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Note

Analysis of urea compounds by high-performance liquid chromatography

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During the course of an investigation on structure–property relationships of polyurethanes, it became desirable to have methods for the determination of free monomeric diisocyanates, low-molecular-weight carbamates and low-molecular-weight ureas because they affect the sequential distribution of chains and the reaction rates of the alcohol and the amine in polyurethane-forming reactions. In previous papers we reported quantitative analyses of several carbamates derived from phenyl isocyanate, 2,4-toluenediisocyanate, and 4,4′-diphenylmethane diisocyanate, and those of free toluene diisocyanate in polyurethane prepolymers by high-performance liquid chromatography (HPLC)[1,2].

In this work, the analysis of urea compounds has been investigated in order to obtain further information to provide a basis for the characterization of polyurethane networks.

EXPERIMENTAL

Urea compounds

Urea compounds were prepared from isocyanates and amines. The urea compounds were recrystallized three times and their purities were checked by elemental analysis, melting point determinations and IR spectroscopy. Table I shows the structures of the urea compounds used and their abbreviations.

Apparatus

A Hitachi Model 634 high-performance liquid chromatograph (Hitachi, Tokyo, Japan) with a 254-nm UV detector and an RI detector was used. A 500 × 2.1 mm I.D. stainless-steel column was packed with Hitachi Gel 3010 (styrene–divinylbenzene porous polymer). The packing was swollen in methanol and packed by a high-pressure slurry technique.

Procedure

The urea compounds were dissolved in dimethylacetamide at concentrations ranging from $1 \cdot 10^{-3}$ to $2.5 \cdot 10^{-1}$ mol/l. A 3.0-μl volume of the solution was injected into the column via a septum by means of a microsyringe. Methanol and mixtures of methanol and water were used as the mobile phase at a flow-rate of 1.0 ml/min at 21°C. The measurement was repeated five times for each sample solution.

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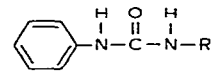
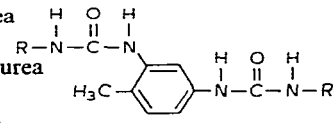
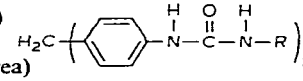
TABLE I
UREA COMPOUNDS STUDIED

Compound	*Structure*	*R*	*Abbreviation*
1-Phenyl-3-propylurea		$-CH_2CH_2CH_3$	PnPU
1-Phenyl-3-isopropylurea		$-CH(CH_3)_2$	PiPU
1-Phenyl-3-butylurea		$-CH_2CH_2CH_2CH_3$	PnBU
1-Phenyl-3-isobutylurea		$-CH_2CH(CH_3)_2$	PiBU
Dipropyl-4-methyl-*m*-phenylenediurea		$-CH_2CH_2CH_3$	TnPU
Diisopropyl-4-methyl-*m*-phenylenediurea		$-CH(CH_3)_2$	TiPU
Dibutyl-4-methyl-*m*-phenylenediurea		$-CH_2CH_2CH_2CH_3$	TnBU
Dipropyl-4,4′-methylenedi(phenylurea)		$-CH_2CH_2CH_3$	MnPU
Diisopropyl-4,4′-methylenedi(phenylurea)		$-CH(CH_3)_2$	MiPU
Dibutyl-4,4′-methylenedi(phenylurea)		$-CH_2CH_2CH_2CH_3$	MnBU

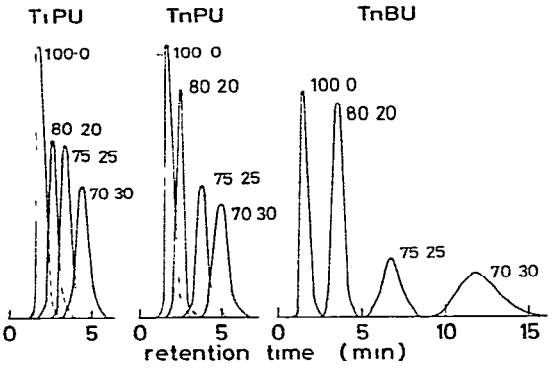
Peak heights and peak areas of the urea compounds in the chromatograms were determined manually. Calibration graphs were prepared by plotting peak height and peak area against urea concentration.

RESULTS AND DISCUSSION

The column packing was a porous styrene–divinylbenzene copolymer, which is used for adsorption chromatography with methanol and *n*-hexane mobile phases and has a good resolution for the separation of aromatic compounds[3]. However, methanol was inadequate as a mobile phase for analysing the phenylurea series in this study. Although the retention times of the phenylurea series increased in the order PiPU < PiBU < PnPU < PnBU, the chromatogram of a mixture of the compounds showed one peak with a retention time of 2 min, indicating that these compounds were not separated under the conditions used. In previous work[1], low-molecular-weight carbamates were separated satisfactorily with methanol–water (80:20) as the mobile phase. Therefore, the addition of water to methanol as the mobile phase was examined in this work.

