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INTERFACE ADSORPTION AND REPRODUCIBILITY OF RETENTION INDICES IN GLASS CAPILLARY COLUMNS WITH DIMETHYLPOLY-SILOXANE STATIONARY PHASES CROSS-LINKED BY γ-IRRADIATION

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SUMMARY

The influence of the interphase adsorption on the retention of polar and non-polar analytes analyzed in glass capillary columns with the non-cross-linked and γ-irradiated cross-linked OV-1 stationary phase has been studied. The separation systems are characterized by a low contribution of adsorption to the retention of polar analytes, which is for film thicknesses above 0.3 μm less than 0.3% and upon cross-linking the stationary phase decrease further by 10–90% of this value. This enables high reproducibility of retention indices of the substances analyzed. The invariant retention indices of polar analytes in systems with both types of OV-1 stationary phases correspond to 0.4 i.u.

INTRODUCTION

Adsorption of chromatographed substances at the surface of phase boundaries, liquid–gas and liquid–solid support (capillary column wall), can have a decisive influence on retention characteristics as well as their reproducibility and on the possibility to identify the substances analyzed on the basis of retention data. This is connected with the fact that classical methods for expressing the analyte retention in qualitative analysis, the retention index and the retention ratio do not take into account their dependence on the properties of the support and the amount of stationary phase in the column.

In an earlier paper Berezkin and Korolev[1] reported the rôle of adsorption at the phase boundaries in fused-silica capillary columns coated with non-polar non-cross-linked and cross-linked polysiloxane stationary phases using benzoyl peroxide as a cross-linking initiator. They found that by cross-linking the non-polar dimethylpoly-siloxane stationary phase SE-30 the contribution of adsorption to the retention of polar substances increased and their retention indices were higher on the cross-linked

stationary phase than on the non-cross-linked one. The changes in retention have been explained by changes in the interactions of chromatographed substances with the stationary phase resulting from certain changes in the character and composition of the stationary phase due to its cross-linking.

The influence of cross-linking the polysiloxane stationary phases OV-1, OV-1$_{vin}$ and SE-54 while using dicumyl peroxide on the retention indices of polyaromatics and other polar substances in fused-silica capillary columns has been studied by Juutilainen and Enqvist[2]. They found that the polarity and selectivity of the stationary phases studied changed upon cross-linking due to reaction of cumyloxy radicals with the stationary phase, resulting in certain changes in its properties.

Cross-linking of the stationary phases by γ-irradiation seems to be more advantageous since when compared to cross-linking with peroxides there is no change in the stationary phase polarity or in the column resolution power and the column preparation is more simple[3–5].

In this work the influence of interphase adsorption and stationary phase cross-linking on the reproducibility of retention indices of polar and non-polar substances analyzed in glass capillary columns with the non-polar dimethylpolysiloxane phase OV-1 non-cross-linked and cross-linked by γ-irradiation was investigated.

EXPERIMENTAL

The columns (14–19 m × 0.325 ± 0.13 mm I.D.) were prepared from glass capillaries made of sodium–calcium Unihost glass (Kavalier, Teplice, Czechoslovakia) drawn on a laboratory-made drawing device. Their inside surfaces were treated with a slight modification of a procedure according to Grob *et al.*[6].

Capillaries packed to 95% of their volume with 20% analytical grade HCl (Merck, Darmstadt, F.R.G.) and fused under vacuum were leached at 130°C for 16 h. Then they were washed with two capillary volumes of 1% HCl at a linear velocity of approximately 1.5 cm s^{-1}. Immediately afterwards the capillaries were dehydrated at 295°C (temperature programme: 40 to 295°C at 20°C min^{-1}) and in a flow of dry nitrogen of 4–6 cm^3 min^{-1} for 1.5–2.5 h according to the capillary length. After cooling, the capillaries were packed to about 20% of their volume with hexamethyldisilazane (HMDS) (Fluka Chemie, Buchs, Switzerland) and then the HMDS was forced through the column with nitrogen at a linear velocity of approximately 1 cm s^{-1}. The fused capillaries were silanized at 380°C for 2 h in an oven (temperature programme 30 min at 150°C, then increased by 50°C every 15 min up to 380°C). The silanized capillaries were carefully cooled and washed immediately with about 25% of their volume with toluene, methanol and diethyl ether at a linear velocity of about 1.5 cm s^{-1}. On the toluene front usually tiny white crystals or a white precipitate was observed (see ref. 7). After brief passage of nitrogen through the capillaries at the laboratory temperature, they were placed in a gas chromatograph equipped with flame ionization detection (FID) and conditioned for 30–60 min at 250°C until the baseline signal of the detector was stabilized. Some of the capillaries were tested at 110°C by injection of pure 1-octanol and methane. The capacity factor of 1-octanol was 0.04. The stationary phase OV-1 (Supelco, Bonaduz, Switzerland) was coated on the capillary walls statically by means of a 0.06–1.64% (v/v), solution in *n*-pentane. The OV-1 film thickness was calculated from the concentration of its solution and the capillary I.D.[8].

