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Study of Support-Solvent Effects in Thin-Layer Chromatography on Cellulose and Starch

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Abstract

The retention behavior of various classes of homologous compounds has been studied in thin-layer chromatography on cellulose and starch. The effects of the developing solvent composition and of the number of methylene groups in the solute molecule have been discussed using the retention constant, R_M. The results obtained indicate that the particular classes of homologous compounds studied are separated from one another only be virtue of their functional groups and that the compositions of the stationary and the mobile phases on both cellulose and starch are the same. It was also observed that the polarity differences between the mobile and the stationary phase proved to be changed by varying the molar proportions of the developing solvent components. Therefore, depending on their properties, some classes of solutes examined are separated on "normal" phases and the others on "reversed" phases.

INTRODUCTION

In a previous paper (*1*) the relations between the chromatographic retention of various classes of homologous compounds in thin-layer chromatography on cellulose and starch and the thermodynamic properties of the system were investigated. With all classes of the studied compounds, straight lines with the same slopes were obtained when plotting R_M values, measured with the same mixed solvent on cellulose and starch, against the number of

CH_2 groups in the solute molecule. The identity of the slopes means that the composition of the stationary phase formed on cellulose and starch is the same, i.e., for solute i, $K_{Di}^{ST} = K_{Di}^{CE}$ and

$$R_{Mi}^{ST} - R_{Mi}^{CE} = \log\left[(\Phi_m/\Phi_s)^{CE}/(\Phi_m/\Phi_s)^{ST}\right] \qquad (1)$$

where K_{Di} is the distribution constant, Φ_m and Φ_s are the cross-sectional areas of the mobile (m) and the stationary (s) phase, and $R_{Mi} = \log[(1/R_{fi}) - 1]$. The superscripts ST and CE denote starch and cellulose, respectively. If Eq. (1) is correct, it should also apply to the analogous situations in which R_{Mi} is varied as a function of the solvent composition.

The role of the parameters Φ_m/Φ_s and K_D in paper partition chromatography with mixed solvents and controlled proportions of their components was thoroughly investigated by Thoma et al. (*2, 3*). Jandera and Churaček (*4*) derived an equation relating the capacity ratio in liquid–liquid column chromatography with the composition of the mobile phase. Both groups of investigators employed the solubility-parameters theory and arrived at basically the same equation, predicting the R_M and/or the logarithm of the capacity ratio to vary linearly with the mole fraction of the more polar component of the solvent mixture. The aim of this paper is to provide additional evidence for the validity of Eq. (1) in thin-layer chromatography on cellulose and starch layers by measuring R_M as a function of the solvent composition.

EXPERIMENTAL

Cellulose MN300 and rice starch were used for the preparation of thin layers as described earlier (*1*). Straight-chain monocarboxylic acids, α-amino monocarboxylic acids, α-hydroxy monocarboxylic acids, and alkyl-3,5-dinitrobenzoates (alkyl-3,5-DNB) were used as model solute compounds. The developing solvent mixtures employed were composed of *n*-propanol and water with various proportions of the components. The chromatograms were run by the ascending technique in tanks saturated with the solvent vapors at about 22°C. The spots of compounds were detected as described earlier (*1*).

RESULTS AND DISCUSSION

For all classes of solutes and both supports studied, Eq. (2) gives a chromatograms were run and the R_f values determined were averaged. The standard deviation of one determination of R_f was 0.016. Linear relationships were obtained with all solutes examined if R_M was plotted against the mole fraction of water (x_w). Hence we can write

$$R_{Mi} = m_i x_w + \log Q_i \tag{2}$$

where m_i and Q_i are constants.

