent (2). Under some conditions TDI and diphenylmethane 4,4′-diisocyanate (MDI) may hydrolyze to the carcinogenic diamines: 2,4-diaminotoluene (2,4-TDA) and 4,4′-diaminodiphenylmethane (MDA) (2).

Several highly sensitive and selective liquid chromatographic methods have recently been published for the determination of airborne isocyanates. Most of them are based on the reaction of the isocyanate group with a secondary amine that has an aromatic moiety to yield a urea derivative with strong UV-absorbance (3,4,5,6). One published method (7) uses ethanol as reagent to determine aromatic isocyanates as ethyl urethane derivatives; in this case normal phase HPLC is used.

Starting from this reaction with ethanol, we have developed a reversed phase HPLC method for simultaneous determination of TDI and MDI as ethyl urethane derivatives and of carcinogenic amines (MOCA, 2,4-TDA and MDA). Reversed phase HPLC is more convenient to use and more suitable for the amines than normal phase HPLC (7).

Because the formation of the ethyl urethane in pure ethanol takes place rather slowly, it tends to be disturbed by certain compounds occurring in the work environment together with isocyanates. An attempt was therefore made to find a suitable catalyst for the ethyl urethane reaction. The best accelerator of the reaction rate proved to be potassium hydroxide (KOH). When ethanol was made alkaline with potassium hydroxide, the reaction between the isocyanate and ethanol took place immediately and the effects of intefering substances were eliminated.

The method is suitable for other aromatic isocyanates besides TDI and MDI. It has been successfully tested for phenyl isocyanate, triphenylmethane 4,4′,4′′- triisocyanate (Desmodur R) and dimer of 2,4-toluene diisocyanate (Desmodur TT).

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EXPERIMENTAL

Chromatographic apparatus

The liquid chromatograph consisted of an Altex model 110A solvent metering pump, a Rheodyne model 7120 injector with 50 ul loop, a Radial Compression module for Radial PAK columns (Waters Associates, Milford, MA, U.S.A.) and a variable Wavelength Pye Unicam LC-UV-detector with 8 ul cell. Radial PAK C_{18} column with particle diameter 10 um and I.D. 8 mm (Waters Ass.) was used.

Chromatographic conditions

A mixture of acetonitrile, tetrahydrofuran and water (3:3:4) was used as eluent. For the determination of aromatic amines it was buffered to pH^* 5.5-6.0 with acetate. (pH^*= apparent pH value measured with glass electrode)

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Chemicals

Acetonitrile and tetrahydrofuran of HPLC grade were obtained from Rathburn Chemicals (Walkerburn, Peedleshire, Scotland). Water was doubly distillated. Acetic acid, sodium acetate and phosphoric acid were purchased from E. Merck (Darmstadt, G.F.R.). Potassium biphosphate was purchased from May & Baker Ltd, (Dagenham., England). Toluene diisocyanate (TDI), a mixture of 80 % 2,4 TDI and 20 % 2,6 TDI, was purchased from Fluka AG, (Buchs, Switzerland) and Diphenylmethane-4,4′-diisocyanate (MDI) from ICN Pharmaceuticals, (Plainview, N. Y., U.S.A.). 4,4′-Diaminodiphenylmethane (MDA) and 2,4-diaminotoluene (TDA) were purchased from Fluka AG. Dimer of 2,4-toluene-diisocyanate (Desmodur TT) and triphenylmethane 4,4′,4"-triisocyanate (Desmodur R) were obtained from Bayer AG (Leverkusen, G.F.R.). Ethanol 99,5 % was obtained from Alko Oy (Rajamäki, Finland) and potassium acetate from J.T. Baker (Gross Gerau, G.F.R.). Dibutyltin dilaurate was a product from Merck-Schuchardt. Methylene chloride and potassium hydroxide were products from E. Merck. DABCO (diethylenetriamine), Desmorapid PP (a mixture of organic amines), TEA (triethylamine) and 3,3′-dichloro-4,4′-diaminodiphenylmethane (MOCA) were obtained from polyurethane manufacturing plants. The indicator paper was Merck Universal Indikator Papier.