The coated columns were conditioned at 250°C for 2 h and then tested. The tested columns were fused under vacuum and irradiated with the ^{60}Co γ-radiation in doses of 4–10 Mrad at a radiation intensity of *ca.* 0.16 Mrad h^{-1}. The irradiated columns were washed with 10 ml of dichloromethane at a flow-rate of about 4 ml h^{-1}, dried under a flow of nitrogen, conditioned at 250°C for 15 h and tested. The film thickness of cross-linked OV-1 was calculated from that of non-cross-linked OV-1 and the cross-linking percentage determined from the difference in capacity factors of *n*-decane in non-irradiated and irradiated columns. At the given doses of γ-radiation an average 97% cross-linking of OV-1 was obtained. The efficiency of the columns with film thickness 0.3–0.6 μm reached an average of 3200 theoretical plates per metre for the capacity factor, $k > 5$ (*ca.* 90% of the theoretical efficiency).

All the chromatographic measurements were carried out with a 4200 Fractovap gas chromatograph (Carlo Erba, Milan, Italy) with FID. The column thermostat temperature was calibrated by a platinum resistance thermometer with an accuracy of $\pm$ 0.1°C. The carrier gas, pure hydrogen, was applied at a linear flow-rate of 35 cm s^{-1}. The test mixture comprised *n*-decane, *n*-undecane, *n*-dodecane, 1-octanol, 2,6-dimethylaniline, 2,6-dimethylphenol and naphthalene in benzene, concentration 0.5–1.0 mg cm^{-3}. Methane was injected on the column simultaneously with 0.05 μl of the test mixture using a 1-μl syringe (Hamilton, Bonaduz, Switzerland) at the splitting ratio of 1:50. No asymmetry of the peaks of the tested analytes was observed on any of the columns studied (Fig. 1). Retention times were measured with an accuracy of 0.1 s on a SP 4100 programmable integrator (Spectra Physics). Columns with non-cross-linked and cross-linked OV-1 were tested at 110°C by measuring the capacity factors and retention indices of the analytes. The capacity factors were measured with an accuracy of $\pm$ 0.5% and retention indices on the columns with film thickness up to 0.1 μm with an accuracy characterized by a standard deviation of up to 0.8 i.u. and of 0.1 i.u. for the columns with higher OV-1 film thickness. All the calculations were carried out with a TI-59 programmable calculator (Texas Instruments).

RESULTS AND DISCUSSION

For separation systems in which polar substances are analyzed on non-polar stationary phases using gas–liquid chromatography, adsorption interactions of the analytes at the liquid phase–capillary surface boundary are characteristic[1,9]. The value of the analyte retention index is a linear function of the reciprocal of the amount of liquid phase in the column or the reciprocal of the thickness of the stationary phase film or of the capacity factor of the analyte with negligible adsorption at the system interphase[9]

