

Review

Retention in reversed-phase chromatography: partition or adsorption?

Anant Vailaya¹, Csaba Horváth*

Department of Chemical Engineering, Yale University, New Haven, CT 06520-8286, USA

Received 15 May 1998; received in revised form 10 August 1998; accepted 10 August 1998

Abstract

A unified framework within the hermeneutics of the solvophobic theory is employed for the treatment of experimental data with nonpolar and weakly polar substances in reversed-phase chromatography (RPC), oil–water partitioning and adsorption on activated charcoal from dilute aqueous solution. This approach sheds light on the energetic similarities between such processes driven by the hydrophobic effect. Among several stationary phase models that have been proposed in the literature for the physical representation of alkyl-silica bonded phases, the isolated solvated hydrocarbon chains model is adopted for the retention in RPC since it represents most closely the stationary phase configuration and is not based a priori on a partition or adsorption mechanism as some other models are for the retention in RPC. Using the fundamental framework of the solvophobic theory, the free energy change per unit nonpolar surface area for octanol–water and hexadecane–water partitioning, retention in RPC as well as adsorption on activated charcoal from dilute aqueous solution at 25°C are evaluated and they are found to be in good agreement with the corresponding experimental data. Furthermore, such quantities are very similar for all the above mentioned processes involving aqueous solution, in contradistinction to the predictions by the lattice theory. From the results it follows that these apparently disparate processes are subject to the same physicochemical principle. The present study demonstrates the capability of the solvophobic theory in describing the energetics of processes involving hydrophobic interactions, and exposes the difficulties in distinguishing between partition and adsorption mechanisms in RPC by using partition models based on the lattice approach. It is concluded that a clear distinction between partition and adsorption in RPC of nonpolar elutes is not apparent from thermodynamic analysis. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Retention mechanisms; Adsorption model; Solvophobic theory; Partition model; Reversed-phase chromatography; Hydrophobic effect

Contents

1. Introduction	2
2. Partition model	3
3. Adsorption model	4
4. Stationary phase models in reversed-phase chromatography	4

*Corresponding author. Tel: +1-203-432-4357; Fax: +1-203-432-4360; E-mail: csaba.horvath@yale.edu

¹ Present address: Merck Research Laboratories, Rahway, NJ 07065, USA

4.1. Liquid hydrocarbon partition model	4
4.2. Liquid-crystalline hydrocarbon partition model	6
4.3. Amorphous-crystalline hydrocarbon partition model	6
4.4. Adsorptive hydrocarbon monolayer model	7
4.5. Isolated solvated hydrocarbon chains model	8
5. Solvophobic theory	9
5.1. Oil–hydroorganic liquid and oil–water partitioning	9
5.2. Retention in reversed-phase chromatography	10
5.3. Adsorption on activated charcoal from dilute aqueous solution	11
6. Prediction of RPC retention data	12
7. Thermodynamic similarity of RPC retention, partitioning and adsorption	15
7.1. Retention in RPC and partitioning between hydroorganic liquid and hexadecane phases	15
7.2. Data analysis based on lattice theory	16
7.3. Data analysis by group molecular parameters	18
7.4. Evaluation of group molecular parameters by the solvophobic theory	20
7.5. Chain length of the alkyl ligates in RPC	23
8. Conclusions	24
Acknowledgements	25
References	25

1. Introduction

Reversed-phase chromatography (RPC) has been the most widely used branch of high-performance liquid chromatography (HPLC) for the analysis and purification of a wide variety of substances [1]. Most commonly RPC employs microparticulate alkyl-silica bonded phases [2], such as octadecylated silica, which offer high separation efficiency combined with unparalleled convenience, versatility and reproducibility. The use of bonded hydrocarbonaceous stationary phases of different provenance and a variety of hydroorganic eluents to modulate retention offers a broad range of operating conditions to separate mixtures containing small or large molecules of different polarity [3]. As a result, RPC is employed in science and technology for numerous applications such as pollution control, food, clinical and pharmaceutical analysis, downstream processing, measurement of physicochemical properties of drugs as well as the separation of peptides, proteins and nucleic acids.

It is believed that the mechanism of solute retention in RPC is governed by hydrophobic interactions between the elute molecules and the stationary phase proper. Such interactions [4,5] are of great technological significance and play an important role in determining the architecture and dynamics of biological systems [6]. Therefore, it behoves us to reinvestigate the physicochemical phenomena underlying RPC and thus facilitate an improvement in the

selectivity and separation efficiency of the chromatographic system. Furthermore, a thorough understanding of the retention behavior in RPC would enhance the scope and applications of molecular chromatography [7] to extract physicochemical and molecular information from retention data.

