Journal of Chromatography, 176 (1979) 43–53

CHROM. 11,873

EXPERIMENTAL STUDY OF SELECTIVITY AND COLUMN EFFICIENCY IN CLATHRATE CHROMATOGRAPHY USING WERNER COMPLEXES AS CLATHRATE HOST COMPONENTS

I. RELATIONSHIP BETWEEN SELECTIVITY OF $Ni(NCS)_2$(4-METHYLPYRIDINE)$_4$ · GUEST CLATHRATE SORBENTS AND COMPOSITION OF THE MOBILE PHASE

JANUSZ LIPKOWSKI, MARIA PAWŁOWSKA and DANUTA SYBILSKA
Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw (Poland)
(First received October 10th, 1978; revised manuscript received March 9th, 1979)

SUMMARY

The chromatographic properties of the clathrates formed by $Ni(NCS)_2$ (4-methylpyridine)$_4$, treated as representative of a large group of Werner MeX_2A_4 complexes able to form clathrates, were studied. As the compounds to be separated, *o*-, *m*- and *p*-isomers of dinitrobenzene and bromonitrobenzene were used. It was found that the volume of the channels in the zeolite-like Ni $(NCS)_2$(4-methylpyridine)$_4$ structure can vary by about 50%, owing to dilatation of the crystal structure related to the composition of the guest, and can be easily controlled by using a suitable composition of the mobile phase. The selectivity of chromatographic sorption as a function of dilatation–contraction of the clathrate structure and competitive clathration of the mobile phase components (elution power) is discussed.

INTRODUCTION

Since the first paper on the separation of isomers using clathrate-type compounds as stationary phases was published[1], many different mixtures have been separated by this chromatographic method[1–5]. Trial and error approaches have usually been made in order to find suitable conditions for selectivity and the column parameters necessary for the satisfactory solution of a given separation problem. Recently, knowledge of the physico-chemical properties and the structure of several $Ni(NCS)_2$(4-MePy)$_4$* clathrates was greatly increased as is described below in more detail. This progress made possible a detailed study of correlations between the structural properties of the clathrates and their chromatographic parameters.

The aim of this work was to study the correlations mentioned above from the

* 4-MePy = 4-methylpyridine.

point of view of optimization and design of liquid chromatographic separations of isomers using clathrate-forming Werner complexes.

EXPERIMENTAL

Reagents

All reagents and solvents were of analytical-reagent grade. 4-Methylpyridine contained less than ca 5% of 3-methylpyridine as the main impurity.

Apparatus

The detection system used was the Kemula apparatus[6] for chromatopolarography combined with a Radelkis OH 101 d.c. polarograph. X-ray diffractograms were taken using a Rigaku-Denki powder diffractometer (Cu K_α).

Preparation of the sorbents

The clathrate sorbents, which are virtually insoluble in the mobile phases used, were precipitated by simultaneous, slow pouring of aqueous $Ni(NCS)_2$ (0.2 *M*) and methylpyridine into a suitable mixture of organic solvent, NH_4SCN and water. The precipitate was then kept in the mother liquor at 25 ± 0.1° for 24 h before use. Acetone (Ac), ethanol(Et), *n*-propanol (Prop) and ethylene glycol (Glyc) were used as organic solvents. Their concentrations (percent by volume) and the 4-methylpyridine content in the mobile phase (mother liquor) are indicated in the text (see Tables I and II) in the form, *e.g.*, Ac 27/3.4, denoting $Ni(NCS)_2(4\text{-MePy})_4 \cdot G$* sorbent equilibrated with aqueous acetone (27%, v/v) containing (3,4% ,v/v) of 4-methylpyridine.

Chromatographic experiments

Glass columns (40 × 6 mm I.D.) were prepared by slurry packing; the particle size was ≤15 μm. Mother liquors were always used as mobile phases and the flow-rate of the mobile phase was 10 ml/h.

The capacity factors (k') were calculated from the equation

$$k' = \frac{V_R - V_0}{V_0}$$

where V_R is the retention volume and V_0 is the volume of the mobile phase in the column calculated as the retention volume of furazol (N-5-nitro-2-furfurylidene-3-amine-2-oxazolidone).

Analytical determinations

The procedure for phase analysis of the substances used has been published previously[7]. Lattice parameters of the clathrates were determined from X-ray diffractograms and refined by using the least-squares method.

The density of the clathrates was measured by pyknometry, slightly modified to avoid weighing of dried material; it has been demonstrated[8] that drying affects the composition and structure of the clathrates.

