RETENTION OF SOME HETEROCYCLIC AMINES ON MIXED STATIONARY PHASES CONTAINING NICKEL(II) SCHIFF BASE CHELATES

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Stationary phases composed of squalane and some nickel(II)-β-keto amine complexes were prepared and used for the separation of complex mixtures of pyridines. The resolution achieved on short classical columns was comparable with that obtained on capillary columns.

Pyridine bases are present in coal tar and tobacco smoke. They are also responsible for the smell and taste of milk products[1]. Trace amounts of these compounds can only be determined using highly efficient separation methods[2,3].

Complex mixtures of heterocyclic amines were resolved by gas-liquid chromatography using largely two-component stationary phases[3,4]. Our gas chromatographic study[5–8] warranted the assumption that separation of heterocyclic amines with boiling points approaching each other closely may be improved by engaging complexation phenomena in the gas-liquid chromatographic treatment, viz. by using nonpolar liquid phases containing coordinatively unsaturated nickel(II) chelates. Formation of reversible adducts on mixed stationary phases of this kind has been proved for aliphatic alcohols, amines, ketones and esters[5–7]. The optimum mass fraction of nickel(II) Schiff base chelate in the stationary phase was found to be 0·03–0·08. According to Conder[9], the role of interfacial adsorption in the GC column increases considerably as the solute concentration approaches zero. In view of the small volume of data available concerning the effect of interfacial sorption on the gas chromatographic retention of pyridines[10,11], we devoted our study to such interactions occurring during their retention on stationary phases containing nickel(II) chelates.

The net retention volume of solute V_N can be related to the partition between the gas-liquid phases and to the adsorption at the gas-liquid interface according to Martin's equation[12]

$$V_N = K_L V_L + K_A A_L , \qquad (1)$$

where V_L and A_L are the volume of the stationary phase and the carrier gas-stationary

liquid interface area per gramme of solid support, respectively, and K_L and K_A are the partition and adsorption coefficients of the solute at the gas-liquid interface, respectively.

EXPERIMENTAL

Pyridine, picolines and lutidines (Merck, Darmstadt, F.R.G.) in 0·1% benzene or cyclohexane solutions served as the solutes. Solid nickel(II) tetradentate Schiff base chelates (Table I) were synthesized, purified and characterized following methods described by Holm[13]. The particle size distribution of the solid chelates was examined on a polarizing microscope (Carl Zeiss, Oberkochen, F.R.G.) using liquid paraffin A as the optical solvent and cellulose triacetate as the fiber. The particle diameters lay in the regions of 0·5—2·0 μm (90%), 2·1—5·0 μm (8%) and 5·1—8·0 μm (2%).

Gas chromatographic measurements with packed columns were conducted on a Model 504 gas chromatograph (MERA-ELWRO, Wrocław, Poland) equipped with a flame ionization detector and a Model 5503 digital integrator (KABIDEZ, Warsaw, Poland). The column, injection port and detector temperatures were 100, 200 and 200°C, respectively. Helium was used as the carrier gas at a flow rate of 55 ml min^{-1}. The solute mixture was injected in the form of rarefied vapours using a 10 μl gas-tight syringe (Hamilton, Bonaduz, Switzerland).

Glass and, occasionally, stainless steel columns (1·3 m × 0·4 cm i.d.) were used. The column packings were Chromosorb P DMCS (80/100 mesh) (John Manville, Denver, U.S.A.) and Polsorb C (0·15—0·20 mm) (ZOCh, Lublin, Poland). The packing was coated with the stationary phase by the following procedure. 1·5 g (corresponding to 5% loading) of squalane, $d = 0{\cdot}81$ g cm^{-3} at 20°C (BDH, Poole, U.K.) and 75 mg of the nickel(II) chelate of choice (5 wt. % with respect to squalane) were dissolved in 100 ml of dry chloroform accomodated in a 250 ml round-bottomed flask. Solid support (30 g) was added and the whole was agitated to become homogeneous. Thereafter, chloroform was removed by means of a rotary vacuum evaporator.

Supports with squalane loadings of 5, 10, 15, 20, and 25% containing nickel(II) chelate in a constant amount ($w = 0{\cdot}0476$) were prepared. Prior to gas chromatographic measurements, the packed columns were weighed and conditioned for 12 h at 120°C under a constant helium stream at a flow rate of 5 ml min^{-1}.