For all classes of solutes and both supports studied, Eq. (2) gives a family of straight lines intersecting at the same point. An example is shown in Fig. 1. The intersecting point coordinates are (x_o, R_{MO}). The family of straight lines obtained is represented by the equation

$$R_{Mi} - R_{MO} = m_i(x_w - x_o) \tag{3}$$

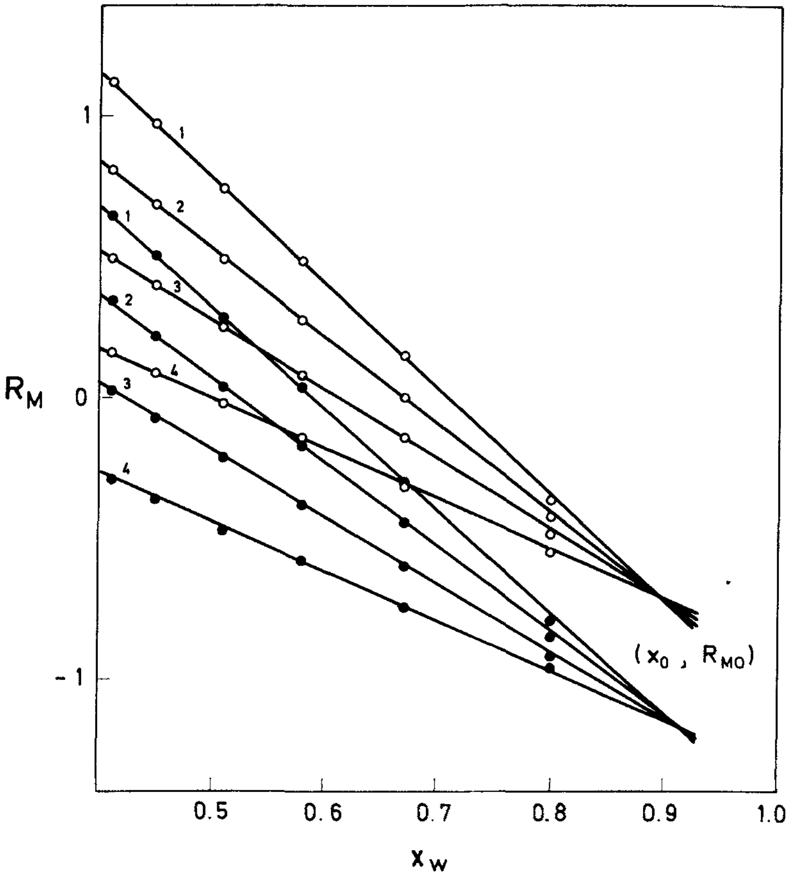

FIG. 1. Plots of R_M versus mole fraction of water (x_w) for α-hydroxy monocarboxylic acids on cellulose (●) and starch (○) layers. (x_o, R_{MO}) = intersecting point coordinates. Solvent: *n*-propanol–water. Designation of samples as in Table 1.

The following relation is generally valid:

$$R_{Mi} = \log K_{Di} + \log \Phi \tag{4}$$

where $\Phi = \Phi_s/\Phi_m$. The linearity between R_{Mi} and x_w is not sufficient evidence for the constancy of Φ with varying x_w (*2*) because, if we suppose $\log K_{Di}$ and $\log \Phi$ are linear functions of x_w, then, for homologous compounds and the given solvent components, the $\log K_{Di}$ function also forms a family of straight lines with intersecting point coordinates (x_o, $\log K_{D0}$), whereas $\log \Phi$, at coordinate x_o, is equal to $\log \Phi_0$. Thus we can write

$$\log K_{Di} - \log K_{D0} = a_i(x_w - x_o) \tag{5}$$

and

$$\log \Phi - \log \Phi_0 = b(x_w - x_0) \tag{6}$$

where a_i and b are constants. Introducing Eqs. (5) and (6) into Eq. (4) and rearranging, we obtain