* G = Guest.

RESULTS AND DISCUSSION

Clathrate sorbents

As shown by X-ray powder diffraction patterns tetragonal $I4_1/a$, the β-phase of $Ni(NCS)_2(4\text{-MePy})_4$, results if the procedure described under *Preparation of the sorbents* is followed, with the use of 4-methylpyridine at concentrations of 1.5–7.5% and the organic solvent at concentrations of 18–60%. The β-structure, recently determined by De Gil and Kerr[9], is of the "zeolite" or channel type. However, this structure, which consists of discrete host complex molecules packed together by means of weak Van der Waals forces, is "sensitive" to sorption of the guest, *i.e.*, it swells on absorbing more guest[8]. It can be seen from the data in Table I that the lattice parameters of the sorbent can easily be changed over a wide range simply by varying the concentrations of 4-methylpyridine and organic solvent in the liquid phase, which is in an equilibrium state with respect to the solid clathrate. The differences, which reach about 17% of the molar volume of the clathrate, are very significant if expressed as differences in the molar "free volume" of the sorbent, *i.e.*, the volume not occupied by the host. If the molar volume of the host in its non-clathrate α-modification (430 cm^3) is subtracted from the molar volume of the clathrate sorbents, the free volume varies from 47 to 90 cm^3/mole. It seems that there is no significant change in the c/a axial ratio and the swelling of the clathrate can be regarded as "proportional" in all three dimensions.

From studies of sorption isotherms in the β-$Co(NCS)_2(4\text{-MePy})_4$ phase, Allison and Barrer[10] found an analogy to the water–montmorillonite system. Stepwise or inflected isotherms were obtained and a modified Langmuir model proposed:

$$\theta = \frac{p}{K + p}\left[\frac{N_1 + F(x)}{N_1 + N_2}\right]$$

where

K = equilibrium constant;
p = external pressure of the sorbate;
N_1 = number of sites initially present;
N_2 = the maximum extra number of sites generated;
$F(x)$ = an equilibrium number of sites generated, dependent on external pressure of the sorbate and temperature.

It seems reasonable to assume a similar model for sorption equilibria in the system β-$Ni(NCS)_2(4\text{-MePy})_4$ + 4-methylpyridine + organic solvent. At high solvent but low 4-methylpyridine concentrations in the mobile phase there is one molecule of the solvent absorbed per molecule of the host. At higher concentrations of 4-methylpyridine there is one molecule of 4-methylpyridine absorbed in addition. Within these limiting values, the equilibria are more complex. For example, in the ranges of 2.3–7.6% of 4-methylpyridine and 27–60% of acetone, one molecule of 4-methylpyridine substitutes *ca.* 0.8 molecule of acetone[11]. This can be interpreted on the basis of the model described above, *e.g.*, five molecules enter into sites occupied by five molecules of acetone substituting them, but at the same time an additional site is generated and one molecule of acetone remains absorbed. Experimentally found data for the mean molecular weight of the guest in the clathrate (Table II) serve to define the overall sorption of all components of the mobile phase.

TABLE I

LATTICE PARAMETERS AND POROSITY OF β-Ni $(NCS)_2$ $(4\text{-MePy})_4 \cdot G$ CLATHRATES
G = 4-MePy + (acetone or ethanol or *n*-propanol or ethylene glycol).

