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SELECTIVITY AND EFFICIENCY OF SEPARATION OF ISOMERS OF ORGANIC ACIDS BY CLATHRATE CHROMATOGRAPHY

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SUMMARY

Chromatographic separations of *o*-, *m*- and *p*-nitrobenzoic acids, *o*-, *m*- and *p*-nitrocinnamic acids and α- and β-naphthoic acids were studied as a function of pH of the mobile phase in the range 5.2–9.0 and using the β-$Ni(NCS)_2(4\text{-methylpyridine})_4$ clathrate as the solid stationary phase. The experimental distribution coefficients (k) were combined with dissociation constants (K_a) and pH values in order to evaluate the coefficient of distribution of undissociated acid molecules (k^c), which is otherwise unobtainable because the solid phase is unstable at pH < 5.2.

The selectivity observed follows the order typically observed for the β-phase: $k_p > k_m > k_o$ for disubstituted benzene derivatives, $k_\beta > k_\alpha$ for monosubstituted naphthalenes and $k_{trans} > k_{cis}$ for ethylene derivatives.

Despite the relatively low efficiency observed in separations of the acids, good separations of isomers were attained owing to high selectivity of the clathration.

INTRODUCTION

The use of β-$Ni(NCS)_2(4\text{-MePy})_4$* clathrates as stationary phases to separate mixtures of different types of isomers in chromatographic systems with polar mobile phases has been demonstrated with numerous examples[1]. The compounds to be separated can have different functional groups, provided that they do not destroy the host complex, *e.g.*, strongly acidic or alkaline media decompose the $Ni(NCS)_2(4\text{-MePy})_4$ complex.

It seemed interesting to investigate whether the high selectivity of clathration towards mixtures of isomers[2] could be used for chromatographic separations of isomers of organic acids. We therefore investigated the practical pH limits for using β-$Ni(NCS)_2(4\text{-MePy})_4$ clathrate sorbents in liquid chromatographic separations of dissociating compounds and studied the retention mechanism, *e.g.*, in order to establish whether the β-clathrate absorbs molecules only or also ionic species.

Systematic studies or liquid chromatographic separations of *o*-, *m*- and *p*-nitrobenzoic acids, *o*-, *m*- and *p*-nitrocinnamic acids and α- and β-naphthoic acids were performed with the pH of the mobile phase varying from 5.2 to 9.0.

* 4-MePy = 4-methylpyridine.

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EXPERIMENTAL

Reagents

The substances to be separated and the solvents were of analytical-reagent grade and their physical constants agreed with those reported in the literature. 4-Methylpyridine contained 0.3% of 3-methylpyridine but did not contain 2,5-dimethylpyridine in amounts detectable by gas chromatography.

Apparatus

The chromatographic experiments were carried out using the Kemula chromato-polarographic apparatus[3]. Diffusion currents for the reduction of nitro compounds eluted from the chromatographic column were recorded using an LP-7 polarograph (Laboratorni Přistroje, Prague, Czechoslovakia) at a constant potential of −0.9 V against the mercury pool anode.

Naphthoic acids were detected by a.c. polarography in an assembly identical with that applied for the detection of methylnaphthalenes[4] using a Radelkis universal OH-105 polarograph at a constant potential of −0.6 V against the mercury pool anode.

Procedure

The sorbent was prepared from the clathrate β-Ni(NCS)$_2$(4-MePy)$_4$ · (4-MePy)$_{0.7}$ by the method described elsewhere[5].

The aqueous methanol (30–70%) mobile phase solutions contained ammonium thiocyanate (0.1–0.2 mole/dm^3), 4-methylpyridine (0.1–1.0 mole/dcm^3) and a suitable amount of mandelic or acetic acid for pH control.

Samples of 5–50 μl of solutions containing $5 \cdot 10^{-3}$–$2 \cdot 10^{-2}$ mole/dm^3 of the compounds to be analysed were injected with a Hamilton microsyringe. The mobile phase was not deaerated and the elution flow-rate was 8.0 cm^3/h.