$$R_{Mi} = \log K_{D0} + \log \Phi_0 + (a_i + b)\,(x_w + x_o) \tag{7}$$

Comparing Eqs. (3) and (7), we have

$$R_{M0} = \log K_{D0} + \log \Phi_0 \tag{8}$$

and

$$m_i = a_i + b \tag{9}$$

Now, Eq. (7) can be written in the form

$$R_{Mi} = m_i x_w + \log K_{D0} + \log \Phi_0 - m_i x_o = m_i x_w + \log Q_i \tag{10}$$

where

$$Q_i = \frac{K_{D0}\Phi_0}{10^{m_i x_o}}$$

Comparing Eqs. (10) and (4), we have

$$K_{Di} = \frac{Q_i}{\Phi}\,10^{m_i x_w} = \frac{K_{D0}\Phi_0}{\Phi}\,10^{m_i(x_w - x_o)} \tag{11}$$

On the other hand, if we suppose that Φ is constant and independent of x_w, then $\Phi = \Phi_0$. Hence, Eq. (7) can be expressed as

TABLE 1

Linear Relation between Retention and Solvent Composition for Acids Examined on Cellulose and Starch. Values of m_i, $\log Q_i$, and R_{M0}

		Cellulose		Starch		Cellulose		Starch	
No.	Acid	m_i	$\log Q_i$	m_i	$\log Q_i$	x_0	R_{M0}	x_0	R_{M0}
				Unsubstituted Acids					
1	Acetic	−3.64	1.90	−3.86	2.53				
2	Propionic	−2.98	1.31	−3.17	1.90				
3	*n*-Butyric	−2.30	0.70	−2.42	1.22	0.91	−1.39	0.91	−0.97
4	*n*-Valeric	−1.67	0.13	−1.74	0.61				
5	Caproic	−1.03	−0.47	−1.04	−0.03				
				Monoxydroxy Acids					
1	Glycollic	−3.66	2.16	−3.76	2.67				
2	Lactic	−3.02	1.58	−3.12	2.09				
3	2-Hydroxy-*n*-butyric	−2.38	1.00	−2.48	1.52	0.90	−1.14	0.90	−0.71
4	2-Hydroxy-*n*-valeric	−1.71	0.41	−1.78	0.89				
				Monoamino Acids					
1	Glycine	−3.68	2.46	−3.76	2.98				
2	α-Alanine	−2.96	1.81	−3.16	2.44				
3	α-Amino-*n*-butyric	−2.48	1.38	−2.43	1.79	0.90	−0.84	0.90	−0.40
4	Norvaline	−1.72	0.71	−1.70	1.13				
5	Norleucine	−1.05	0.10	−1.00	0.50				

$$R_{Mi} = \log K_{D0} + \log \Phi + m_i(x_w - x_o) \tag{12}$$

and

$$K_{Di} = K_{D0}\, 10^{m_i(x_w - x_o)} \tag{13}$$

Both suppositions give a linear relation between R_{Mi} and x_w. The values of m_i, log Q_i, x_o, and R_{M0} for the series of homologous acids studied are presented in Table 1.

The fact that lines for particular homologous compounds intersect at the same point proves that there is the same distribution constant, K_{D0}, for all homologs at x_o for a given chromatographic system. However, this is possible only when the retention of solute is based on the mechanism of liquid–liquid partition. Therefore, the particular classes of homologous compounds studied are separated from one another only by virtue of their functional groups, i.e., on the basis of their different K_{D0} values. On the other hand, R_{M0}^{CE} and R_{M0}^{ST} values are reached at the same x_o value on both cellulose and starch layers; that means that the compositions of the stationary and the mobile phases on cellulose and starch are the same.