Clathrate sorbent	*a (Å) (±0.02)*	*c (Å) (±0.02)*	*Molar volume (cm^3) (±1.6)*	*Molar volume of pores (cm^3) (±1.6)*
Ac 18/1.5	17.01	23.22	505.8	75.8
Ac 18/2.3	17.04	23.31	509.3	79.3
Ac 18/3.4	17.10	23.36	514.4	84.4
Ac 18/5.1	17.10	23.40	515.2	85.2
Ac 18/7.6	17.17	23.45	520.6	90.6
Ac 27/1.5	16.82	22.59	481.0	51.0
Ac 27/2.3	16.87	22.78	487.9	57.9
Ac 27/3.4	16.99	23.23	504.6	74.6
Ac 27/5.1	17.12	23.41	516.7	86.7
Ac 27/7.6	17.13	23.41	517.4	87.4
Ac 40/1.5	16.77	22.65	479.7	49.7
Ac 40/2.3	16.88	22.88	491.0	61.0
Ac 40/3.4	16.87	23.11	495.4	65.4
Ac 40/5.1	17.02	23.18	505.7	75.7
Ac 40/7.6	17.17	23.48	521.0	91.0
Ac 60/1.5	16.75	22.69	478.2	48.2
Ac 60/2.3	16.78	22.72	481.8	51.8
Ac 60/3.4	16.79	22.66	480.5	50.5
Ac 60/5.1	16.88	22.85	490.4	60.4
Ac 60/7.6	17.07	23.27	510.4	80.4
Et 18/1.5	17.09	23.26	511.6	81.6
Et 18/2.3	17.11	23.29	513.4	83.4
Et 18/3.4	17.18	23.41	520.1	90.1
Et 18/5.1	17.19	23.45	521.8	91.8
Et 18/7.6	17.15	23.45	519.6	89.6
Et 27/1.5	17.07	23.14	507.5	77.5
Et 27/2.3	17.11	23.22	512.0	81.0
Et 27/3.4	17.15	23.28	515.5	85.5
Et 27/5.1	17.12	23.28	513.9	83.9
Et 27/7.6	17.14	23.26	514.7	84.7
Et 40/1.5	16.99	23.02	500.4	70.4
Et 40/2.3	17.02	23.08	503.3	73.3
Et 40/3.4	17.08	23.17	509.1	79.1
Et 40/5.1	17.10	23.17	510.2	80.2
Et 40/7.6	17.12	23.22	512.3	82.3
Et 60/1.5	17.00	23.06	501.6	71.6
Et 60/2.3	17.09	23.12	508.4	78.4
Et 60/3.4	17.13	23.13	511.1	81.1
Et 60/5.1	17.09	23.11	508.4	78.4
Et 60/7.6	17.08	23.17	509.1	79.1
Glyc 18/3.4	17.14	23.40	517.4	87.4
Glyc 18/7.6	17.17	23.46	520.7	90.7
Glyc 27/3.4	17.03	23.29	508.5	78.5
Glyc 27/7.6	17.16	23.47	520.8	90.8
Glyc 40/3.4	17.04	23.35	510.1	80.1
Glyc 40/7.6	17.21	23.34	520.2	90.2

TABLE I *(continued)*

Clathrate sorbent	*a (Å) (±0.02)*	*c (Å) (±0.02)*	*Molar volume (cm^3) (±1.6)*	*Molar volume of pores (cm^3) (±1.6)*
Prop 18/3.4	17.09	23.18	509.8	79.8
Prop 18/7.6	17.14	23.39	517.5	87.5
Prop 27/3.4	16.93	22.86	493.4	63.4
Prop 27/7.6	17.11	23.23	511.8	81.8
Prop 40/3.4	16.82	22.78	485.2	55.2

Clathrate selectivity as a function of eluent composition

On the basis of the X-ray diffraction data described above, one might expect a great dependence of the chromatographic properties of the clathrates on their lattice constants, that is, the size of the cages. This is indeed so, as illustrated in Fig. 1.

It can be seen (Figs. 2 and 3) that the mobile phase composition influences not only the absolute, but also the relative capacity factors and that the selectivity ($\alpha = k_2'/k_1'$) is related to the eluent composition.

A special feature of these diagrams is that the capacity factor (k') plotted against 4-methylpyridine concentration has a maximum at a value of the latter which depends on the solvent concentration. In other words, there is an optimum for chromatographic separation that may be related to structural factors. However, there is no obvious relationship between the size of the cage and the capacity factor that might

TABLE II

DENSITY (d), MOLECULAR WEIGHT (M_{Cl}) OF CLATHRATE AND MEAN MOLECULAR WEIGHT (M_G) OF THE GUEST IN CLATHRATE

Clathrate sorbent	*d (g/cm^3)*	M_{Cl}	M_G
Ac 18/1.5	1.2962	655.6	108.4
Ac 18/7.6	1.2687	660.5	113.3
Ac 27/3.4	1.2164	613.8	66.6
Ac 27/5.1	1.2561	649.0	71.8
Ac 27/7.6	1.2578	650.8	103.6
Ac 40/3.4	1.2107	559.8	52.6
Ac 40/5.1	1.2365	625.3	78.1
Ac 60/1.5	1.2396	592.8	45.6
Ac 60/3.4	1.2618	606.3	59.1
Ac 60/7.6	1.2104	617.8	70.6
Et 18/1.5	1.2585	643.8	96.6
Et 18/7.6	1.2921	671.4	124.2
Et 27/1.5	1.2337	626.1	78.9
Et 27/2.3	1.2643	647.3	101.1
Et 27/3.4	1.2474	643.0	95.8
Et 27/5.1	1.2368	635.6	88.4
Et 40/2.3	1.2477	627.9	80.7
Et 40/3.4	1.2747	648.9	101.7
Et 60/1.5	1.1949	599.4	52.2
Et 60/7.6	1.1949	608.1	60.9