Preparation of the absorption solution

About 2 g of potassium hydroxide was dissolved in 1 l 99,5 % ethanol.

Preparation of the standard solutions

Stock solutions of the aromatic isocyanates and amines were prepared in dry methylene chloride: about 10 mg of the pure isocyanate or amine was dissolved in 50 ml methylene chloride. A suitable aliquot of the solution was added to 20 ml of the absorption solution where the isocyanates immediately reacted to form the corresponding ethyl urethanes. The amines remained in nonionized state. Hydrochloric acid (37 %) was carefully added to the standard solution until indicator paper showed the apparent pH of the solution to be between 3 and 5. (At lower pH the amines, especially MOCA which is very sensitive, may be precipitated.) KCl-precipitate was separated out by filtration or centrifucation and washed with ethanol. The clear standard solution together with the washing solution was evaporated to dryness at 35°C under vacuum. The residue was dissolved in 1 ml of ethanol:water (1:1) or 1 ml eluent, and 20-50 ul of the solution was injected into the chromatograph.

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Air sampling and sample preparation

The air was sampled through an impinger bottle filled with 20 ml absorption solution at a rate of 1 to 2 l/min. Sampling time was 15-30 min. To investigate the collection efficiency, sampling was done through two impingers connected in series each filled with 20 ml of absorption solution. Since no isocyanates or amines were detected in the second impinger at a flow rate of 1 or 2 l per min, the single impinger can reliably used to collect the air samples. The sample solutions were handled as described above for the sandard solutions.

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RESULTS

Experimental conditions affecting the ethylurethane reaction.

Certain organic amines (TEA, DABCO and Desmorapid PP) used as catalysts in the polyurethane industry were found to interfere with the formation of ethyl urethanes. TEA (triethylamine) and Desmorapid PP (a mixture of organic amines) are used as catalysts in the production of MDI-based polyurethanes and DABCO (diethylenetriamine) is a catalyst used in the production of TDI-based polyurethanes.

When MDI or TDI (10 ug) was added to ethanol solution (20 ml) containing these amines up to the concentration of 50 ug/ml, the HPLC-ethyl urethane peaks obtained from the solutions were smaller than those obtained from pure ethanol.

The MDI ethyl urethane peak obtained from ethanol containing TEA was reduced by 30 %, and when Desmorapid PP was present in ethanol no MDI ethyl urethane peak was found at all. The TDI ethyl urethane peak obtained from ethanol containing DABCO was reduced by 70 %.

Since both TDI- and MDI ethyl urethanes are formed rather slowly (FIG. 1), there obviously had been enough time for interfering reactions to occur. A suitable catalyst for the ethyl urethane reaction was therefore sought.

Of all the compounds tested the best accelerator of the reaction rate proved to be a simple base: potassium hydroxide (KOH). The TDI and MDI ethyl urethanes formed immediately in ethanol made alkaline with KOH (FIG. 1). The isocyanate ethyl urethanes formed quantitatively even