When experimentally obtained R_M values were plotted against the number of CH_2 groups (n) in acid molecules, a family of straight lines intersecting at the same point was also obtained (Fig. 2). The intersecting point coordinates are (n_o, R_{M0}). This linear free energy relationship for particular lines can be expressed as

$$R_{Mx} = m_x n + \log q_x \tag{14}$$

and for the families of lines as

$$R_{Mx} - R_{M0} = m_x\ (n - n_o) \tag{15}$$

where m_x and q_x are constants dependent on the solvent composition. In such a case the cross-sectional areas ratio, Φ, is independent of the number of CH_2 groups in solute molecule, thus

$$R_{Mx} = \log K_{D0} + \log \Phi + m_x(n - n_o) \tag{16}$$

and

$$K_{Dx} = \frac{q_x}{\Phi}\, 10^{m_x n} = K_{D0}\, 10^{m_x(n - n_o)} \tag{17}$$

The values of m_x, log q_x, n_o, and R_{M0} for the series of homologous acids studied are shown in Table 2. It can be seen from Tables 1 and 2 that R_{M0} values in Eqs. (3) and (15), for the corresponding series of acids studied, are equal. Therefore,

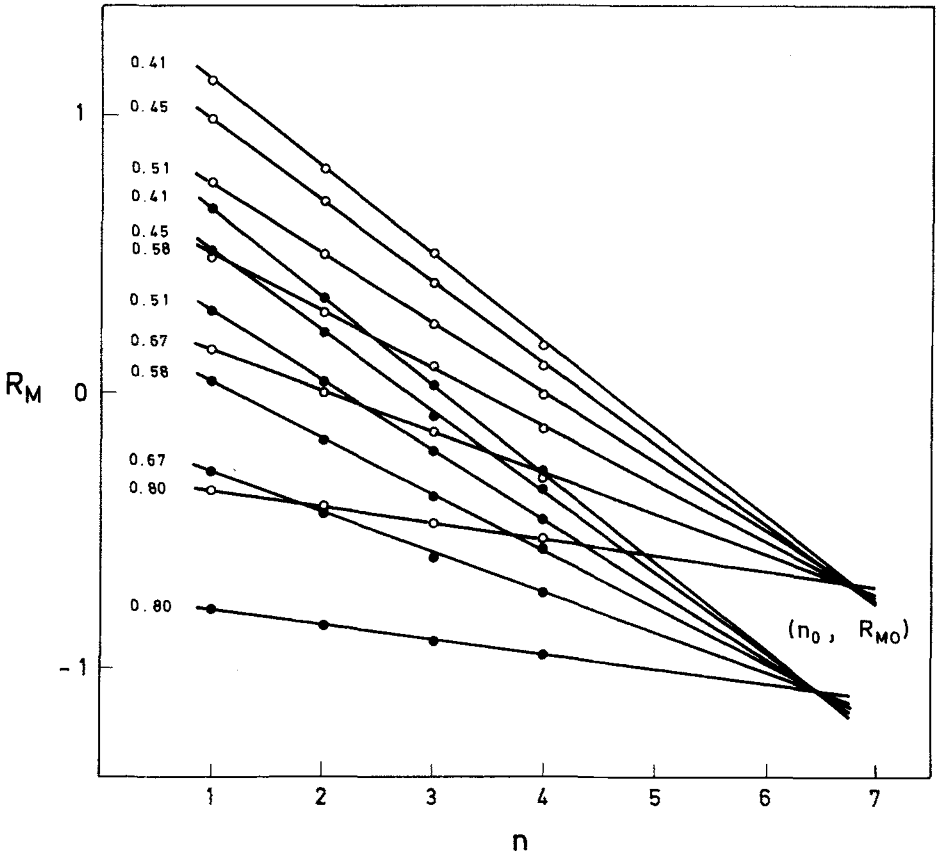

FIG. 2. Plots of R_M versus number of methylene groups (n) in α- hydroxy monocarboxylic acid molecule for mole fractions of water studied on cellulose (●) and starch (○) layers. (n_o, R_{Mo}) = intersecting point coordinates. Solvent: n-propanol–water. Designation of water mole fractions as in Table 2.