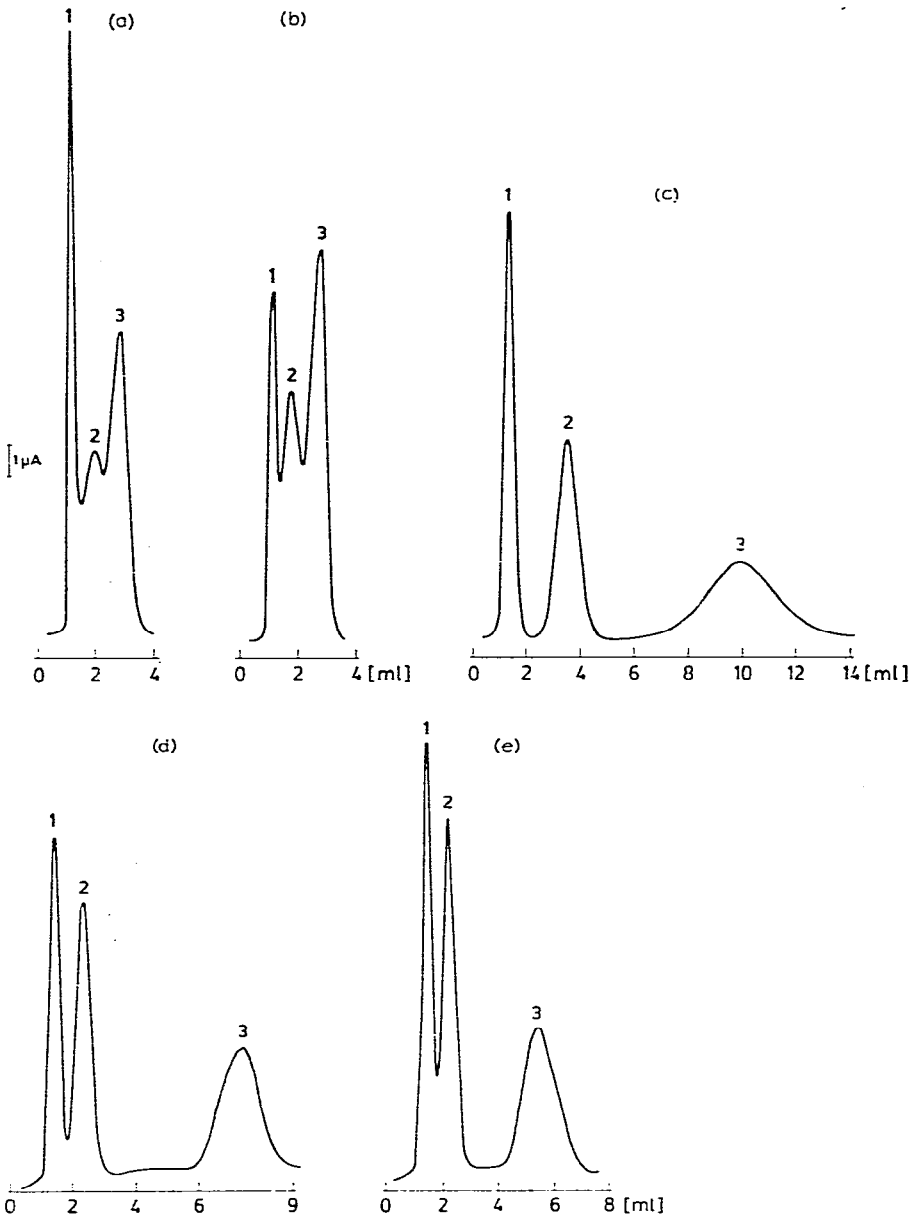

Fig. 1. Dependence of chromatographic separation of dinitrobenzene isomers on clathrate structure and mobile phase composition. Peaks 1, 2 and 3 represent *o*-, *m*- and *p*-dinitrobenzene, respectively. Column, 40 × 6 mm I.D.; d_p < 15 μm; flow-rate, 10 ml/h. (a) Ac 27/1.5, V_{mol} = 481 cm³; (b) Ac 27/2.3, V_{mol} = 488 cm³; (c) Ac 27/3.4, V_{mol} = 505 cm³; (d) Ac 27/5.1, V_{mol} = 516 cm³; (e) Ac 27/7.6, V_{mol} = 517 cm³.

