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Study of the Homologous Retention Increment in Flat-Bed Chromatography

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Abstract

Relations between the chromatographic retention of various classes of homologous compounds in flat-bed chromatography systems and the thermodynamic properties of the latter were investigated. Provided the retention of solute (i) is due to liquid-liquid partition, the R_{Mi} is related to the partial molar excess Gibbs free energies of the solute in the mobile and stationary phases, G^E_{im} and G^E_{is}, by $R_{Mi} = [(G^E_{im} - G^E_{is})/2.3RT] - \log A$, where R and T are the universal gas constant and the absolute temperature of the system, respectively, and $A = d_m M_s \phi_m / d_s M_m \phi_s$, d, M, and ϕ denoting the densities, molar masses, and cross-sectional areas of the mobile and the stationary phases, m and s, respectively. The difference in the partial molar excess Gibbs free energies per solute methylene group in the mobile and in the stationary phase, related with the R_M increment per methylene group of solute by $G^E_m(CH_2) - G^E_s(CH_2) = 2.3RTR_M(CH_2)$, can be used to characterize the difference in the polarities of the phases.

INTRODUCTION

The homologous retention increment has been a subject of interest to chromatographers since the earliest times of the development of chromatographic techniques. Most of the empirically observed regularities between the chromatographic behavior of homologous compounds and their physicochemical and/or structural properties can readily be interpreted in terms of a

homologous increment. Naturally, the first fundamental papers on this topic come from the area of flat-bed chromatography (*1–4*). However, the significance of the homologous retention increment is quite general in chromatography. Actually, the commonly recognized advantages of using the carbon number concept (*5–10*) and/or retention index systems (*11*) to process gas chromatographic retention data are largely due to an approximate constancy and additivity of the logarithms of retention increment per a methylene group of solute on a given stationary phase at fixed conditions. The possibility of employing different classes of reference compounds in the above systems was shown to be based on the same argument (*12*). The homologous retention increment was also found to be a possible measure of the polarity of gas chromatographic stationary phases (*13–15*).

The aim of this paper is to present thermodynamically rigorous relations between chromatographic retention data measured in flat-bed liquid–liquid systems and the properties of the latter, and to discuss the possibility of using the homologous R_M increment as a criterion of the polarity of the phases in flat-bed systems.

THEORETICAL

Providing chromatographic retention is based on the mechanism of liquid–liquid partition, the standard Gibbs function of the transition of 1 mol of solute i from a standard state of pure solute at infinite dilution in the stationary phase (s) to a standard state of pure solute at infinite dilution in the mobile phase (m) at the temperature (T) and pressure in the system, i.e., the standard molar Gibbs function of desorption, ΔG^*_{di}, is given by

$$\Delta G^*_{di} = RT \ln (\gamma^*_{is} x_{is} / \gamma^*_{im} x_{im}) \tag{1}$$

where γ^*_{is}, γ^*_{im}, x_{is}, and x_{im} are the Henry-law activity coefficients and the mole fractions of the solute in the stationary phase and in the mobile phase, respectively, R being the perfect-gas constant. At very low solute concentrations, both γ^*_{is} and γ^*_{im} approach unity, and

$$G^*_{di} = RT \ln (x_{is}/x_{im}) = RT \ln (\gamma^\circ_{im}/\gamma^\circ_{is}) = G^E_{im} - G^E_{is} \tag{2}$$

where γ°_{im} and γ°_{is} are the Raoult-law activity coefficients of the solute in the mobile and stationary phases, and G^E_{im} and G^E_{is} are the corresponding partial molar excess Gibbs functions, respectively. Provided the solute distribution constant, K_{Di}, is defined as

$$K_{Di} = \frac{n_{is}V_m}{n_{im}V_s} = \frac{x_{is}d_sM_m}{x_{im}d_mM_s} = \left(\frac{1}{R_{fi}} - 1\right)\frac{\phi_m}{\phi_s} \qquad (3)$$

where n_{is} and n_{im} are the mole numbers of the solute present in the volumes V_s and V_m of the stationary and the mobile phase, respectively; d, M, and ϕ are the densities, molar masses, and cross-sectional areas of the phases; and R_{fi} is the retardation factor of the solute, Eqs. (2) and (3) yield