Retention in RPC has received extensive theoretical treatment over the past twenty years. The solvophobic theory [8] proposed in 1976 provided the first rigorous treatment of the retention energetics in RPC. The theory is based on a thermodynamic cycle [9] and views the retention in RPC as association in vacuo of the elute with the stationary phase and subsequent transfer of participating species into the mobile phase. In the ensuing period, several theories based on various partition and adsorption models have been put forward. Most of them are lattice theories [10–12] based on statistical thermodynamics that *ab ovo* imply a partition mechanism to treat elute retention in RPC. Another theoretical approach [13–15] is based on classical thermodynamics and combines partition and displacement models.

A simplified version of the solvophobic theory was employed recently to re-examine a large body of RPC retention data in view of the individual roles played by the mobile and the stationary phases [16]. These were established by analyzing thermodynamic data obtained with the retention on C₁₈ bonded phase in gas chromatography and the transfer of nonpolar solutes from the hydroorganic liquid to the gas

phase. The solvophobic theory led to the introduction of the free energy change per unit water accessible nonpolar surface area as a physicochemical parameter which characterizes the ability of the chromatographic media to selectively distinguish between nonpolar homologues in RPC. This parameter was evaluated in the entire range of the organic modifier concentration and the calculated values were found to be in excellent agreement with those obtained from RPC experimental data.

This investigation was prompted by some criticism regarding the application of the solvophobic theory to retention in RPC [11,12,17]. Nevertheless, our goal was not only to dispel some of the confusion enshrouding the solvophobic hermeneutics but to go further and formulate a unified theoretical framework for processes involving the hydrophobic effect, such as RPC retention, oil–water partitioning and adsorption on activated charcoal from dilute aqueous solution, as well as to answer the age-old question asked by many chromatographers, i.e., whether partition or adsorption mechanism governs retention in RPC. In practice, partition and adsorption processes are quite different and this justifies an inquiry into the nature of retention in RPC within such a classification. In the physical sense, adsorption of a substance from a solution takes place at the solid–liquid interface whereas partition involves its transfer from the bulk solution into another immiscible solvent. As the chromatographic surface in RPC can be highly solvated by components of the mobile phase it is most likely that the mechanism of retention cannot be ascribed to either of the above two processes alone in their ideal form. It is therefore not surprising that much of the ongoing debate concerning retention mechanisms in RPC has arisen from the employment of different stationary phase models in the theoretical treatment and from the way the retention data is analyzed [11,12,15,17–23].

Our approach to explicating the mechanistic aspects of RPC retention entails the analysis of the adsorption, partition and RPC retention of nonpolar substances based on fundamental thermodynamic principles. The conventional partition and adsorption models will be employed in the light of the simplified solvophobic theory to treat oil–hydroorganic liquid as well as oil–water partitioning and adsorption on activated charcoal from dilute aqueous solution, respectively. On the other hand, in order to

find an answer to the question about the retention mechanism in RPC, the isolated solvated hydrocarbon chains model will be adopted within the hermeneutics of the solvophobic theory. Furthermore, experimental data obtained with such apparently disparate processes involving essentially nonpolar and weakly polar substances will be analyzed and the results compared with the theoretical predictions. The results are expected to shed light on the relative significance of adsorption and partition in determining the energetics of RPC retention.

2. Partition model

The equilibrium constant for partitioning between an organic and an aqueous phase is an important physicochemical parameter for characterizing the hydrophobicity of environmental and pharmaceutical substances [24]. The molecular details of the partition process is illustrated schematically in Fig. 1. It entails creation of a solute-sized cavity in the organic phase, transfer of a solute molecule from the aqueous phase into the organic phase and closing of the cavity left behind by the solute molecule in the aqueous phase. The partition model assumes that the two phases are mutually insoluble, which may not be the case. For instance, in octanol–water partitioning which is a widely used partition system, the aqueous and organic phases are mutually saturated. The concentration of octanol in the saturated aqueous phase is very low and its presence does not measurably affect the thermodynamic parameters obtained from direct measurements of partitioning. Conversely, a large concentration of water is present in the organic phase which could produce noticeable differences between thermodynamic parameters of solutes in water saturated compared with anhydrous

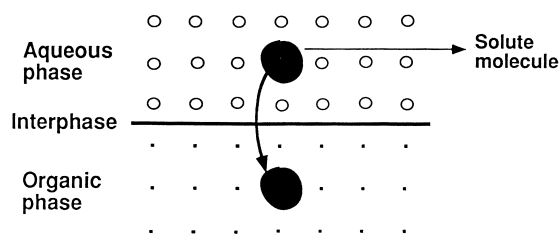


Fig. 1. Schematic illustration of partitioning between an aqueous and an organic phase.