serve for selecting an optimal sorbent. The other factor that has to be involved is the competition of the mobile phase components with respect to the clathration of isomers to be analysed. The following discussion is based on the assumptions that:

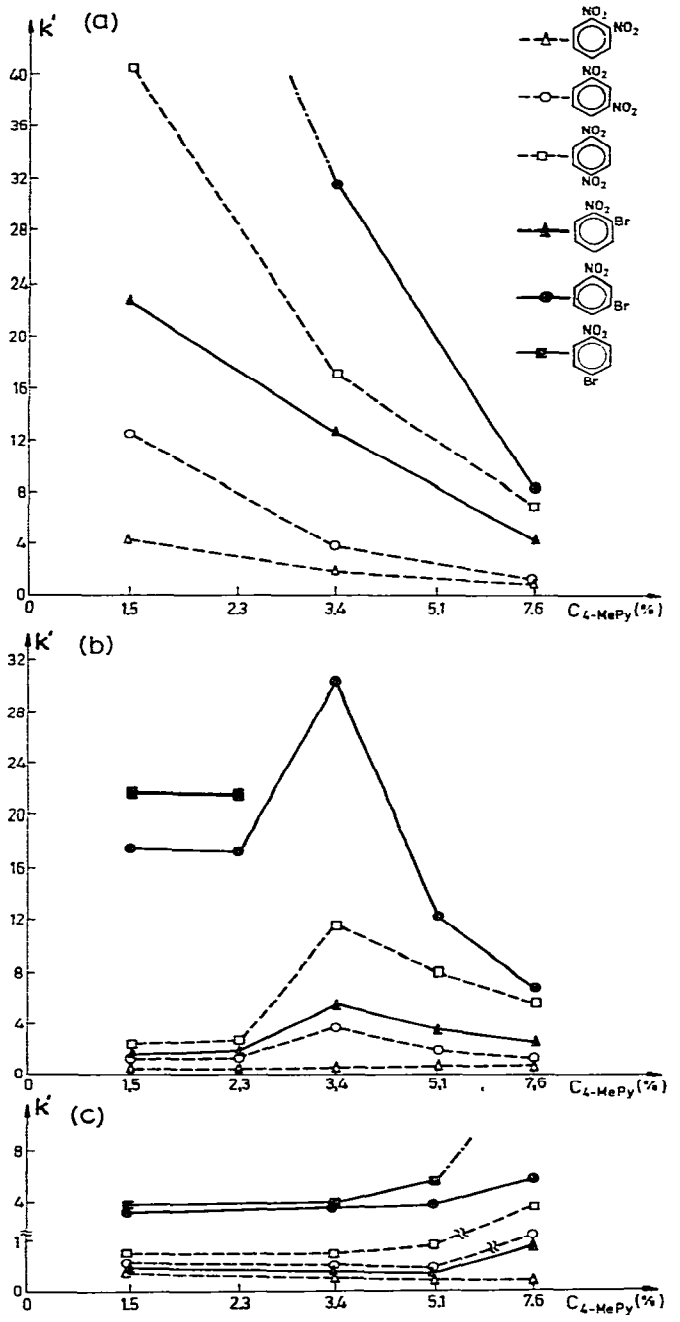

Fig. 2. Dependence of k' on mobile phase composition. Column, 40 × 6 mm I.D.; $d_p \leqslant 15\,\mu$m; flow-rate, 10 ml/h. (a) 18% acetone; (b) 27% acetone; (c) 40% acetone.

(a) dilatation of the clathrate host structure increases the capacity factor for the absorption of benzene derivatives and