$$I_i = I_{0i} + a_i\frac{1}{k_{st}} \tag{1}$$

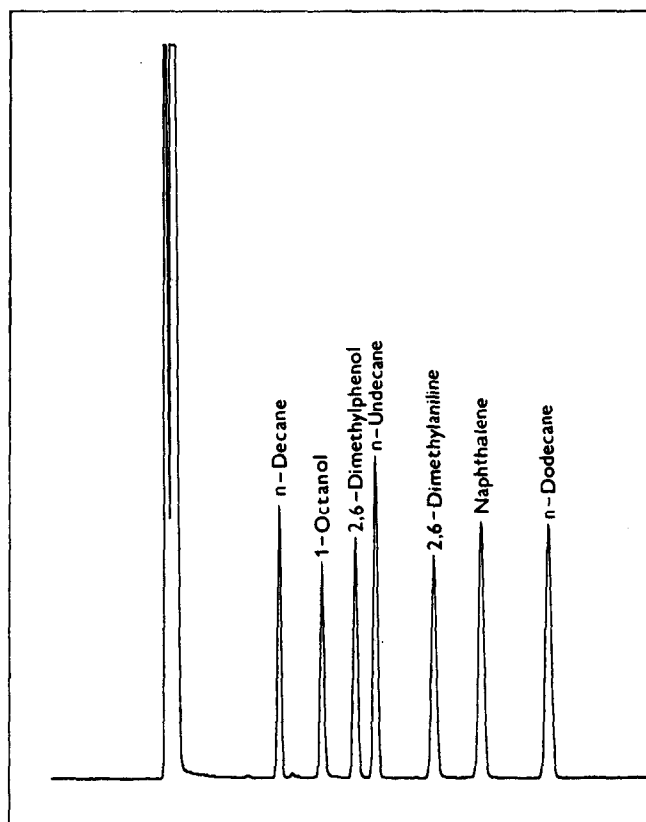$$I_{0i} = 100\left[z + \frac{\log(K_i/K_z)}{\log(K_{z+1}/K_z)} \right] \tag{2}$$

Fig. 1. Chromatogram of the tested substances on a column with cross-linked OV-1, film thickness, d_f = 0.310 μm.

where I_i is the retention index of the substance analyzed, I_{0i} the invariant retention index of the substance analyzed independent of adsorption in the chromatographic system, K_i the absorption coefficient of the substance analyzed, k_{st} the capacity factor of the standard substance whose adsorption in the system is negligible, and z is the number of carbon atoms of the n-alkane used as a comparison standard.

The contribution of adsorption to the retention of the substances studied in the systems with the non-cross-linked OV-1 stationary phase was calculated from

$$k_i = A_i + r_{0i}k_{st} \tag{3}$$

where k_i is the capacity factor of the substance analyzed, A_i the contribution of adsorption to its retention and $r_{0i} = t'_{Ri}/t'_{Rst}$ is the invariant retention ratio of the substance analyzed independent of adsorption.

In accord with previous studies[1,9], n-decane was selected as the analyte with negligible adsorption in the given system. Fig. 2 presents the dependence of the measured capacity factors of n-decane at 110°C in ten capillary columns with 0.325 $\pm$
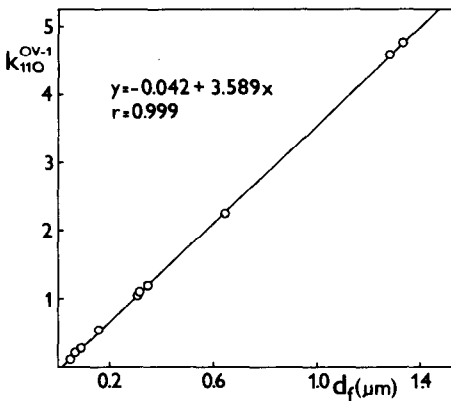
Fig. 2. Dependence of the capacity factor for *n*-decane on non-cross-linked OV-1 with different film thicknesses.

0.13 mm I.D. and different contents of the non-cross-linked phase OV-1 on the stationary phase film thickness; a very good correlation is seen. The capacity factor as an expression of the liquid phase content can be measured more easily and with higher accuracy; the film thickness is one of the basic parameters of the column.

The contribution of adsorption to the retention was calculated from the measured capacity factors of the analytes studied (1-octanol, 2,6-dimethylphenol, 2,6-dimethylaniline, naphthalene), k_i, and *n*-decane as the standard with negligible adsorption in the system, k_{st}, at 110°C by linear regression from eqn. 3 (Tables I and II).