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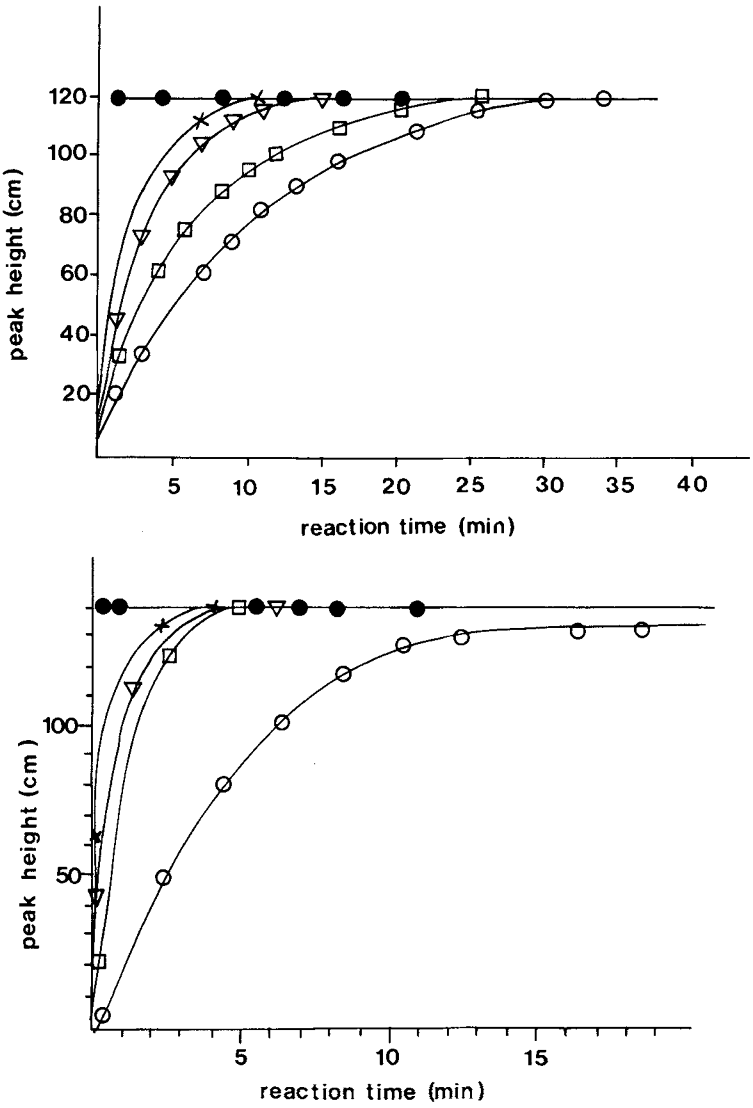

FIGURE 1

The reaction rate of TDI ethyl urethane (A) and MDI ethyl urethane (B). The HPLC-peak height versus reaction time of TDI (A) and MDI (B) in ethanol

○ in the absence of catalyst
□ in the presence of 0.01 % dibutyltin dilaurate
▽ in the presence of 0.1 % dibutyltin dilaurate
X in the presence of 0.1 % potassium acetate
● in the presence of 0.02 % potassium hydroxide

in the presence of TEA, DABCO and Desmorapid PP. Dibutyltin dilaurate, a catalyst commonly used in urethane technology, and potassium acetate also had satisfactory good catalyzing effects, (FIG. 1) but they did not counteract the interfering reactions.

Even a small amount of KOH in ethanol is enough to catalyze the ethyl urethane reaction, but when air samples are taken it is necessary to add considerable more KOH to the ethanol since atmospheric carbon dioxide precipitates some of the KOH to potassium carbonate.

The aromatic amines 2,4-TDA, MDA and MOCA did not interfere with the formation of ethyl urethanes. This was proved experimentally by adding TDI or MDI to ethanol that contained these aromatic amines in amounts up to 5 equivalents of the isocyanate added. The reaction rates of TDI and MDI followed the same curve as their reaction rates in pure ethanol when the amines were not present. In ethanol with KOH the ethyl urethanes formed immediately.

Selection of the RPLC conditions

Acetonitrile, tetrahydrofuran and water (3:3:4) as mobile phase gave good isocratic separation and short elution times for the isocyanates (FIG. 2). The tetrahydrofuran (THF) was found to improve the shapes of the peaks when Rad PAK C_{18} columns were used. In later experiments with another commercial column (ODS-Hypersil) THF had no apparent effect on the shapes of the peaks, and mere acetonitrile and water was used as eluent.

For the determination of the amines (2,4-TDA, MDA and MOCA) the mobile phase was buffered with acetate.