RESULTS AND DISCUSSION

The experimental k' values indicated that the practical lower pH limit for chromatography on β-Ni(NCS)$_2$(4-MePy)$_4$ is 5.2. This result was obtained by studying the retention of non-dissociating dinitrobenzenes. From the data in Table I one can draw the conclusion that the lower pH limit of chromatographic activity of the system studied is not related to the molecular stability of the host complex but rather to the stability of the β-Ni(NCS)$_2$ (4-MePy)$_4$ · G clathrate crystalline phase (G = guest molecule). This structure is stable when filled with the guest component, which, in the case under consideration, consists of 4-methylpyridine and added methanol. Simple calculations of the molar percentage of non-protonated 4-methylpyridine in the mobile phases (a), (b) and (c) in Table I (pK_a of 4-methylpyridine = 6.02) give *ca.* 0.035 *M* in (a) and *ca.* 0.14 *M* in (b). The concentration of 4-methylpyridine in the mobile phase necessary to make the Ni(NCS)$_2$ (4-MePy)$_4$ chromatographically active has been reported as *ca.* 0.04 *M*[5]. It seems clear that protonated 4-methylpyridine may not be clathrated or form ion pairs (*e.g.*, with CH_3COO^-).

Fig. 1 illustrates the dependence of the equilibrium distribution coefficients (k) of *o*-, *m*- and *p*-nitrobenzoic and *trans-o*-, *-m*- and *-p*-nitrocinnamic acids on the pH of

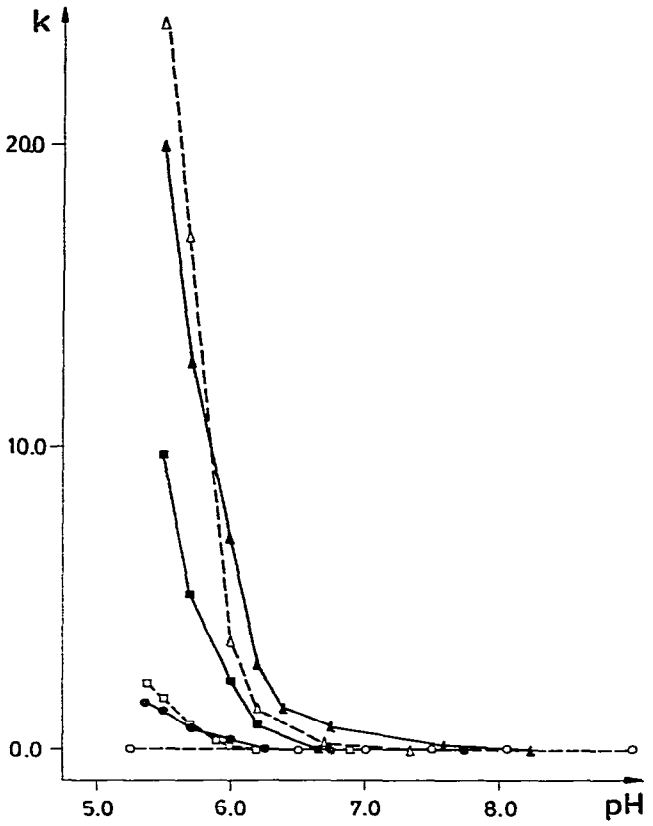


Fig. 1. Plot of distribution coefficients (k) of *o*- (○), *m*- (□) and *p*-nitrobenzoic acid (△) and *o*- (●), *m*- (■) and *p*-nitrocinnamic acid (▲) (in the *trans* configuration) *versus* pH of the mobile phase. Mobile phase: 0.1 *M* NH_4SCN–0.2 *M* 4-MePy in 30% aqueous methanol. The pH of the solutions were adjusted by the adding acetic acid.

the mobile phase. It is clear that at pH > pK_a + 2 (corresponding to practically complete dissociation) the distribution coefficients are zero. In other words, clathrate sorbents are able to absorb molecular rather than ionic species and, as in extraction or adsorption reversed-phase chromatography, with a decrease in pH an increase in the distribution coefficients is observed. By using the known equation[6,7]

$$k^{\circ} = k\,(1 + 10^{pH - pK_a})$$

the distribution coefficients of the undissociated part of the acids (k°) can be calculated (Table II). The high values obtained for this quantity, compared with the k values of non-dissociating dinitrobenzenes (measured under the same experimental conditions), should be mentioned.