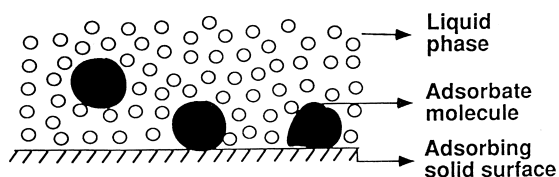


Fig. 2. Schematic illustration of adsorption from aqueous solution on an adsorptive solid surface.

octanol [25]. In this study, we shall analyze partition data obtained indirectly with pure aqueous and octanol phases [25,26].

Unlike the octanol–water system, in which the mutual solubility of the two solvents is quite high, the hexadecane–water system can be regarded as a system of two pure solvents. Hence, in our analysis we shall employ hexadecane–water partition coefficients measured by directly using the shake–flask method [27].

3. Adsorption model

Adsorption from aqueous solutions on activated charcoal has been widely used for the removal of soluble organic contaminants from water supplies. Unlike partitioning, the process of adsorption is a surface phenomenon which occurs at the solid–liquid interface. The solute molecules or adsorbates migrate from the liquid phase to the interface (the adsorptive monolayer) and displace the physically adsorbed molecules of the solvent. Such a displacement or competitive adsorption process dominates the retention in liquid–solid chromatography with polar adsorbents [28,29]. In this study, we chose to analyze adsorption data [30] obtained on activated charcoal in dilute aqueous solution since this process like RPC is governed by the hydrophobic effect and employs hydrophobic solid surfaces. Fig. 2 illustrates the adsorption model which views the above adsorption process as an association in aqueous solution of the adsorbate with the activated charcoal.

4. Stationary phase models in reversed-phase chromatography

The structure and composition of bonded phases in the presence of different eluents have been subjects

of much study and discussion in the past [3,10,11,19,20,23,31–33]. Upon perusal of the literature, we have identified five stationary phase models which have been proposed to describe retention in RPC. The models are listed in Table 1 along with the theories that adopt them to treat RPC retention. Each of these models represents a different view of the retention mechanism in RPC. In the following, we review and briefly describe the characteristic features of each model.

4.1. Liquid hydrocarbon partition model

The simplest model of alkyl-silica bonded stationary phases is an amorphous bulk liquid hydrocarbon layer over the surface of the silica support, with ordinary bulk phase partitioning between the organic (stationary) phase and a hydroorganic (mobile) phase as the retention mechanism. Thus on the molecular level, eluite retention in RPC involves the creation of eluite-sized cavity in the liquid-like stationary phase, the transfer of the eluite into the cavity and the subsequent closing of eluite-sized cavity in the mobile phase. This is illustrated schematically in Fig. 3a and the lattice model, which has been employed by the mean field lattice approach [12] to represent the amorphous bulk liquid hydrocarbon layer over the siliceous chromatographic surface in RPC, is shown in Fig. 3b.

The liquid hydrocarbon layer model was first proposed by Lochmüller and Wilder [23] to describe the sorptive behavior of alkyl-silica bonded stationary phases in RPC. They conjectured that bonded alkyl chains in methanol–water mobile phases might associate with each other due to hydrophobic interactions to form ‘liquid-droplet’ like clusters. Such clusters could afford a bulk liquid-like environment for the partitioning of small eluites. To test their hypothesis, they determined the partition coefficients and selectivities at 25°C for methyl-substituted benzene solutes in a partition system containing alkane and methanol–water (1:1, v/v) as the two liquid phases and compared the partition data to retention data obtained at 25°C using methanol–water (1:1, v/v) as the eluent. It was concluded from the analysis of the data that RPC stationary phases with dodecyl and longer bonded chains exhibit a liquid-like behavior with the eluite molecule being completely embedded in the liquid-droplet. On the other

Table 1
Models for bonded stationary phases in reversed-phase chromatography employed by various theories