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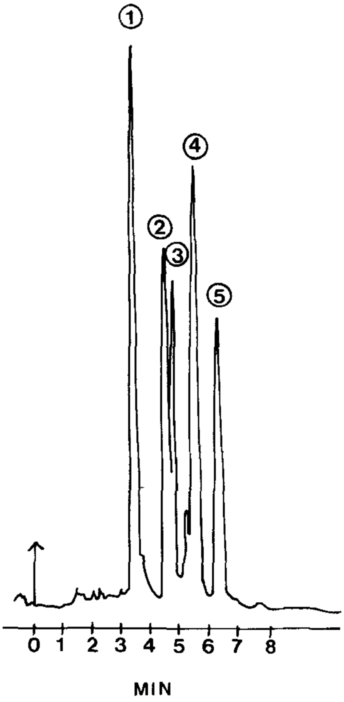

FIGURE 2

Chromatogram of the ethyl urethane derivatives of the aromatic isocyanates: 1. Phenyl isocyanate, 2. Toluene diisocyanate (TDI), 3. Dimer of 2,4-TDI (Desmodur TT), 4. Diphenylmethane 4,4′-diisocyanate (MDI), 5. Triphenylmethane 4,4′,4″-triisocyanate (Desmodur R.) Conditions: Rad PAK C_{18} column; tetrahydrofuran, acetonitrile and water (3:3:4) as mobile phase; flow rate 1 ml/min; sample volume 50 ul; detection at 245 nm and 0.04 AUFS.

The retentions of MDA and TDA increased drastically when the pH decreased (FIG. 3). This phenomenon, which is in contradiction to the ion suppression theory (8), is possibly caused by the ability of acetate to form ion pairs.

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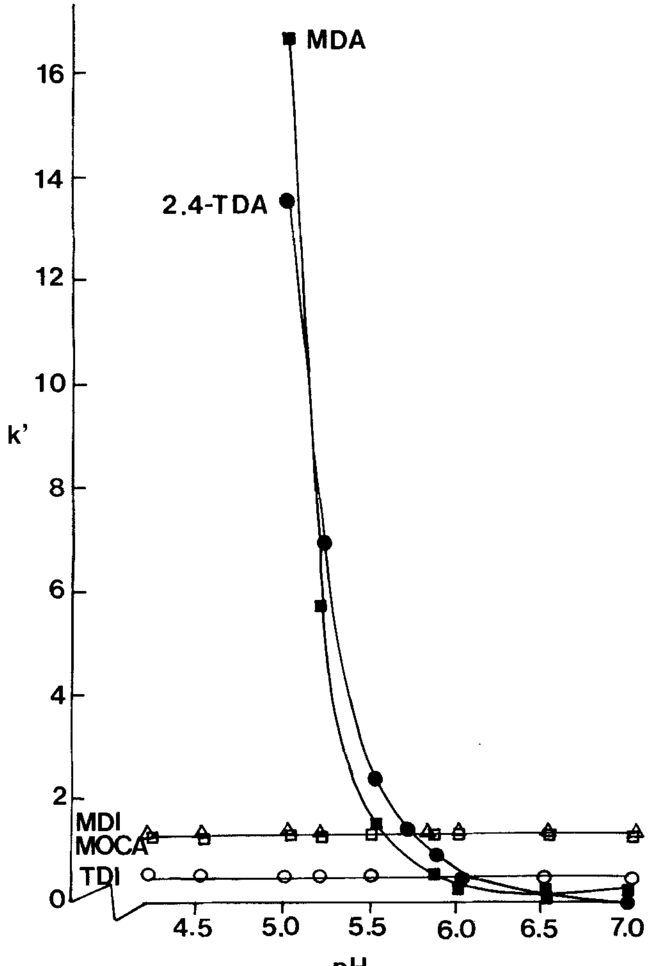

FIGURE 3

Plot of the capacity factor (k') versus pH* of the eluent: tetrahydrofuran, acetonitrile and water (3:3:4) buffered with 0.005 M acetate.