$$G^*_{di} = RT \ln \frac{K_{Di}d_mM_s}{d_sM_m} = 2.3RTR_{Mi} + RT \ln \frac{\phi_m d_m M_s}{\phi_s d_s M_m} \qquad (4)$$

where (*3*) $R_{Mi} = \log [(1/R_{fi}) - 1]$. Applying Martin's (*2, 16*) theorem of the additivity of ΔR_M values corresponding to the individual groups constituting the solute molecule, it is possible to write for a monofunctional straight-chain compound $i = CH_3(CH_2)_nX$:

$$\Delta G^*_{di} = \Delta G^*_d\ (CH_3) + n\Delta G^*_d(CH_2) + \Delta G^*_d(X) \qquad (5)$$

and

$$d\Delta G^*_{di}/dn = \Delta G^*_d(CH_2) = G^E_m(CH_2) - G^E_s(CH_2) = 2.3RT(dR_{Mi}/dn)$$
$$= 2.3RTR_M(CH_2) \qquad (6)$$

The quantity $G^E(CH_2)$ is a measure of the nonideality of a dilute solution of a unit of nonpolar mass (a methylene unit) in the given liquid, or, in other words, a measure of the reluctance of the liquid to accommodate a CH_2 unit, thus constituting a thermodynamically defined criterion of the polarity of the liquid (*17–22*). Hence, denoting the polarities of the mobile phase and the stationary phase by P_m and P_s, we have

$$P_m - P_s = 2.3RTR_M(CH_2) = \Delta G^*_d(CH_2) \qquad (7)$$

According to Eq. (7), positive values of $\Delta G^*_d(CH_2)$ refer to reversed-phase chromatography systems. In systems with $\Delta G^*_d(CH_2) = 0$, the solute components theoretically can be separated from each other only by virtue of their functional groups, without any separation of the individual homologues. The concept specified by Eqs. (5) and (6) are in accord with the commonly recognized fact that, in a given system, plots of R_{Mi} versus carbon number for different series of homologous solute compounds form a family of parallel straight lines. Relatively few exceptions to this rule have been reported (*23,*

24). Relation (4) formally conforms with that presented by Bate-Smith and Westall (*3*):

$$_{i}u_i = RT \ln K_{Di} = bR_{Mi} + k$$

where k and b are constants.

EXPERIMENTAL

Cellulose MN300 (Macherey-Nagel Co., Düren, G.F.R.) and rice starch (Carlo Erba, Milan, Italy) were used for the preparation of thin layers. The cellulose layers were prepared according to the recommendations given by the producers, and the starch layers were prepared by the procedure described by Petrović and Petrović (*25*). The layers were dried in air (relative humidity of 30–40%) at room temperature (about 22°C) for 24 h before use.

Straight-chain monocarboxylic acids, α-amino monocarboxylic acids, α-hydroxy monocarboxylic acids, and alkyl-3,5-dinitrobenzoates, were used as model solute compounds. The developing systems employed with the acids and dinitrobenzoates were *n*-propanol–2 mol/dm^3 aqueous ammonia (7:3) and dioxane–water–methyl ethyl ketone (34:35:8), respectively. The chromatograms were run by the ascending technique in chambers saturated with the solvent vapors at 22 ± 0.5°C.

The spots of carboxylic acids, amino acids, hydroxy acids, and dinitrobenzoates were detected with an 0.5% ethanolic solution of 2,7-dichlorofluoresceine (in UV light), ninhydrin reagent, glucose–aniline reagent, and an 0.015% methanolic solution of Rhodamine B (in UV light), respectively.

RESULTS AND DISCUSSION

At least nine chromatograms were run with each series of compounds studied, and the R_f values determined for each solute compound were averaged. The standard deviation of one determination of R_f was 0.02. The R_M values of the individual solute compounds are plotted against the number of methylene groups in Figs. 1 and 2. It is apparent from the figures that with all the classes of compounds studied the plots are straight lines. The parallelism of the straight lines for amino, hydroxy, and nonsubstituted carboxylic acids in a given solvent–sorbent system can be looked upon as evidence that the values of $\Delta G_d^*(CH_2)$ are indeed practically constant and additive. With systems comprising the propanol–aqueous ammonia solvent (Fig. 1), the slopes of R_M versus CH_2 number and thus the corresponding $\Delta G_d^*(CH_2)$ values are negative, indicating that $P_m < P_s$ in these cases (cf. Eq.