In the columns with the non-cross-linked stationary phase OV-1 the adsorption contribution changes from 0.0006 to 1.04% in the film thickness range 0.092–0.647 μm. It increases with decreasing thickness of the phase film and with increasing polarity of the analytes studied. In the columns with the cross-linked phase the contribution of adsorption is 10–90% lower than that of the non-cross-linked phase. In the film thickness range 0.081–0.642 μm the contribution of adsorption reaches a maximum at 0.85% and decreases with increasing film thickness. Low values of the adsorption contribution to the retention of polar analytes in glass capillary columns coated with the non-polar phase OV-1 show that the method of surface deactivation of glass capillaries used leads to suppression of the adsorption and the method of cross-linking the stationary phase by γ-irradiation also suppresses the adsorption.

Even if the interphase adsorption in the systems studied is relatively small, it is reflected characteristically in the values of the retention indices of the analytes. Table III presents the measured retention indices of the analytes studied and the capacity factors of *n*-decane in ten capillary columns with different film thicknesses (0.047–1.336 μm) of the non-cross-linked phase OV-1. The graphic representation of the dependences $I_i = f(d_f)$, $I_i = f(k_{st})$ or $I_i = f(1/k_{st})$ (Fig. 3) shows the characteristic asymptotic decrease in retention indices of the analytes with increasing film thickness of the stationary phase as the result of suppression of the adsorption of polar analytes on the surface of the non-polar stationary phase–capillary column wall boundary. The character of this dependence is related to the polarity of the substances analyzed, the retention index of 2,6-dimethylphenol decreasing by 6.3 i.u. and of naphthalene by 0.5

P. FARKAŠ et al.

TABLE I

CONTRIBUTION OF ADSORPTION TO THE RETENTION OF THE ANALYTES STUDIED AND THE VALUES OF THE COEFFICIENTS OF EQN. 3 FOR NON-CROSS-LINKED OV-1

DMP = Dimethylphenol; DMA = dimethylaniline.

Analyte	$d_f = 0.092 \ \mu m$		$d_f = 0.305 \ \mu m$		$d_f = 0.648 \ \mu m$		A_{0i}	r_{0i}	r	$d_f = 0.500 \ \mu m^a$	
	k_i	$A_i \ (\%)$	k_i	$A_i \ (\%)$	k_i	$A_i \ (\%)$				k_i	$A_i \ (\%)$
1-Octanol	0.393	1.04	1.426	0.29	3.090	0.11	0.0041	1.361	0.999	2.387	0.17
2,6-DMP	0.474	0.44	1.727	0.12	3.746	0.06	0.0021	1.651	0.999	2.893	0.07
2,6-DMA	0.669	0.61	2.440	0.17	5.287	0.08	0.0041	2.330	0.999	4.084	0.10
Naphthalene	0.784	0.005	2.860	0.001	6.212	0.0006	0.00004	2.739	0.999	4.796	0.0008
n-Decane k_{st}	0.285	–	1.046	–	2.267	–	–	–	–	1.751	–

^{a} Hypothetical column with film thickness of 0.500 μm.

TABLE II

CONTRIBUTION OF ADSORPTION TO THE RETENTION OF THE ANALYTES STUDIED AND THE VALUES OF THE COEFFICIENTS OF EQN. 3 FOR CROSS-LINKED OV-1

Analyte	$d_f = 0.081 \ \mu m$		$d_f = 0.309 \ \mu m$		$d_f = 0.642 \ \mu m$		A_{0i}	r_{0i}	r	$d_f = 0.500 \ \mu m^a$	
	k_i	$A_i \ (\%)$	k_i	$A_i \ (\%)$	k_i	$A_i \ (\%)$				k_i	$A_i \ (\%)$
1-Octanol	0.351	0.85	1.390	0.22	2.073	0.10	0.0030	1.364	0.999	2.392	0.13
2,6-DMP	0.422	0.09	1.681	0.02	3.721	0.01	0.0004	1.654	0.999	2.896	0.01
2,6-DMA	0.595	0.12	2.372	0.03	5.249	0.01	0.0007	2.333	0.999	4.086	0.02
Naphthalene	0.693	–	2.786	–	6.163	–	−0.0037	2.742	0.999	4.797	–
n-Decane k_{st}	0.252	–	1.021	–	2.248	–	–	–	–	1.751	–

^{a} Hypothetical column with film thickness of 0.500 μm.

i.u. At film thicknesses higher than 0.3 μm the retention indices of the analytes are practically independent of the stationary phase film thickness (standard deviation of retention indices, $s = 0.1$ i.u.).