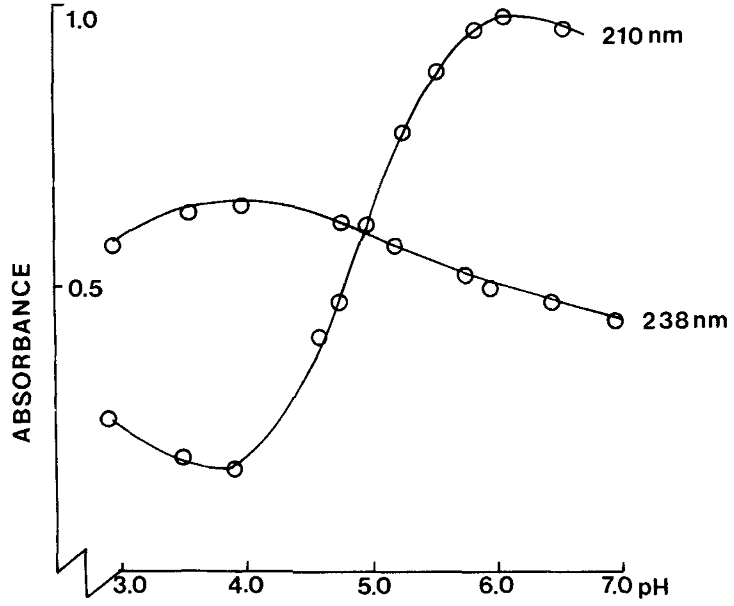

FIGURE 4

*The UV absorbance of 2,4-TDA versus pH*of the eluent.*

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A phosphate buffer was also tested for the pH control of the eluent. No significant changes in retention times were obtained in the pH*range 4.5-7.0.
Below pH*4.5 a slight reduction was found in the retention of both 2,4-TDA and MDA. 2,4-TDA and MDA behaved according to the mechanism of ion suppression proposed for weak bases (8), but for the regulation of the retention times the phosphate buffer was not suitable.

The pH had a clear effect on the intensity of the UV-absorbances of 2,4-TDA and MDA (FIGS 4 and 5), but no effect on the UV absorbances of MOCA, TDI ethyl urethane or MDI ethyl urethane. The UV-absorbances of 2,4-TDA and

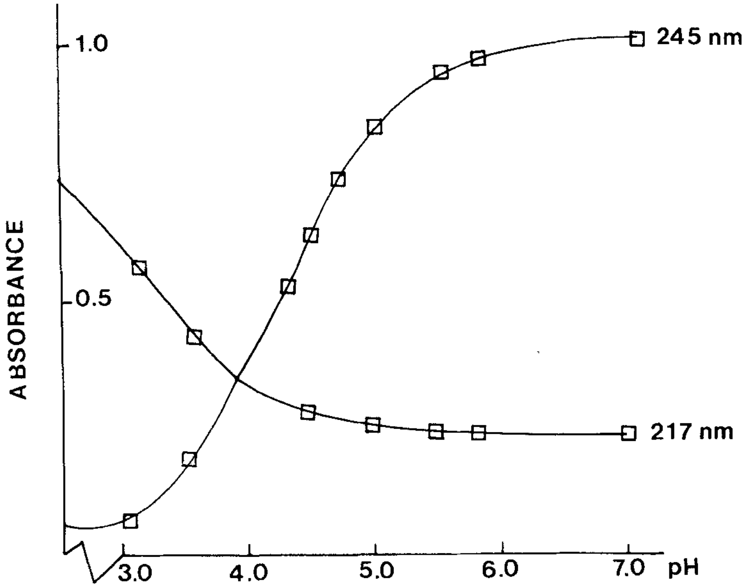

FIGURE 5

The UV absorbance of MDA versus pH*of the eluent.