The amount of the mixed squalane–chelate stationary phase deposited on the solid support surface was determined based on thermogravimetric measurements performed in air using a Paulik–Paulik–Erdey type derivatograph (MOM, Budapest, Hungary); the instrument was

TABLE I

Schiff base nickel(II) chelates studied

Systematic name	Abbreviation
N,N′-Ethylene-bis(acetylacetoneimine)Ni(II)	Ni(en)AA
N,N′-Trimethylene-bis(acetylacetoneimine)Ni(II)	Ni(tm)AA
N,N′-Phenylene-bis(acetylacetoneimine)Ni(II)	Ni(ph)AA

operated at 20—1 000°C applying a heating rate of 5 K min^{-1}; 50 mg of sample was used. The specific surface area of the prepared GC packings was determined by the BET method from nitrogen adsorption-desorption isotherm measurements, performed on a Sorptomatic 1800 instrument (Carlo Erba, Italy).

The dead time values t_M were calculated for each column using an iterative method[14]. All the other calculations such as of the adjusted retention times t'_R or the net retention volume V_N relied on four individual values of the uncorrected retention time t_R for each set of solutes and column.

Capillary gas chromatographic experiments were performed on a Chrom 4 gas chromatograph (Laboratorní přístroje, Prague, Czechoslovakia) equipped with a flame ionization detector, using a stainless steel WCOT capillary column (25 m × 0·26 mm i.d.) containing a 0·3 μm film of a squalane + 0·1% KOH phase (Cormay, Lublin, Poland). Helium served as the carrier gas; inlet pressure 30 kPa, split ratio 1 : 100. Column, injection port and detector temperatures were 100, 210 and 210°C, respectively. The efficiency of the column was tested with a mixture of 2,6-dimethylphenol and 2,4-dimethylaniline, whose capacity ratios k were 2·7 and 2·9, respectively. In the experimental conditions used, the number of effective theoretical plates was 30 000 for 2,6-dimethylphenol.

RESULTS AND DISCUSSION

The relative retention $r_{i,s}$ was calculated for each solute as

$$r_{i,s} = t'_{R,i}/t'_{R,s}\,, \qquad (2)$$

where $t'_{R,i}$ and $t'_{R,s}$ are the adjusted retention times of solute (i) and 3-methylpyridine (s), respectively.

The relative retention values $r_{i,s}$ were obtained for pyridines on three stationary phases which contained equal amounts of nickel(II) chelates differing slightly in their molecular structure. The data, given in Table II, indicate that substituted methylpyridines are not eluted from the one-component stationary phase (squalane) in order of their boiling points. 3-Methylpyridine and 2,3-dimethylpyridine remain unresolved by this phase. With Ni(en)AA or Ni(ph)AA added to squalane, the separation of the 3-methylpyridine/4-methylpyridine, 3-methylpyridine/2,3-dimethylpyridine and 3-methylpyridine/2,4-dimethylpyridine pairs is incomplete. However, with stationary phases containing Ni(tm)AA or Ni(ph)AA, the retention order of the 3-methylpyridine/2,3-dimethylpyridine pair is reversed and their peaks are resolved. In general, satisfactory separation of all pyridine pairs is only achieved when using the Ni(tm)AA chelate (Fig. 1).

We assume that present in the squalane stationary phase, the nickel(II) chelates act as strong Lewis acids for pyridines, and this leads to the formation of adducts of different stability[7]. The separating efficiency for pyridines can be related to the structure and amount of the nickel(II) chelate added to the liquid phase. This is consistent with the result of this study, changes in the type of alkyl bridge linking

TABLE II

Relative retention $r_{i,s}$ of pyridines. Columns containing squalane mixed with different nickel(II) chelates, support type: Chromosorb P DMCS, stationary phase loading: 20%, mass fraction of the chelate 0·0476, t_c = 100°C. Other GC conditions see Experimental

Solute	$r_{i,s}$[a]			
	Squalane[b]	Ni(en)AA[c]	Ni(tm)AA	Ni(ph)AA
Pyridine	0·24	0·31	0·37	0·46
2-Methylpyridine	0·73	0·63	0·57	0·47
3-Methylpyridine	1·00	1·00	1·00	1·00
4-Methylpyridine	1·63	1·00	1·09	1·00
2,3-Dimethylpyridine	1·00	1·00	0·78	0·46
2,4-Dimethylpyridine	2·16	1·75	1·54	1·00
		t'_R, min		
3-Methylpyridine	10·87	6·42	6·31	6·36

[a] See Eq. (2); [b] one component stationary phase; [c] see Table I.