(b) absorption of 4-methylpyridine and an aliphatic solvent (the components

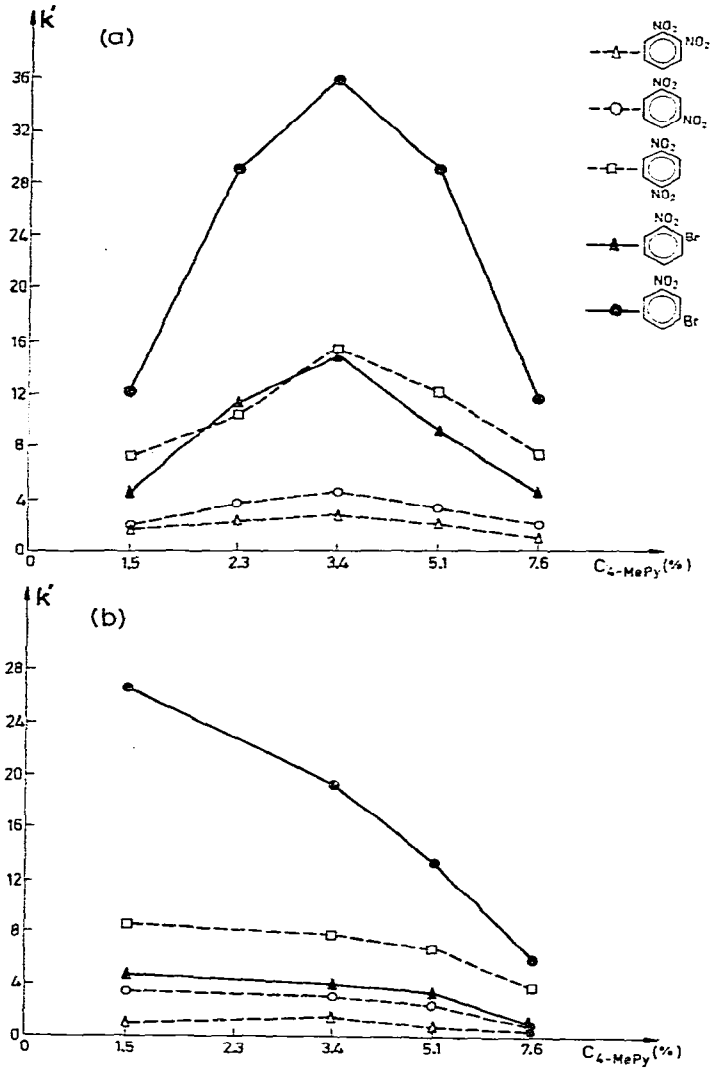

Fig. 3. Dependence of k' on mobile phase composition. Column, 40 × 6 mm I.D.; $d_p \leq 15\,\mu m$; flow-rate, 10 ml/h. (a) 18% ethanol; (b) 40% ethanol.

of the mobile phase) compete with the absorption of the compounds being analysed and causes a decrease in k'.

The example in Fig. 2 demonstrates these two effects. Maxima are present at $c_{solv} = 27\%$ and $c_{4\text{-MePy}} = 3.4\%$ (Fig. 2b). At $c_{solv} = 18\%$ the maxima are presumably shifted to $c_{4\text{-MePy}} < 1.5\%$ (Fig. 2a) and at $c_{solv} = 40\%$ to $c_{4\text{-MePy}} > 7.6\%$ (Fig. 2c). At any given concentration of the solvent, the cage size increases with increasing concentration of 4-methylpyridine. At the same time, competitive clathration of 4-methylpyridine becomes more important, because of its higher concentration in the liquid phase. Thus, in the increasing part of the $k' = k'(c_{4\text{-MePy}})$ relationship, the dominating factor is lattice dilatation favourable for absorption of the analysed guests. It might be suggested that dilatation is equivalent to the generation of absorption sites.

After the maximal number of the sites has been reached, competitive clathration of 4-methylpyridine becomes predominant and k' decreases as $c_{4\text{-MePy}}$ increases. On going to higher concentrations of aliphatic solvents, it should be noted that lattice contraction occurs (see Table I). To generate absorption sites a higher 4-methylpyridine concentration is necessary, *e.g.*, with 40% acetone a higher concentration of 4-methylpyridine is required than at 27% acetone. Similarly, at 18% acetone the maximal number of absorption sites appears at $c_{4\text{-MePy}} < 1.5\%$.

Let us assume that sorption of the guest (G) follows a substitution mechanism:

$$KP + G \rightleftharpoons KG + P \text{ or } KR_x + G \rightleftharpoons KG + xR$$

where KP, KR_x and KG denote clathrates with 4-methylpyridine (P), solvent (R) or sorbate (G) as the guest, respectively. Then, equilibrium constants can be written as

$$c_{PG} = \frac{[KG]\,[P]}{[KP]\,[G]} \quad \text{or} \quad c_{RG} = \frac{[KG]\,[R]^x}{[KR_x]\,[G]}$$

Taking into account that

$$\frac{[KG]}{[G]} = k' \quad \text{and} \quad [KP] + [KR_x] = [K]$$

the following equations can be derived:

$$k' = \frac{c_{RG} \cdot \frac{1}{\kappa} \cdot [K]}{[P] + \frac{1}{\kappa} \cdot [R]^x} \quad \text{or} \quad k' = \frac{c_{PG}\,[K]}{[P] + \frac{1}{\kappa} \cdot [R]^x}$$

where $\kappa = [KP]\,[R]^x/[KR_x]\,[P]$ is the equilibrium constant for substitution of solvent (R) with 4-methylpyridine (P) in the clathrate.