No.	Stationary phase model	Theory	Description of stationary phase model	References
I	Liquid hydrocarbon partition	Lattice theory	Bonded chains form a bulk liquid hydrocarbon layer over siliceous surface; Retention governed by partition mechanism.	Lochmüller and Wilder [23], Dorsey and Dill [12], Carr et al. [19]
II	Liquid-crystalline hydrocarbon partition	Lattice theory	Bonded chains form a liquid-crystalline hydrocarbon layer over siliceous surface; Retention governed by partition mechanism.	Martire and Boehm [53]
III	Amorphous-crystalline hydrocarbon	Lattice theory	Bonded chains form an amorphous-crystalline partition hydrocarbon layer over siliceous surface; Retention governed by partition mechanism.	Dill [11]
IV	Adsorptive hydrocarbon monolayer	Lattice theory	Bonded chains interact laterally with the tips forming an adsorptive monolayer surface; Retention governed by adsorption mechanism.	Dill [11]
V	Isolated solvated hydrocarbon chains	Solvophobic theory	Elutes associate with solvated bonded chains; Retention governed by the magnitude of contact area upon binding.	Horváth, Melander and Molnár [8]

hand, lower RPC selectivity values for bonded phases with chain length less than 12 carbon atoms were attributed to an adsorption mechanism or some blend of adsorption and quasi-liquid partition be-

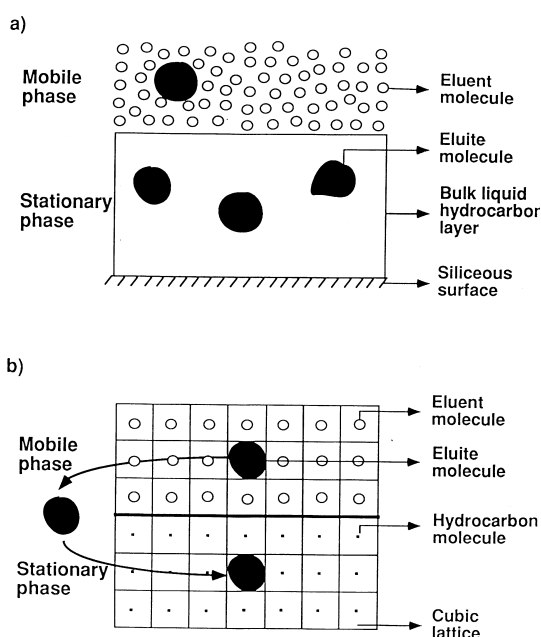


Fig. 3. Schematic illustration (a) and lattice model (b) of the retention in RPC according to the partition mechanism where the alkyl-silica bonded phase is modelled by a bulk liquid hydrocarbon layer into which the elute partitions.

havior owing to incomplete utilization of the total elute molar volume in the interaction with the bonded phase. Lochmüller and Wilder further noted that for larger solutes, selectivities of partition systems are not achieved even with octadecyl bonded phases due to the inability of the stationary phase to permit a complete three-dimensional interaction with elute molecules. In sum, according to the liquid hydrocarbon layer model, the partition-like behavior of RPC is observed only with the retention of small nonpolar elute molecules by stationary phases employing alkyl-bonded chains longer than 12 carbon atoms.

Carr and co-workers [19] found that the energetics of the transfer of alkylbenzenes from hydroorganic liquid phase to bulk hexadecane was similar to that of RPC retention in the organic modifier range from 0–70%. They concluded that the retention of small nonpolar elutes in RPC is governed by the partition mechanism with bulk liquid hexadecane serving as a good representative of the stationary phase characteristics for describing the retention behavior in the above range of organic modifier concentration. At higher organic modifier concentrations, retention behavior in RPC deviated from partition like behavior and this was attributed to a shift to adsorption mechanism, to the sorption of organic modifier by the stationary phase or simply to experimental artifacts. By examining the methylene group selec-

tivities, i.e., the ratio of equilibrium constants of two homologues differing by a single methylene unit, for the transfer from the mobile phase to the gas phase as well as from the gas phase to bulk liquid hexadecane, the authors further concluded that the contribution of the stationary phase to methylene selectivity in RPC is significantly greater than that of the mobile phase. Recently, Carr and co-workers have used the liquid hydrocarbon model to distinguish between adsorption and partition in monomeric and polymeric hydrocarbonaceous stationary phases [17,22].

Another approach by Park et. al [18,20] is based on bulk liquid hydrocarbon model and employs the UNIFAC method for activity coefficient determination to examine the effect of carbon number and type of functional group of the elutes, the composition of the mobile phase as well as the chain length of alkyl-silica bonded stationary phases in RPC. According to this approach, the partition coefficients and methylene group selectivities of hydrocarbonaceous solutes are evaluated for their transfer from the hydroorganic phase to neat hydrocarbon phase, such as hexadecane, octane, butane or benzene. The thermodynamic data so determined is believed to represent retention on hexadecyl-, octyl-, butyl- and phenyl-silica bonded stationary phases in RPC, respectively. However, it was found that the calculated methylene group selectivities increased in the order of hexadecane < octane < butane, opposite to the experimental observation in RPC with the corresponding alkyl bonded stationary phases. The treatment provides only a qualitative description of retention in RPC owing to the failure of UNIFAC to predict accurately the activity coefficients.