$$m_i(x_w - x_o) = m_x(n - n_o) \tag{18}$$

The equality expressed by Eq. (18) is possible only when the cross-sectional areas ratio, Φ, is not a function of x_w. Also, log Φ is not simply equal to R_{Mo} (*2*, *3*). Under such circumstances, $R_{M0}^{\mathrm{ST}} - R_{M0}^{\mathrm{CE}} = \log\ (\Phi^{\mathrm{ST}}/\Phi^{\mathrm{CE}})$ and $Q_i^{\mathrm{ST}}/Q_i^{\mathrm{CE}} = q_x^{\mathrm{ST}}/q_x^{\mathrm{CE}} = \Phi^{\mathrm{ST}}/\Phi^{\mathrm{CE}}$. By processing the appropriate experimental data for acids, it was found that $\Phi^{\mathrm{ST}}/\Phi^{\mathrm{CE}} = 3, R_M(\mathrm{OH}) = 0.232$, and $R_M(\mathrm{NH_2}) = 0.54$. These values are very close to those obtained previously (*1*).

The slope m_i on both cellulose and starch is actually the same for acids

TABLE 2

Linear Relation between Retention and Number of Methylene Groups in Acids Molecule for Water Content (x_w) Used. Values of m_x, log q_x, and R_{M0}

	Cellulose		Starch		Cellulose		Starch	
x_w	m_x	log q_x	m_x	log q_x	n_o	R_{M0}	n_o	R_{M0}
			Unsubstituted Acids					
0.41	−0.32	0.73	−0.35	1.30				
0.45	−0.30	0.57	−0.32	1.11				
0.51	−0.26	0.31	−0.28	0.84				
0.58	−0.21	0	−0.23	0.51	6.6	−1.41	6.5	−0.99
0.67	−0.15	−0.38	−0.17	0.11				
0.80	—	—	−0.07	−0.50				
			Monohydroxy Acids					
0.41	−0.32	0.98	−0.32	1.45				
0.45	−0.29	0.80	−0.30	1.28				
0.51	−0.25	0.53	−0.26	1.01	6.6	−1.10	6.8	−0.74
0.58	−0.20	0.23	−0.21	0.70				
0.67	−0.15	−0.14	−0.15	0.30				
0.80	−0.06	−0.73	−0.06	−0.30				
			Monoamino Acids					
0.41	−0.32	1.27	−0.34	1.81				
0.45	−0.29	1.08	−0.31	1.60				
0.51	−0.25	0.82	−0.26	1.28	6.6	−0.82	6.4	−0.39
0.58	−0.20	0.53	−0.22	1.04				
0.67	−0.15	0.14	−0.16	0.63				
0.80	−0.06	−0.45	−0.06	0.02				

containing the same number of CH_2 groups; that means that for a series of n-alkyl homologous compounds of different functionalities, constant m_i depends only on the number of CH_2 groups in a solute molecule. The difference in the partial molar excess Gibbs free energies per solute methylene group in the mobile and the stationary phase is equal to m_x, i.e.,

$$m_x = \Delta G_d^*(CH_2) = 2.3RTR_M(CH_2) = P_m - P_s$$

where P_m and P_s are the polarities of the mobile and the stationary phase, respectively (*1*). Negative m_x values show that the polarity of the stationary phase is greater than the polarity of the mobile phase, i.e., $P_m < P_s$. There is a linear dependence between m_i and n (Fig. 3a) and between m_x and x_w (Fig. 3b). The values of both slopes are equal to an m_i increment per solute methylene group.