Comparing the two expressions for k', it should be noted that the relationship $k' = k'([P], [R])$ is essentially of the same type in both instances; c_{RG}, c_{PG}, κ and K are constant. The equations can be considered as analogous to Snyder's[12] and Soczewiński's[13] equations:

$$k' = \frac{B}{[x]^n}$$

but the elution factor in clathrate chromatography is the sum of $[P] + 1/\kappa \cdot [R]^x$.

If only κ and x are really constant, the experimental results for $k' = k'([P], [R])$ are relatively clear and understandable, as in Fig. 2. Moreover, the curves in Figs. 2a and 3b, even if they refer to different solvents, are in fact similar, because they correspond to similar values of $1/\kappa \cdot [R]^x$ in both instances[11].

However, the problem is more complex. x may even assume negative values, *i.e.*, absorption of 4-methylpyridine causes additional sorption and not elimination

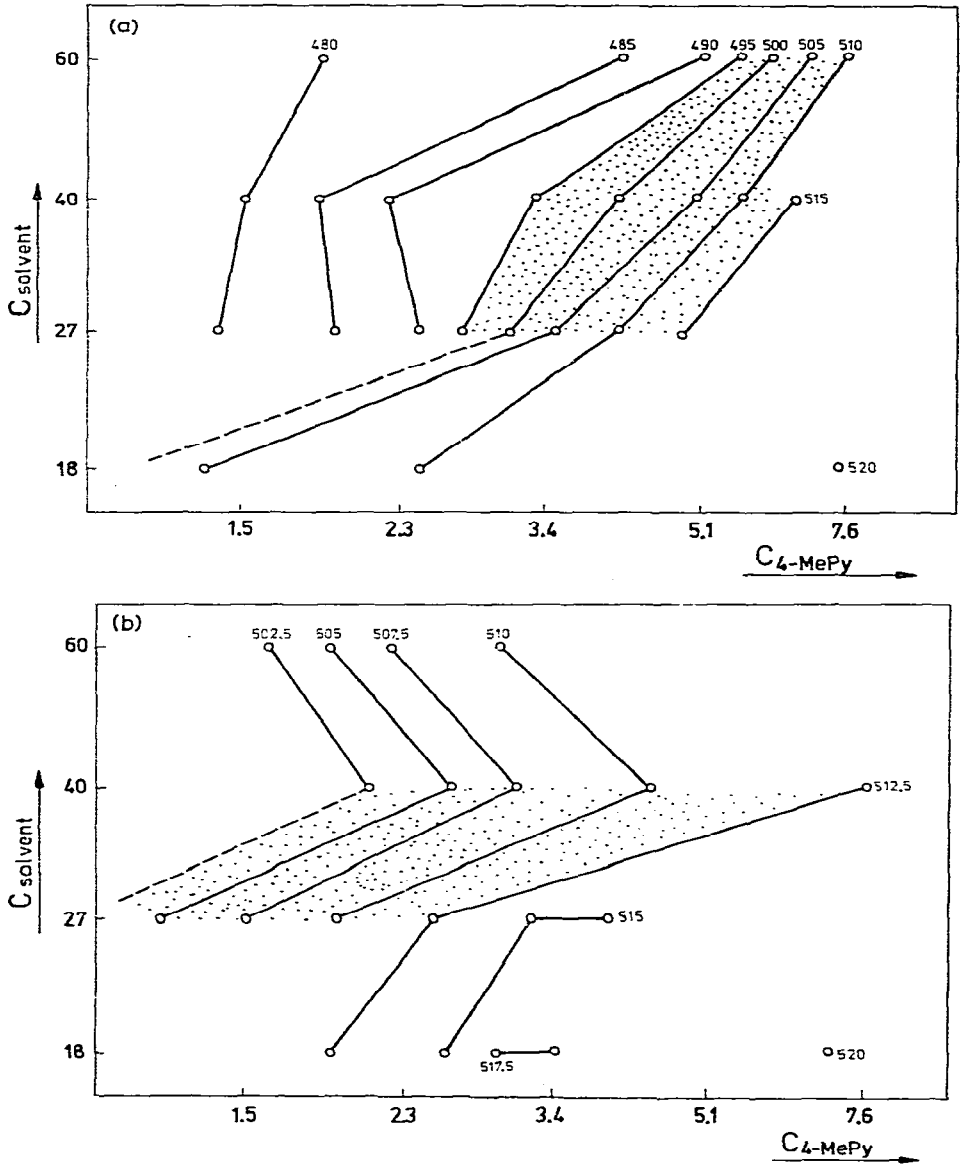

Fig. 4. Layer diagram of molar volume of clathrate sorbents plotted against 4-methylpyridine ($c_{4\text{-MePy}}$) and solvent ($c_{solv.}$) concentrations in the mobile phase. In the shaded regions a substitution mechanism, $KR_x + P \rightarrow KP + xR$, is valid; x is approximately constant (see text). (a) solvent = acetone, $x \approx 0.8$; (b) solvent = ethanol, $x \approx 2$.

of the solvent in the clathrate, because of lattice dilatation. The equations given above for k' have no meaning in such instances. The applicability of the above considerations is illustrated in Fig. 4.

If the equilibrium were of the type $KR_x + P \rightleftharpoons KP + xR$, the molar volumes should be constant and appear as parallel straight lines on the diagrams. This is the case only within narrow limits and the explanation given above is valid only in these

ranges. Finally, it must be mentioned that the solubility of the sorbate in the mobile phase influences the k' values: a higher concentration of organic solvent in the mobile phase results in smaller k' values. This effect is not selective, of course, and appears as a uniform lowering of the $k' = k'([P])$ curves on going to higher [R] values.

Summarizing the above considerations, the following conclusions can be drawn:

(1) Selectivity of clathration is determined by the host structure in the clathrate, defining shape of the cage–absorption sites. The β-structure studied in this work is "para-selective" with respect to mixtures of isomers of disubstituted benzene derivatives.