The use of mandelic instead of acetic acid (as the pH controlling agent) results in a slight decrease in the measured k values of the acids but the pH dependences of k are strictly analogous.

TABLE I

DEPENDENCE OF CAPACITY FACTORS (k') OF DINITROBENZENES (DNB) ON THE TOTAL 4-METHYLPYRIDINE CONCENTRATION IN THE MOBILE PHASE, THE pH BEING KEPT CONSTANT AT 5.2

Mobile phase: 0.1 *M* NH_4SCN–4-MePy in 30% aqueous methanol; the pH was kept constant at 5.2 by addition of suitable amount of acetic acid. Column dimensions: 35 × 5 mm I.D.

Mobile phase	*Total concentration of 4-MePy in the mobile phase (mole/dm³)*	k'			*Solid stationary phase*
		o-DNB	*m-DNB*	*p-DNB*	
a	≤0.21	0.0	0.0	0.0	Inactive α-$Ni(NCS)_2(4\text{-MePy})_4$
b	0.84	1.4	2.7	10.5	Active sorbent β-$Ni(NCS)_2(4\text{-MePy})_4 \cdot G$
c	≥0.84	$0 < k' < 1.4$	$0 < k' < 2.7$	$0 < k' < 10.5$	Active sorbent β-$Ni(NCS)_2(4\text{-MePy})_4 \cdot G$

TABLE II

DISTRIBUTION COEFFICIENTS (k^c) CALCULATED FOR UNDISSOCIATED ACID MOLECULES, EXPERIMENTAL DISTRIBUTION COEFFICIENTS ($k^{5.5}$) TAKEN AT pH 5.5 AND DISSOCIATION CONSTANTS (GIVEN AS pK_a) OF NITROBENZOIC (NB) AND NITROCINNAMIC (NC) ACIDS AND $k^{5.5}$ OF DINITROBENZENES (DNB)

Mobile phase and column dimensions as in Fig. 1.

Compound	pK_a	$k^{5.5}$	k^c
o-NB	2.17	0.0	
m-NB	3.49	1.7	175
p-NB	3.43	24.0	2845
o-NC	4.15	1.3	30
m-NC	4.12	9.8	245
p-NC	4.05	20.0	585
o-DNB	–	6.0	–
m-DNB	–	11.0	–
p-DNB	–	42.0	–