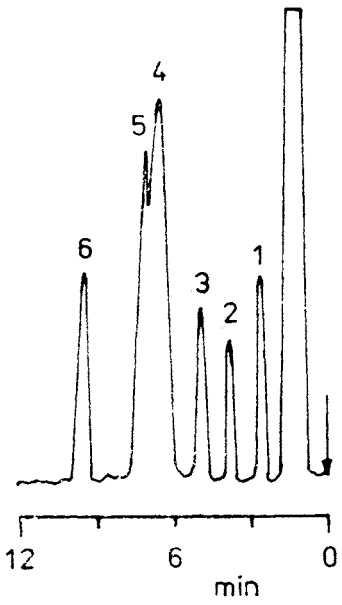

FIG. 1

Isothermal separation of pyridines on squalane–Ni(tm)AA mixed stationary phase deposited on Chromosorb P DMCS. Degree of coating 20%, chelate mass fraction of the stationary phase 0·0476, t_c = 100°C, other conditions as given in the Experimental. Peaks: 1 pyridine, 2 2-methylpyridine, 3 2,3-dimethylpyridine, 4 3-methylpyridine, 5 4-methylpyridine, 6 2,4-dimethylpyridine

of the nitrogen donor atoms in the nickel(II) chelate molecule (Table I) giving rise to an increase in the relative retention of pyridine on the one hand and to a decrease in the relative retention of 2-methylpyridine or 2,4-dimethylpyridine on the other hand (Table II).

From our previous study[6] we concluded that the amount of nickel(II) chelate in squalane cannot be varied over a wide region without affecting negatively the chromatographic resolution of electron-rich compounds. So, it was of interest to examine whether changes in the stationary phase loading or in the kind of the support material affect the retention when using nickel(II) chelate-containing mixed stationary phases.

Equation (*1*) can be written in the form

$$V_N/V_L = (K_A A_L)/V_L + K_L \qquad (3)$$

from which it is apparent that changes in the adsorption effect on the gas-liquid interface ($K_A A_L$ contribution) and the partition coefficient K_L can be conveniently evaluated if the function $V_N/V_L = f(A_L/V_L)$ is linear. In order to ascertain whether Eq. (*3*) holds true we measured the area A_L of the gas-liquid interface for the Polsorb C support[15] loaded with increasing quantities of the squalane–Ni(tm)AA chelate mixed stationary phase (Table III). The calculated K_A and K_L coefficients for the pyridines studied are given in Table IV. It is apparent that the difference between the K_L values of the partly resolved (Fig. 1) 3-methylpyridine and 4-methylpyridine is very small. In this case we suppose that although dissolution in the liquid phase contributes, it is adsorption and association that play the major part in the retention.

TABLE III

Characteristic values of Polsorb C coated with squalane + Ni(tm)AA chelate mixed stationary phase. Mass fraction of chelate in the mixture wtih squalane $w = 0{\cdot}0476$

w_L[a] %	A_L[b] $m^2\ g^{-1}$	V_L[b] $cm^3\ g^{-1}$
0	11·7	0
5	8·2	0·062
10	7·8	0·123
15	6·8	0·185
20	6·1	0·246
25	5·3	0·308

[a] Stationary phase loading determined from TG data; [b] gas-liquid surface area determined by BET method; [c] stationary phase volume calculated at 20°C.

A sharp increase in K_A is observed with the substitution of the pyridine molecule by methyl groups. The exceedingly high K_A value for 4-methylpyridine can be related to its highest dipole moment ($8{\cdot}7 \cdot 10^{-30}$ C m) causing a high specific interaction at the gas-liquid interface during its retention. Andersons and coworkers[10] found the highest K_A value for 3-methylpyridine on Apiezon M and concluded that heterocyclic amines possess a very low surface activity at this liquid phase.