Table IV presents the measured retention indices of the analytes in ten capillary columns with different film thicknesses of OV-1 cross-linked by γ-irradiation at doses of 4.0–10.0 Mrad. Irradiation doses in the range 4–8 Mrad practically do not influence the value of the retention index. However, at a dose of 10 Mrad an increase in retention by 0.5–1.0 i.u. was observed with increasing polarity of the analytes. Therefore, only the data obtained on the columns irradiated with doses of 4–8 Mrad were evaluated.

During conditioning of the columns with OV-1 cross-linked by γ-irradiation at 250°C, the retention indices of the analytes decrease slightly with time, by 0.1–0.3 i.u. After 15 h of conditioning the retention practically did not change and, therefore, the data obtained after 15 h of conditioning at 250°C were selected for evaluation.

The dependence of the retention indices of the analytes studied on the film thickness of the cross-linked OV-1 phase is illustrated in Fig. 4. For film thicknesses above 0.08 μm the dependences are similar to those for non-cross-linked OV-1. For film thicknesses less than 0.08 μm a remarkable decrease in the retention indices of the analytes is observed with decreasing film thcikness. This shows that the thin films of cross-linked and non-cross-linked OV-1 have different characters.

From the measured retention indices of the analytes and their capacity factors on the given columns with film thicknesses above 0.08 μm, the values of the invariant retention indices, I_{0i}, and adsorption coefficients, a_i, were calculated by linear regression from eqn. 1. The data for the calculation were tested for irrelevant values according to Grubbs[10] and these values were excluded from the set (one value for 1-octanol, two values for 2,6-dimethylphenol and one value for naphthalene, all on non-cross-linked OV-1). The invariant retention indices were calculated with an accuracy (Table V) characterized by a standard deviation, $s = 0.1$–0.2 i.u. within the interval, $L_{1,2}$ $(I_{0i}) \pm 0.2$–0.5 i.u. at a significance level of 95% ($\alpha = 0.05$). The correlation coefficients, r, show that the correlation of the dependence $I_i = f(1/k_{st})$ is high. For naphthalene analyzed on non-cross-linked OV-1, the low value of the

TABLE III

RETENTION INDICES AND CAPACITY FACTORS OF THE ANALYTES STUDIED ON COLUMNS WITH DIFFERENT FILM THICKNESSES OF NON-CROSS-LINKED OV-1

| Column	I_{110}				k_{st110}	d_f (μm)
					$\overline{n\text{-}Decane}$	
	1-Octanol	2,6-DMP	2,6-DMA	Naphthalene		
1	1052.2	1089.4	1141.8	1167.2	0.127	0.047
2	1052.1	1084.5	1141.0	1167.5	0.215	0.070
3	1053.3	1084.1	1141.2	1167.5	0.285	0.092
4	1051.7	1084.1	1140.7	1167.4	0.551	0.156
5	1051.3	1083.2	1140.3	1167.1	1.046	0.305
6	1051.1	1083.2	1140.5	1167.4	1.092	0.314
7	1051.0	1083.1	1140.4	1167.2	1.183	0.351
8	1051.2	1083.1	1140.3	1167.1	2.267	0.647
9	1051.1	1083.0	1140.1	1166.9	4.570	1.283
10	1051.1	1083.2	1140.2	1167.1	4.758	1.336
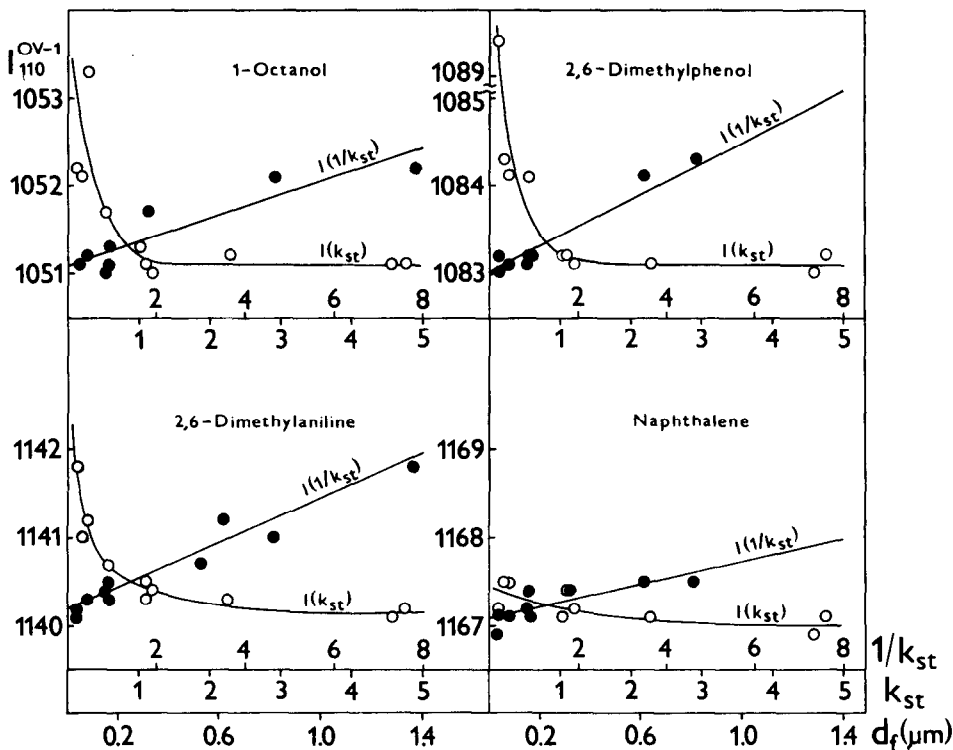
Fig. 3. Dependences of retention indices of the analytes studied on non-cross-linked OV-1 with different film thicknesses.