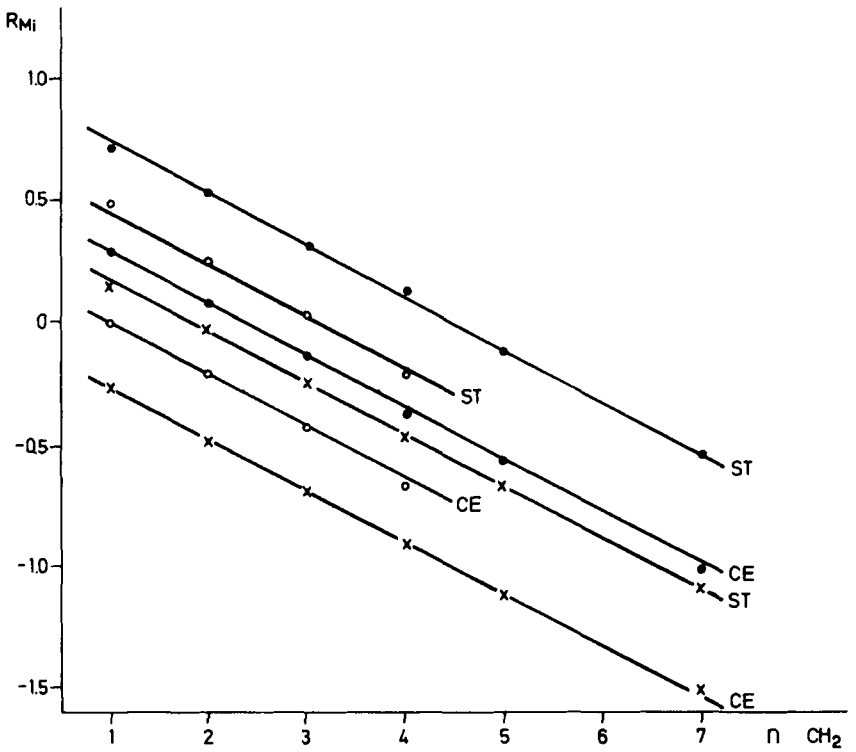

FIG. 1. Plots of R_{Mi} versus number of solute methylene groups (nCH_2) for n-alkyl monocarboxylic acids (+) and the corresponding α-hydroxy (○) and α-amino (●) acids on starch (ST) and cellulose (CE). Solvent: n-PrOH–2 mol/dm^3 aq. NH_4OH (7:3).

7). On the other hand, in the systems with dioxane–water–methyl ethyl ketone (Fig. 2), the positive slope shows that $P_m > P_s$, a situation typical of reversed phase chromatography.

Figure 1 shows that the slopes of the straight lines for the three groups of acids on cellulose and starch are practically the same with the given solvent, and an analogous situation also applies to the system with the dioxane–water–methyl ethyl ketone solvent (Fig. 2). The identity of slopes on cellulose and starch is interesting; in the light of Eqs. (6) and (7), this identity means that the values of $P_m - P_s$ are the same with cellulose and starch. As the composition of the mobile phase and, consequently, the value of P_m is the same in both systems, it follows that there must also be the same composition of the stationary phases. If this is true, then (a) the difference $R_{Mi}^{ST} - R_{Mi}^{CE}$ for any solute compound is given, according to Eq. (4), by $\log [(\phi_m/\phi_s)^{CE}/(\phi_m/\phi_s)^{ST}]$, and (b) it must hold that $R_M^{ST}(OH) = R_M^{CE}(OH)$ and $R_M^{ST}(NH_2)$