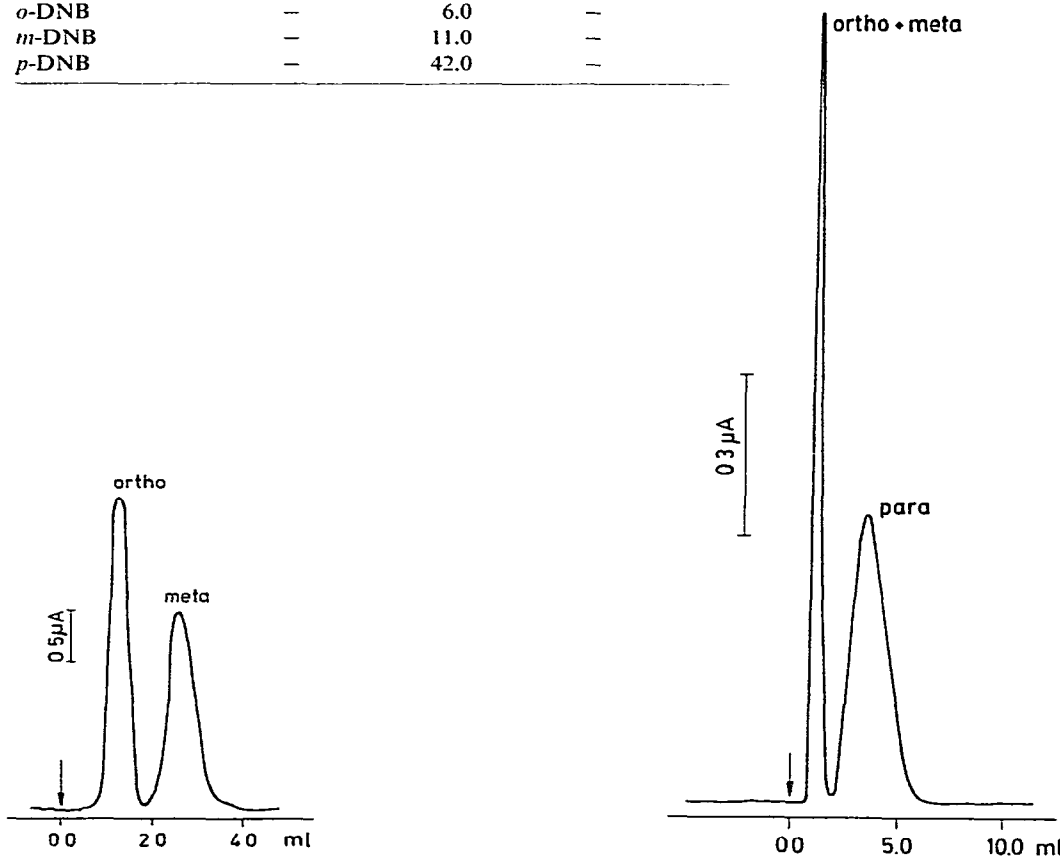
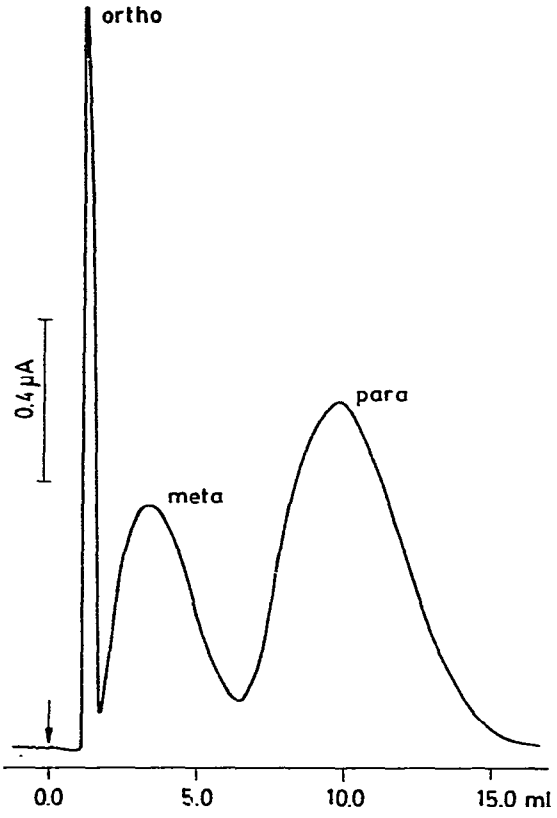


Fig. 2. Elution curves for *o*-, *m*- and *p*-nitrobenzoic acid using different mobile phases. (a) 0.2 *M* NH_4SCN–0.3 *M* 4-MePy–0.28 *M* CH_3COOH in the 40% aqueous methanol (pH 5.25), with column dimensions, 28 × 6 mm I.D.; under the conditions of the experiment $V_{max.}$ (retention volume) of *p*-nitrobenzoic acid is greater than 20 ml. (b) 0.1 *M* NH_4SCN, 0.84 *M* 4-MePy, 0.56 *M* CH_3COOH in the 30% aqueous methanol; column dimensions, 30 × 5 mm I.D.



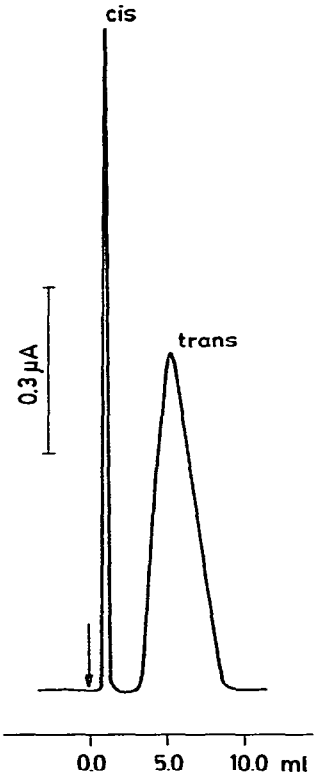


Fig. 3. Elution curve for *o*-, *m*- and *p*-nitrocinnamic acid in the *trans* configuration with 0.1 *M* NH_4SCN–0.84 *M* 4-MePy–0.56 *M* CH_3COOH in 30% aqueous methanol (pH 5.5) as the mobile phase; column dimensions, 65 × 5 mm I.D.