correlation coefficient is connected with a small dependence of the retention index on the stationary phase film thickness. In the case of cross-linked OV-1, the low value of the correlation coefficient, $r = -0.505$, is connected with the fact that the dependence $I = f(k_{st})$ for naphthalene on thin OV-1 films is not described by eqn. 3 so precisely as in the cases of the other analytes studied.

The calculated invariant retention indices of polar analytes, I_{0i}, on non-cross-linked and cross-linked OV-1 differ on average by $\Delta I_{0i} = 0.4$ i.u. and they are systematically higher on cross-linked OV-1. The value of $\Delta I_{0i} = 0.6$ i.u. for naphthalene is slightly higher than expected, which is connected with the above-mentioned character of the dependence $I = f(k_{st})$ for this analyte on non-cross-linked OV-1.

The results obtained show that upon cross-linking OV-1 with γ-irradiation the stationary phase polarity increases only slightly. For comparison, the published invariant retention indices of the same substances measured in fused-silica capillary columns coated with the phase SE-30 cross-linked with benzoyl peroxide[1] were higher by 6.4–14.5 i.u., and the similar retention indices in the fused-silica capillary columns with OV-1 cross-linked with dicumyl peroxide[2] were higher by 6.1–18.1 i.u. as on the non-cross-linked stationary phase.

TABLE IV

RETENTION INDICES AND CAPACITY FACTORS OF THE ANALYTES STUDIED ON COLUMNS WITH DIFFERENT FILM THICKNESSES OF CROSS-LINKED OV-1