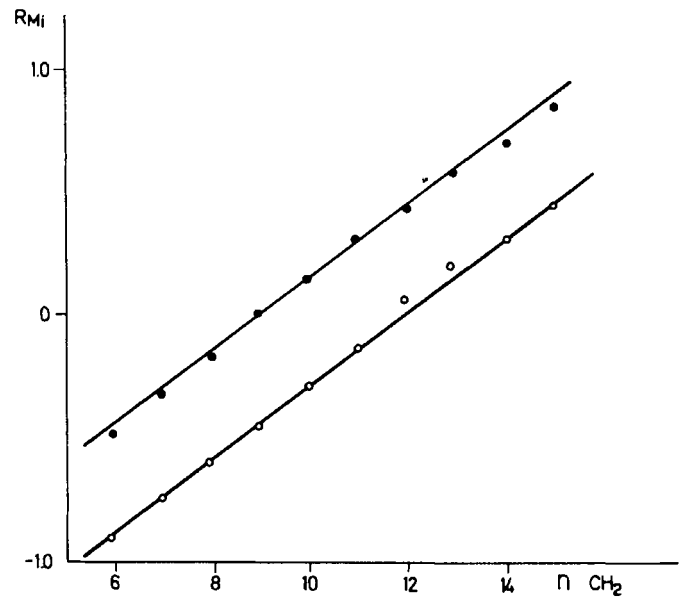

FIG. 2. Plots of R_{Mi} versus number of solute methylene groups (nCH$_2$) for n-alkyl-3,5-dinitrobenzoates on starch (○) and cellulose (●). Solvent: dioxane–H_2O–methyl ethyl ketone (35:35:8).

$= R_M^{CE}(NH_2)$, the superscripts ST and CE denoting starch and cellulose, respectively. By processing the appropriate experimental data it was found that $(\phi_m/\phi_s)^{CE}/(\phi_m/\phi_s)^{ST} = 2.82$ for all the species of acids studied, and $R_M^{ST}(OH) = 0.263 \pm 0.016$, $R_M^{CE}(OH) = 0.263 \pm 0.012$, $R_M^{ST}(NH_2) = 0.552 \pm 0.008$, and $R_M^{CE}(NH_2) = 0.551 \pm 0.008$. Hence the results show

TABLE 1

Values of the Gibbs Free Energy of Desorption per a Solute Methylene Group [$\Delta G_d^*(CH_2)$] and Differences in the Polarities (P) of the Stationary Phases for the Solutes and the Systems Studied

Solutes	$\Delta G_d^*(CH_2)$ (J/mol)		$P_{CE} - P_{ST}$
	Cellulose (CE)	Starch (ST)	
Unsubstituted acids	−1169	−1192	−23
Amino acids	−1214	−1197	17
Hydroxy acids	−1242	−1254	−12
Dinitrobenzoates	841	853	12

that $R_{Mi}^{ST} - R_{Mi}^{CE} = \log[(\phi_m/\phi_s)^{CE}/(\phi_m/\phi_s)^{ST}]$ in the systems with the propanol–aqueous ammonia solvent. Regarding the parallelism of the straight lines for alkyl-3,5-dinitrobenzoates on cellulose and starch (Fig. 2), it can be supposed that there also exists a situation similar to these systems, $R_{Mi}^{ST} - R_{Mi}^{CE}$ amounting to -0.42 and $(\phi_m/\phi_s)^{CE}/(\phi_m/\phi_s)^{ST} = 0.38$ in this case. The above findings are not very surprising when the close chemical similarity and the appreciably different physical patterns of cellulose and starch are considered.

The data on $\Delta G_d^*(CH_2)$ for the individual systems and classes of solute compounds and the differences in the P_s values of different stationary phases (at fixed P_m) are summarized in Table 1.

CONCLUSIONS

The results strongly support the theorem that with *n*-alkyl compounds of different functionalities, chromatographed in a given TLC and/or LC system, the R_M increment per methylene group of the alkyl chain, $R_M(CH_2)$, has practically a constant value. Provided the retention of solute is due to liquid–liquid partition, then $2.3RT[R_M(CH_2)] = G_m^E(CH_2) - G_s^E(CH_2) = P_m - P_s$, where $G_m^E(CH_2)$ and $G_s^E(CH_2)$ are the partial molar excess Gibbs free energies per solute methylene group in the mobile and stationary phases, and P_m and P_s are the polarities of the phases, respectively. In chromatograms run with a given solvent on cellulose and starch layers, the differences in the R_{Mi} values of a given solute compound on cellulose and starch were found to be equal to $\log[(\phi_m/\phi_s)^{Ce}/(\phi_m/\phi_s)^{ST}]$, the compositions of the stationary phases in the cellulose and starch beds being the same.

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