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MDA at 245 nm (a wavelength sensitive enough to determine all the present substances) is shown as a function of the pH*in FIG. 7. The maximum absorbance for MDA is found between pH*5.5 and 7.0, while the absorbance of 2,4-TDA is 75 % of its maximum in this pH range.

The maximum UV absorbance of 2,4-TDA and TDI ethyl urethanes was found at 210 nm. When greater sensitivity was required the RPLC runs were done near this wavelength. It was possible to use the wavelength 220 nm when THF, acetonitrile and water with acetate buffer was used as the eluent.

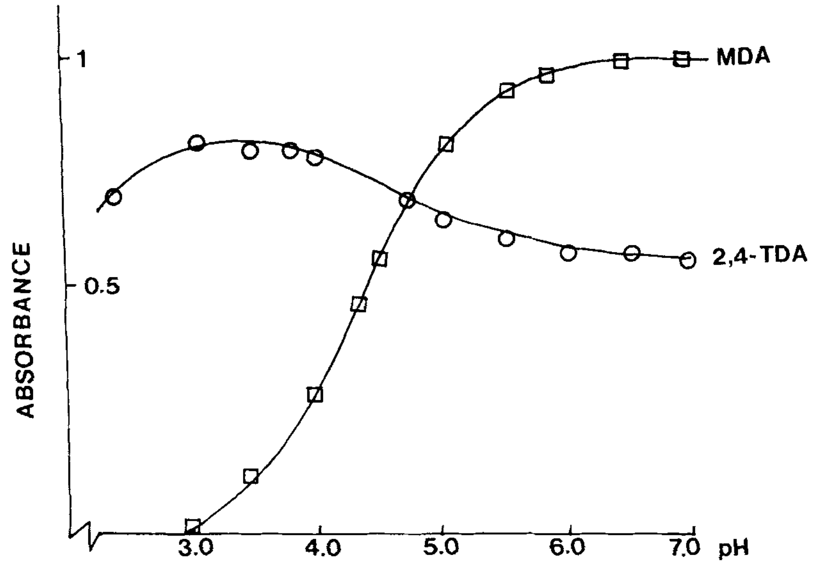

FIGURE 6

The UV absorbances of 2,4-TDA and MDA as function of pH* of the eluent at 245 nm.

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The use of acetate buffered eluent in the pH* range 5.5-7.0 is favored both by the retention behavior and UV-detectability of these aromatic amines. By selecting a suitable pH value in this pH range the elution sequence of the compounds can also be changed (FIG. 7).

Detection limits and molar absorption coefficients

The detection limits of the method at 245 nm, are on the order of about 1 x 10^{-3} - 5 x 10^{-3} mg/m^3 based on 10 l air samples (1 x 10^{-4} - 10 x 10^{-4} ppm at 25°C and 760 mmHg). The exact detection limits of the