(2) The number of effective absorption sites in the clathrate is greatly dependent upon the lattice parameters of the β-structure, which may be varied by clathration of the mobile phase components. By controlling the composition of the mobile phase by varying the concentration of 4-methylpyridine and/or organic solvent, one can produce sorption of the mobile phase components in order to obtain a clathrate sorbent with the desired lattice constants and hence sorption capacity.

(3) Adjustment of the absorption sites in clathrates by controlling the mobile phase composition must involve simultaneous variation of the "elution power" of the mobile phase. Two factors contribute to the elution power: competitive clathration of the mobile phase components and the solubility of the sorbate in the liquid phase. The former can be written as a sum, $[P] + 1/\kappa \cdot [R]^x$; the latter has not been studied in this work as a non-selective, typical effect in liquid chromatography.

In conclusion, β–$Ni(NCS)_2(4\text{-}MePy)_4$ clathrate sorbents have valuable features of high selectivity and versatility of sorption. Optimization of the operating parameters in these liquid chromatographic systems will be the subject of a forthcoming paper.

ACKNOWLEDGEMENTS

The authors are deeply indebted to Prof. W. Kemula for stimulating discussions of this problem. This work was supported within project 03.10.

REFERENCES

1 W. Kemula and D. Sybilska, *Nature (London)*, 185 (1960) 237.
2 D. Sybilska, *Proc. VI Int. Symp. on Chromatography and Electrophoresis*, Presses Academiques Europeennes, Brussels, 1971, pp. 212–221.
3 W. Kemula and D. Sybilska, *Anal. Chim. Acta*, 38 (1967) 97.
4 W. Kemula, D. Sybilska and K. Duszczyk, *J. Microchem.*, 11 (1966) 296.
5 W. Kemula, Z. Borkowska and D. Sybilska, *Monatsh. Chem.*, 103 (1972) 860.
6 W. Kemula, in L. Zuman and I. M. Kolthoff (Editors), *Progress in Polarography*, Wiley-Interscience, New York, 1962, p. 397.
7 W. Kemula, J. Lipkowski and D. Sybilska, *Rocz. Chem.*, 48 (1974) 3.
8 J. Lipkowski and S. Majchrzak, *Rocz. Chem.*, 49 (1975) 1655.
9 E. R. de Gil and T. S. Kerr, *J. Appl. Crystallogr.*, 10 (1977) 315.
10 S. A. Allison and R. M. Barrer, *J. Chem. Soc.*, (1969) 1717.
11 M. Pawłowska, *Thesis*, Warsaw, 1978.
12 L. R. Snyder, *Anal. Chem.*, 46 (1974) 1384.
13 E. Soczewiński, *J. Chromatogr.*, 130 (1977) 23.