| Column	I_{110}				$k_{st\,110}$ n-Decane	d_f (μm)	Radiation dose (Mrad)	Cross-linking (%)
	1-Octanol	2,6-DMP	2,6-DMA	Naphthalene				
1	1053.4	1082.1	1137.5	1160.4	0.126	0.047	4.0	99.2
2	1055.9	1084.0	1141.4	1166.7	0.212	0.069	4.0	98.6
3	1054.8	1085.5	1142.0	1167.3	0.252	0.081	4.0	98.4
4	1052.2	1084.4	1141.3	1167.5	0.534	0.151	6.5	96.9
5	1051.9	1083.7	1141.0	1167.8	1.021	0.309	4.0	97.6
6	1052.0	1083.8	1141.1	1167.8	1.107	0.310	8.0	101.4
7	1052.5	1083.9	1141.1	1167.5	1.167	0.343	6.0	98.6
8	1051.9	1083.6	1140.9	1167.6	2.248	0.642	6.5	99.2
9	1051.6	1083.6	1140.6	1167.4	4.437	1.246	6.5	97.1
10	1052.8	1084.7	1141.8	1168.1	4.558	1.280	10.0	95.8
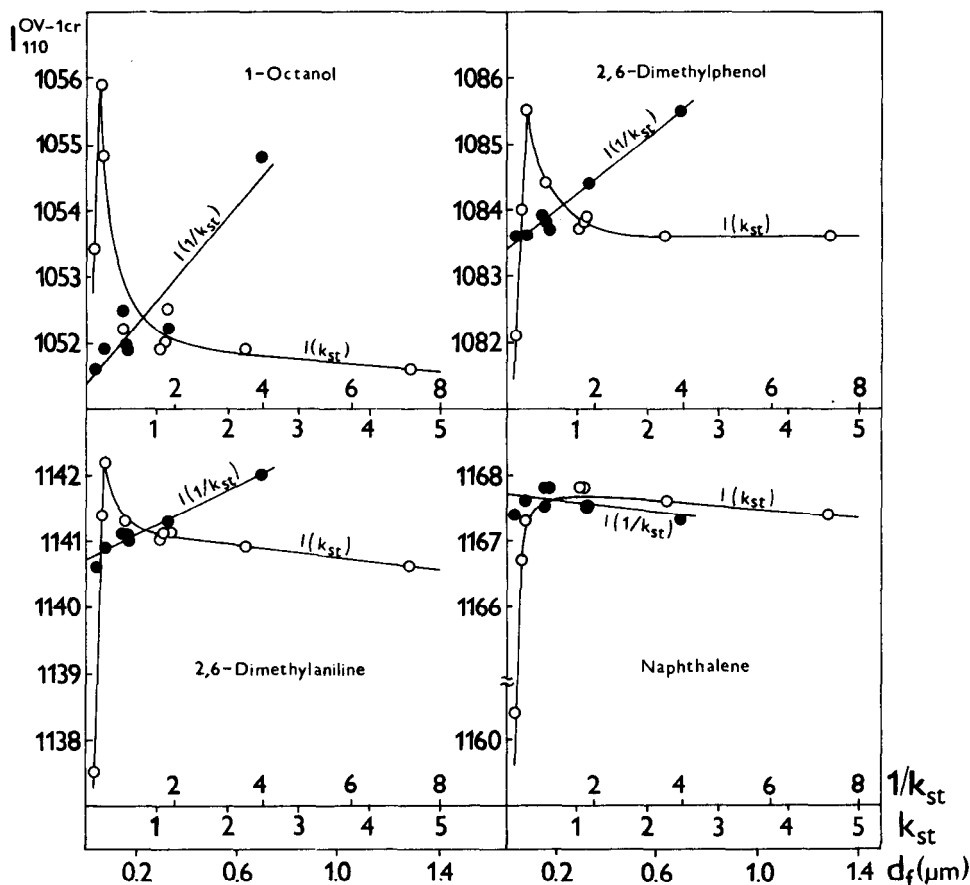
Fig. 4. Dependences of retention indices of the analytes studied on cross-linked OV-1 with different film thicknesses.

TABLE V

INVARIANT RETENTION INDICES OF THE ANALYTES STUDIED ON NON-CROSS-LINKED AND CROSS-LINKED OV-1

Analyte	St. phase[a]	I_{0i}	$S_{(I0i)}$	$L_{1,2(I0i)}$	a_i	r	ΔI_{0i}	$I_{0.5} - I_{0i}$
1-Octanol	OV-1	1051.1	0.1	0.2	0.163	0.917	0.3	0.1
	OV-1 cr.	1051.4	0.2	0.5	0.802	0.941		0.4
2,6-DMP	OV-1	1083.0	0.1	0.2	0.295	0.999	0.4	0.1
	OV-1 cr.	1083.4	0.1	0.3	0.533	0.990		0.2
2,6-DMA	OV-1	1140.2	0.1	0.2	0.208	0.968	0.5	0.1
	OV-1 cr.	1140.7	0.1	0.3	0.332	0.974		0.2
Naphthalene	OV-1	1167.1	0.1	0.2	0.110	0.813	0.6	0.0
	OV-1 cr.	1167.7	0.1	0.3	−0.075	−0.505		0.1

[a] cr. = Cross-linked.

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