The results of alkyl-3,5-DNB (Table 3) show positive values of m_i and m_x

on both cellulose and starch, indicating $P_m > P_s$. Regarding both kinds of solutes, we have an unusual situation: in the same solvent system acids are separated on the "normal" phases and alkyl-3,5-DNB on the "reversed" phases. In order to solve this problem we must take into consideration the range of solvent component proportions in which the separation of all participants would be attained. The satisfactory separations of particular classes of acids and of alkyl-3,5-DNB were attained in the region of water molar concentrations between 0.4–0.8 and 0.9–0.94, respectively, as shown in Fig. 4 for caproic acid (C_5) and amyl-3,5-DNB (C_5 alkyl chain). The solubility of acids in the mobile phase increases and of alkyl-3,5-DNB

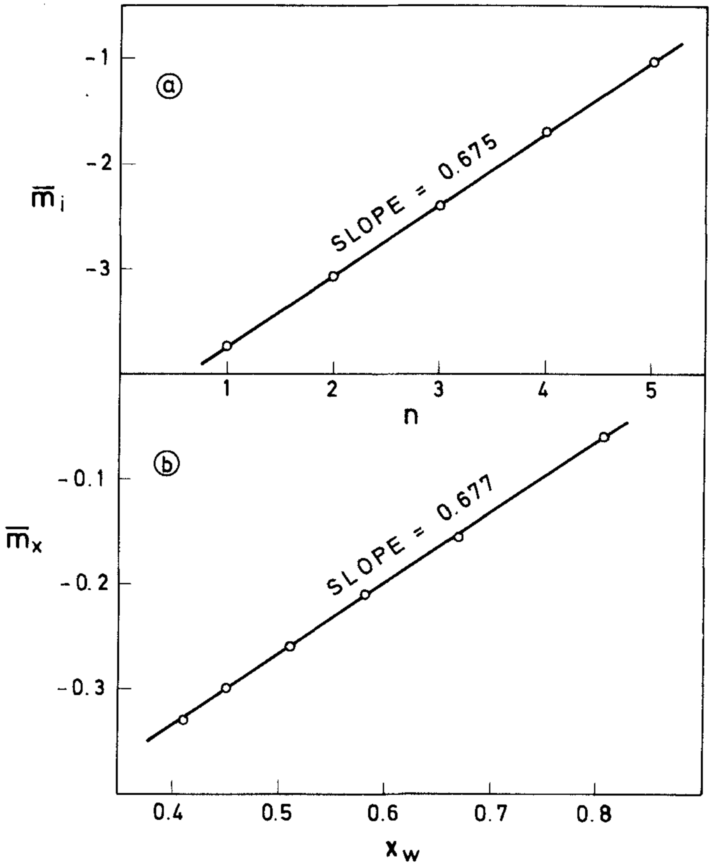

FIG. 3. Plots of (a) means values of slope m_i (Eq. 2) versus number of methylene groups (n) in molecule of acids studied and (b) mean values of slope m_x (Eq. 14) versus mole fraction of water (x_w). Particular m_i and m_x values are presented in Tables 1 and 2, respectively.

TABLE 3

Linear Relation between Retention and Solvent Composition (x_w) and between Retention and Number of Methylene Groups (n) in Aliphatic Chain of Alkyl-3,5-DNB

		Cellulose		Starch		Cellulose		Starch	
Alcohol	n	m_i	$\log Q_i$	m_i	$\log Q_i$	x_0	R_{M0}	x_0	R_{M0}
Pentanol-1	5	19.90	−18.91	20.00	−18.47	0.7	−4.88	0.7	−4.38
Hexanol-1	6	20.55	−19.35	20.63	−18.91				
Heptanol-1	7	21.23	−19.86	21.37	−19.47				
Octanol-1	8	22.05	−20.44	22.10	−19.97				
Nonanol-1	9	22.72	−20.92	22.80	−20.47				
Decanol-1	10	23.39	−21.38	23.50	−20.96				
Undecanol-1	11	24.10	−21.87	24.25	−21.49				
Dodecanol-1	12	24.82	−22.37	24.80	−21.83				
x_w		m_x	$\log q_x$	m_x	$\log q_x$	n_0	R_{M0}	n_0	R_{M0}
0.896		0.136	−1.78	0.135	−1.25	−23	−5.00	−25	−4.56
0.906		0.142	−1.60	0.142	−1.08				
0.916		0.149	−1.43	0.149	−0.91				
0.925		0.156	−1.28	0.155	−0.76				
0.934		0.162	−1.13	0.157	−0.58				
0.943		0.168	−0.99	0.161	−0.42				