Fig. 4. Elution of *cis*- and *trans*-*p*-nitrocinnamic acids with 0.1 *M* NH_4SCN–0.84 *M* 4-MePy–0.56 *M* CH_3COOH in 30% aqueous methanol as the mobile phase (pH 5.5); column dimensions, 32 × 5 mm I.D.

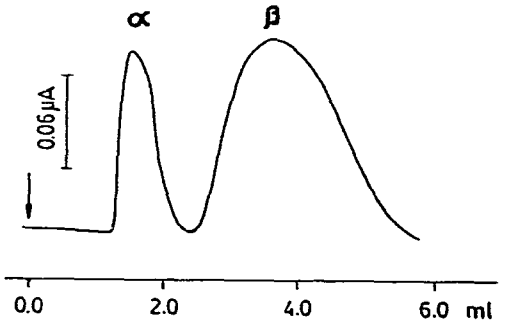


Fig. 5. Elution of α- and β-naphthoic acids with 0.1 *M* NH_4SCN–0.42 *M* 4-MePy–0.14 *M* CH_3COOH in 50% aqueous methanol (pH 5.8) as the mobile phase; column dimensions, 5.7 × 5 mm I.D. A.c. polarographic detection.

Figs. 2–5 show the separations of *o*-, *m*- and *p*-nitrobenzoic, *o*-, *m*- and *p*-nitrocinnamic, *cis*- and *trans*-*p*-nitrocinnamic and α- and β-naphthoic acids.

As a consequence of the fact that β-Ni(NCS)$_2$ (4-MePy)$_4$ · G is able to absorb molecules but not to exchange ions, the selectivity observed (Figs. 2–5) follows the rules previously reported for this solid phase. Thus *o*-, *m*- and *p*-nitrobenzoic and nitrocinnamic acids show $k_p > k_m > k_o$, similarly to dinitrobenzene isomers[8], $k_\beta > k_\alpha$ for naphthoic acids, which is analogous to the results obtained for the separation of nitronaphthalenes[9], and $k_{trans} > k_{cis}$ for *p*-nitrocinnamic acid, which is similar to the result for the selective clathration of azobenzenes[10].

As can be seen in Figs. 2–5, the elution curves of the organic acids have diffuse maxima and efficient separations are due to the high selectivity of the clathration and not to the efficiency of the columns.

REFERENCES

1 D. Sybilska, *Proceedings of the VIth International Symposium on Chromatography and Electrophoresis*, Presses Académiques Européennes, Brussels, 1971, pp. 212–221.
2 W. Kemula, D. Sybilska, J. Lipkowski and K. Duszczyk, *J. Chromatogr.*, 204 (1981), 23–28.
3 W. Kemula, in L. Zuman and I. M. Kolthoff (Editors), *Progress in Polarography*, Wiley-Interscience, New York, 1962, p. 397.
4 W. Kemula, B. Behr, K. Chlebicka and D. Sybilska, *Rocz. Chem.*, 39 (1965) 1315.
5 W. Kemula, D. Sybilska, J. Lipkowski and K. Duszczyk, *Polish J. Chem.*, 54 (1980) 317–324.
6 W. Kemula and H. Buchowski, *J. Phys. Chem.*, 63 (1959) 155–159.
7 E. Soczewiński, *Advan. Chromatogr.*, 5 (1968) 3.
8 J. Lipkowski, M. Pawlowska and D. Sybilska, *J. Chromatogr.*, 176 (1979) 43–53.
9 W. Kemula, D. Sybilska and K. Duszczyk, *Microchem. J.*, 11 (1966) 296.
10 W. Kemula, Z. Borkowska and D. Sybilska, *Monatsh. Chem.*, 103 (1972) 860–866.