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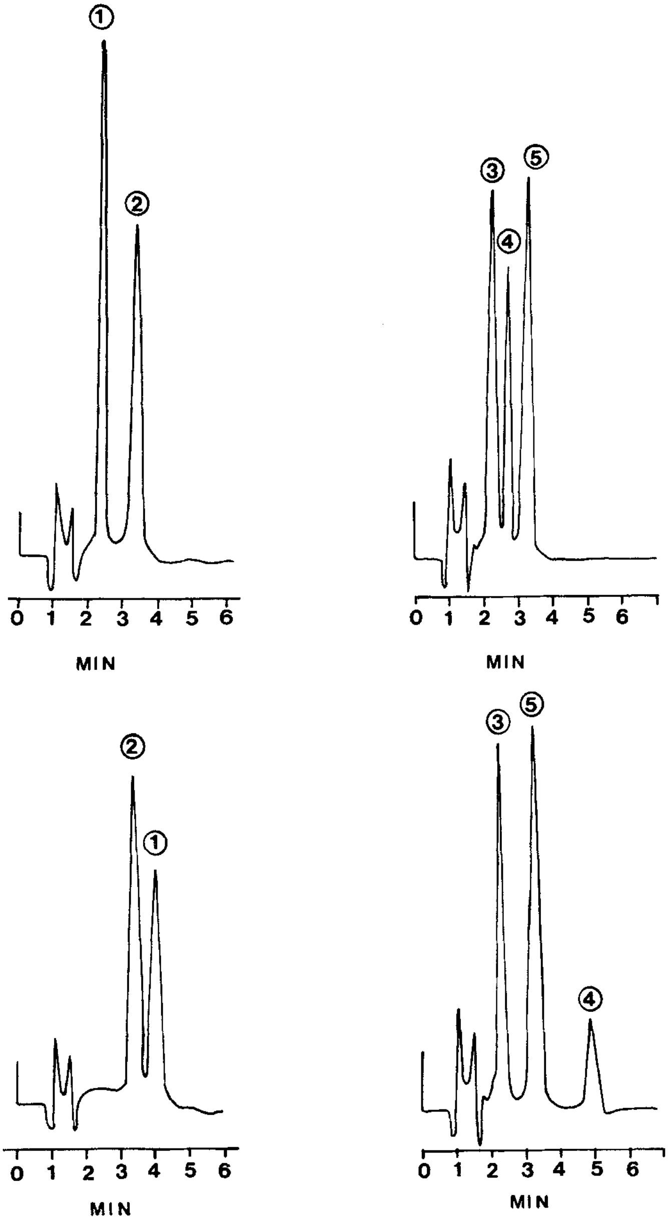

FIGURE 7

The effect of pH* of the eluent on the chromatograms of 1. MDA 2. MDI ethyl urethane 3. TDI ethyl urethane 4. TDA and 5. MOCA.
Eluent: Tetrahydrofuran, acetonitrile and water (3:3:4) buffered with 0.005 M acetate to pH* 5.85 (A) and pH 5.50 (B).

TABLE

The Molar Absorption Coefficients of MDI and TDI ethyl urethanes and Amines at pH* 6.0 :

The compound:	245 nm	220 nm
MDI-ethyl urethane	47.1 x 10^3	28.6 x 10^3
MDA	16.9 x 10^3	-
TDI-ethyl urethane	26.7 x 10^3	36.7 x 10^3
TDA	5.0 x 10^3	11.0 x 10^3
MOCA	13.9 x 10^3	-

amines depend on the pH of the eluent. The molar absorption coefficients are given in the Table.

DISCUSSION

The advantage of this method is its versatility. Many of the most hazardous chemicals present in the occupational environment where polyurethanes are manufactured can be determined simultaneously from the same sample. Most of the previously published HPLC methods for isocyanates emphasize the separation of different isocyanates from each other. Seldom, however, are there more than one or two different isocyanates at the same workplace. The aromatic isocyanates are widely used, while aliphatic isocyanates are usually used only for special purposes. The ethyl urethane derivative is highly stable, and standards and samples can be stored for long periods without deterioration. Ethanol is a readily procurable reagent and since it does not absorb in the ultraviolet region it is superior to amine-based reagents.

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The addition of KOH as catalyst assures that the reaction takes place so rapidly that disturbing side reactions are avoided.

The acetate buffer was found easy to use for regulating the retention of the amines, though the mechanism of its behavior is not yet fully understood. The unreacted silanol groups in the column can adsorb protonated amino groups, an effect which is minimized by phosphate ions (9). Consistent with this the retention times of 2,4-TDA and MDA showed a pH-dependent increase in acetate buffers below pH^* 7.0, whereas the retention times in phosphate buffers were practically constant from pH^* 2.0 to pH 7.0. However, when a mixture of acetate and phosphate buffer was used, both 2,4-TDA and MDA showed the same pH-dependent increase in retention times as in acetate buffer alone. This finding supports the assumption that the pH-dependent retention behavior of aromatic amines in acetate buffer is explained by the ion pair mechanism.

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