decreases with an increase of water content in the mobile phase. The molar water concentration range for the possible separation of alkyl-3,5-DNB is rather narrow (Fig. 4) and in that region the polarity of the mobile phase is greater than of the stationary phase. This means that the variation of the mobile phase composition (or polarity) is not followed by the variation of the stationary phase composition (or polarity) in the same way, i.e., when increasing the water content in the solvent system, the polarity of the mobile phase increases faster than the polarity of the stationary phase. At the point of intersection of the lines in Fig. 4 the polarity of the mobile phase is equal to the polarity of the stationary phase ($P_m = P_s$), leftward from this point $P_m < P_s$, and rightward $P_m > P_s$. The water content at this point, for the solvent system n-propanol–water, is about 63% on both cellulose and starch. By increasing the total polarity of the solvent system, for example, in the solvent system ethanol–water, the retention of the acids decreased and the retention of alkyl-3,5-DNB increased, while the point of intersection moved leftward. It is doubtful whether any definite conclusions about the nature of the stationary phase can be derived only from chromatographic data. It is

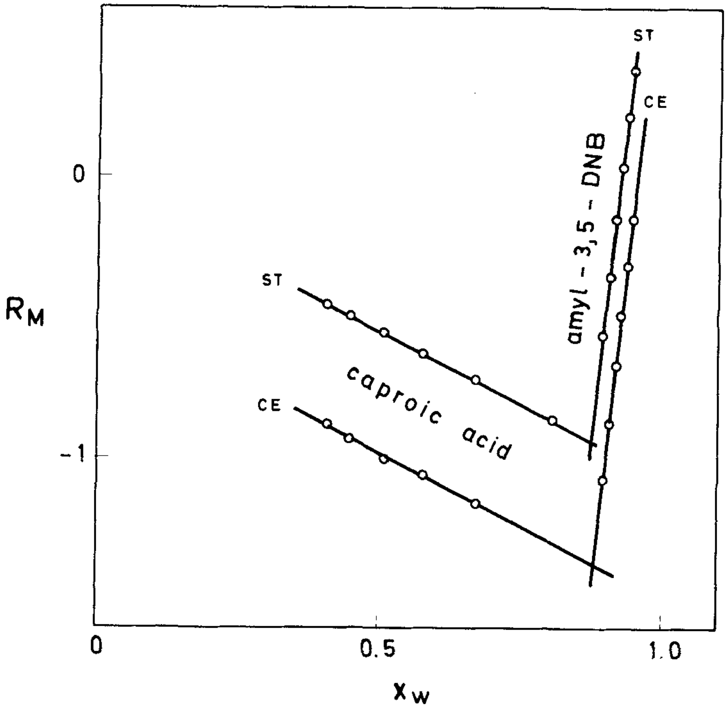

FIG. 4. Plots of R_M versus mole fraction of water (x_w) for caproic acid and amyl-3,5-dinitrobenzoate (DNB) on cellulose (CE) and starch (ST) layers.

possible that: (a) the stationary phase contains both water and organic modifier and that by increasing the water concentration in the mobile phase, the concentration of organic modifier in the stationary phase decreases slower than in the mobile phase, or (b) the polarity of water molecules bonded to the support (H-bonds) is lower than that of free molecules in the mobile phase. To evaluate the mode of stationary phase formation it is necessary to take into consideration the fact that chromatographic processes in thin-layer chromatography take place in an open three-phase system (solid–liquid–gaseous), especially when a conditioning of layers is carried out before developing.

From the experimental data for alkyl-3,5-DNB it was also found that $\Phi^{ST}/\Phi^{CE} = 2.95$ and the m_i increment per solute methylene group (0.66) is actually equal to that obtained for acids (Fig. 3).

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