Fig. 1 shows the effect of the addition of water to methanol as the mobile phase on chromatogram of 4-methyl-*m*-phenylenediureas. With increasing amount of water, the retention time and peak width increased and the peak height decreased. The effect of water on the retention time of TnBU was greater than on those of TiPU and TnPU.

The influence of the water content on the chromatogram of PnPU was similar to that of TnPU, whereas it had a greater effect on the retention time and peak width of MnPU. The decrease in peak height and increase in peak width are attributed to



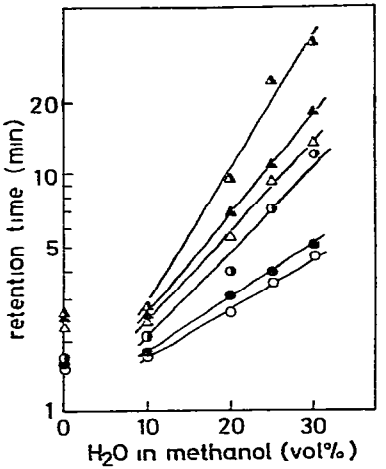


Fig 1 Effect of water in methanol as the mobile phase on the chromatogram of 4-methyl-*m*-phenylenediureas Column, 500 × 2.1 mm I D ; Hitachi Gel 3010; detector, UV, 0 64 AUFS; flow-rate, 1.0 ml/min. Mobile phase methanol–water, 100.0, 80 20, 75·25 and 70·30.

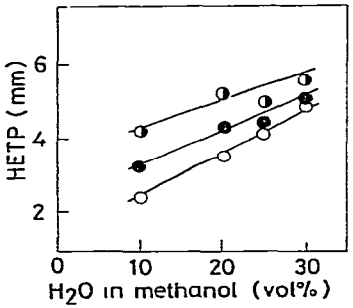
Fig 2. Relationship between logarithm of retention time of 4-methyl-*m*-phenylenediureas and 4,4′-methylenediphenylureas and the concentration of water in methanol as the mobile phase ○, TiPU, ●, TnPU; ◑, TnBU; △, MiPU; ▲, MnPU; ◭, MnBU

the decrease in the solubility of the solute in a mobile phase with increasing amounts of water.

Fig. 2 shows a graph of the logarithm of the retention time of 4-methyl-*m*-phenylenediureas and 4,4′-methylenediphenylureas against the concentration of water in methanol. There is linear relationship. For both series of ureas, isopropylurea was eluted first, then *n*-propylurea and *n*-butylurea last. This behaviour is similar to that of reversed-phase partition chromatography using octadodecylsilane as a packing[4].

Figs 3 and 4 show the effect of water in methanol as the mobile phase on the column efficiency (HETP) and peak resolution (R_s), respectively. The column efficiency decreased with increasing water content and molecular weight of the ureas. In contrast, the resolution improved considerably with increasing water content. These phenomena are attributable to the increase in the resistance to mass transfer in the mobile phase due to the increase in the viscosity resulting from addition of water.

The chromatographic behaviour of the styrene–divinylbenzene porous polymer used as a packing may be interpreted as follows. As polystyrene gel is a non-polar or only slightly polar packing, adsorption phenomena arise from Van der Waal's force interactions. When the mobile phase is a polar solvent, the interaction between the solute and the surface of the stationary phase is strong and elution of the solute is difficult. When the mobile phase is non-polar or weakly polar, the affinity between the stationary phase and the mobile phase is strong and elution of the solute is rapid. In this study, with a mixture of methanol and water as the mobile phase, the methanol, which is less polar than water, is adsorbed by the surface of the polystyrene gel packing, making the surface rich in methanol. As urea compounds are more soluble in methanol than in water, the affinity of ureas for a stationary phase rich in methanol increases with increasing amount of water in the methanol, which increases the reten-



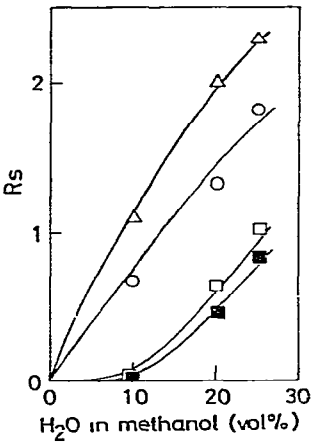


Fig 3. Effect of water in methanol as the mobile phase on column efficiency (HETP) ○, TiPU; ●, TnPU, ◑, TnBU.