Based on the results of our study we assume that the separation of isomers of picoline can be partly improved by the small changes in their gas-liquid adsorption (compare their K_A values). Fig. 2 shows that under isothermal conditions, decrease

TABLE IV

Adsorption (K_A) and partition (K_L) coefficients of pyridine and its derivatives. Column with squalane + Ni(tm)AA chelate mixed stationary phase ($w = 0{\cdot}0476$) deposited on Polsorb C, $t_c = 100°C$. Other GC experimental conditions as given in Experimental

Solute	K_L	$K_A \cdot 10^3$
Pyridine	226·7	432·3
2-Methylpyridine	447·1	1 728·1
3-Methylpyridine	955·2	1 436·1
4-Methylpyridine	1 005·2	3 000·9
2,3-Dimethylpyridine	595·1	2 583·6
2,4-Dimethylpyridine	1 528·6	2 372·1

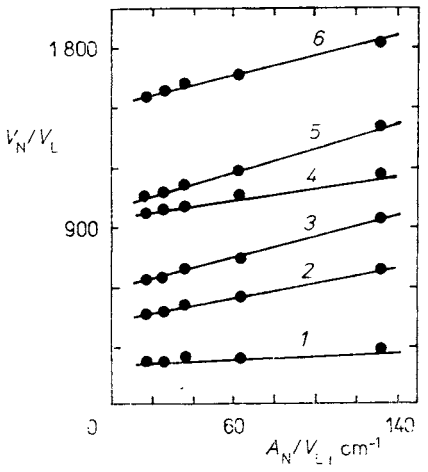

FIG. 2

Plot of V_N/V_L vs A_V/V_L for pyridines and squalane-Ni(tm)AA mixed stationary phase deposited on Polsorb C. Conditions and curve labelling as in Fig. 1

in the stationary phase loading, supported by the increase in the gas-liquid interface area A_L (Table III), brings about a better separation of 3-methylpyridine and 4--methylpyridine. On the other hand, the retention volumes V_N of 3-methylpyridine and 2,3-dimethylpyridine approach each other too closely in such conditions and these isomers cannot be resolved.

The K_A values given in Table IV and the shape of functions shown in Fig. 2 indicate that the isothermal gas chromatographic separation of rarefied pyridine vapours on the squalane–nickel(II) chelate mixed stationary phases is affected strongly by complex adsorption phenomena at the gas-liquid interface. During isothermal GC analysis, the composition of and the degree of a loading by the mixed stationary phase containing a nickel(II) Schiff base should be optimized individually for each set of solutes treated, type of chelate and kind of support. It should be pointed out that increase in the concentration of the injected solutes over the region of 10^{-9} to 10^{-12} g in the dose can affect the GC retention by some adsorption effects at the stationary liquid-solid support interface, as reported recently[10].

It is noteworthy that the squalane–Ni(tm)AA stationary phase offers gas chromatographic performance for the pyridines studied that is comparable with that of capillary WCOT columns, as is documented by Fig. 3. The slight peak tailing in these squalane + KOH capillary columns can be reduced by optimization of the KOH content of the stationary phase. The overall selectivity and time of analysis, however, are nearly equivalent under the conditions used. The use of the Ni(tm)AA-

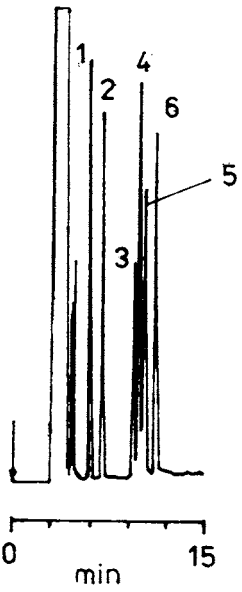

FIG. 3

Capillary gas chromatographic trace of a mixture of pyridines in cyclohexane, obtained at 100°C using squalane + 0·1% KOH stationary phase; conditions as given in the Experimental. Peaks: 1 pyridine, 2 2-methylpyridine, 3 3-methylpyridine, 4 2,3--dimethylpyridine, 5 4-methylpyridine, 6 2,4-dimethylpyridine

-containing packed column (Fig. 1) is convenient for the separation of the 2,3-dimethylpyridine/3-methylpyridine isomer pair at the lowest relative retention (0·78 for squalane–Ni(tm)AA packed column and 1·15 for squalane–KOH capillary column).

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