Fig. 4. Effect of water in methanol as the mobile phase on peak resolution (R_s) ■, PiBU–PnBU, □, PnPU–PnBU; ○, TnPU–TnBU; △, MnPU–MnBU

tion times of the ureas. On the other hand, the viscosity of methanol and the resistance to mass transfer in the column vary on addition of water, and these variations may affect the column efficiency.

The results suggested that urea compounds could be separated by HPLC using the styrene porous polymer and methanol–water as the mobile phase, and quantitative analyses were carried out with methanol–water (75:25) as the mobile phase. Linear relationships were obtained between the peak intensity (peak height and peak area) and the concentration of urea compounds up to $2.5 \cdot 10^{-2}$ mol/l. The slopes and retention times are given in Table II. Fig. 5 shows the separation of several urea compounds under these conditions. For each plot, amounts of the ureas injected in 3-μl volumes gave points lying within $\pm 0.5\%$ of the calibration line. The detection limits of phenylureas, 4-methyl-*m*-phenylenediureas and 4,4′-methylenediphenylureas were $2.4 \cdot 10^{-3}$, $1.7 \cdot 10^{-3}$ and $1.3 \cdot 10^{-3}$ mol/l, respectively. These detection limits

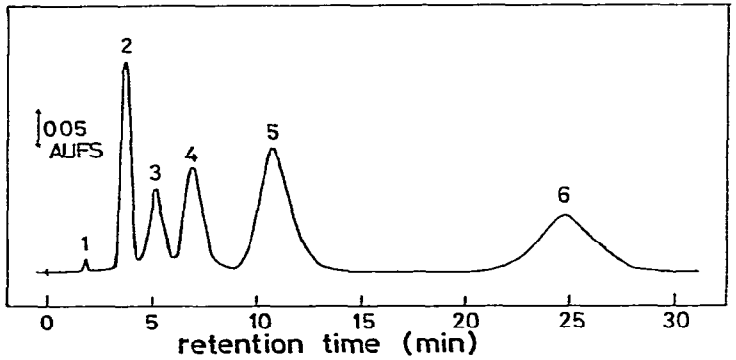


Fig 5 Separation of urea compounds Column 500 × 2 1 mm I D ; Hitachi Gel 3010; mobile phase, methanol–water (75 25); flow-rate, 1.0 ml/min; detector, UV, 0 64 AUFS. Peaks 1 = DMA; 2 = TnPU ($7.1\ 10^{-3}$ mol/l); 3 = PiBU (0.10 mol/l); 4 = TnBU ($6\,6\ 10^{-3}$ mol/l); 5 = MnPU ($4\,0\ 10^{-3}$ mol/l); 6 = MnBU ($6\,6\ 10^{-3}$ mol/l).

TABLE II
SLOPES OF CALIBRATION GRAPHS ($y=ax$) AND RETENTION TIMES FOR UREA COMPOUNDS

Urea	*Retention time**	A_{height}**	A_{area}***
PnPU	4 min 9 sec	5457	425
PiPU	3 min 54 sec	6053	464
PnBU	5 min 42 sec	4431	497
PiBU	5 min 16 sec	4463	459
TnPU	3 min 48 sec	15681	1260
TiPU	3 min 20 sec	16418	1218
TnBU	7 min 3 sec	8555	1252
MnPU	10 min 42 sec	15504	3675
MiPU	9 min 20 sec	18826	4005
MnBU	24 min 53 sec	7306	3873

* Mobile phase, methanol–water (75:25); flow-rate, 1.0 ml/l; detector, UV, 0.64 AUFS.
** Caclulated by peak-height method (mm l/mol).
*** Calculated by peak-area method (cm^2 l/mol).

may be improved by injecting a greater volume of sample solution into the HPLC system and operating at a high sensitivity of the UV detector. To confirm the resolution, mixtures of samples, the concentrations of the components of which had been pre-determined by the weight method, were analysed. The deviations of the concentrations determined from the calibration lines were $\pm 1.7\%$ for phenylureas, $\pm 1.0\%$ for 4-methyl-*m*-phenylenediureas and $\pm 0.7\%$ for 4,4′-methylenediphenylureas using the peak-height method, and $\pm 1.0\%$, $\pm 1.4\%$ and $\pm 1.0\%$, respectively, using the peak-area method.

In conclusion, separation and quantitative analysis of the ureas could be possible by means of HPLC with a styrene–divinylbenzene copolymer and methanol–water (75:25) as the mobile phase. It is suggested that the proposed method involves a process similar to reversed-phase chromatography. The column efficiency (HETP) decreased and the resolution improved on increasing the concentration of water in the mobile phase.

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