4.2. Liquid-crystalline hydrocarbon partition model

Martire and Boehm [10] developed a molecular theory for RPC based on a simple lattice model which takes into account the organization of the stationary phase chains. They considered the changes in the properties of the stationary phases under varying mobile phase conditions, and their influence on the retention behavior in RPC. Two regimes of the eluent composition were identified in which the stationary phase chains were believed to assume different geometric configurations. At high organic modifier concentration, the bonded chains are ex-

pected to be extended and oriented more or less normal to the surface, thus giving it a brush-like appearance and allowing complete penetration by the solvent and solute molecules. In contradistinction, the authors found that with water-rich mobile phases, the stationary phase behaves as a quasi-liquid layer of recumbent alkyl chains that hinder solvent penetration but do not preclude solute penetration. They provided theoretical support for the latter geometric configuration under typical conditions in RPC with commonly used hydroorganic eluents and concluded that the retention process approaches that of classical liquid-liquid partitioning.

Nevertheless, the lattice theory by Martire and Boehm does not model the alkyl-silica bonded phases as a bulk liquid but as a liquid-crystalline hydrocarbon layer, i.e., as an anisotropic condensed phase that has an order intermediate between that of a liquid phase and a crystalline phase. The model draws from related lattice statistics developed to treat among others liquid-crystalline systems [34–37], fatty acid monolayers [38,39] and the amorphous region in diblock copolymers [40]. Eluite retention in RPC is believed to occur via the partition mechanism when the eluite fully penetrates the liquid-crystalline hydrocarbon layer as shown in Fig. 4a. The liquid-crystalline hydrocarbon layer model is a refinement of the bulk liquid hydrocarbon layer model as it includes the stationary phase organization in terms of the bonded chain length, the intrinsic chain stiffness and surface coverage as well as the configuration of the chains in various mobile phases. Fig. 4b illustrates some of the features of the lattice model. By accounting for configurational entropy changes for completely flexible as well as rigid chains and upon equating the chemical potentials in the stationary and mobile phases, the equilibrium constant for distribution of the eluite between the two phases in RPC is derived in terms of binary interaction parameters of the solvents as well as the flexibility of elutes and bonded chains.

4.3. Amorphous-crystalline hydrocarbon partition model

Dill [11,12] proposed the so called ‘interphase’ model for the description of the retention behavior in RPC. He argued that the molecular organization of the bonded phase resembles neither the all-trans

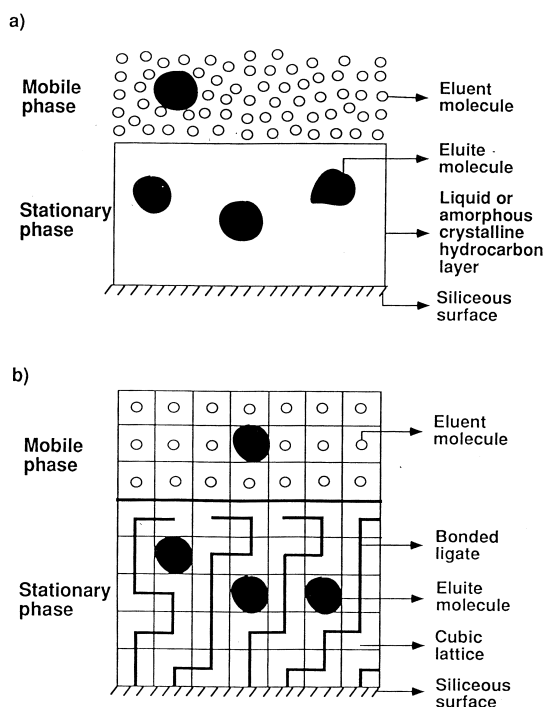


Fig. 4. Schematic illustration (a) and lattice model (b) of the retention in RPC according to the partition mechanism where the alkyl-silica bonded phase is modelled by a liquid-crystalline or an amorphous-crystalline hydrocarbon layer into which the elute partitions.

crystalline state of *n*-alkane chains nor the randomly structured liquid state nor even a liquid-crystalline state of intermediate order. Instead, the chromatographic surface in RPC may be likened to the interphase between lamellar crystalline and adjoining amorphous regions in a semicrystalline polymer [41]. Such a resemblance for hydrocarbon chains in amphiphilic mesophases, such as lipid monolayers and bilayers as well as micelles and microemulsions [42–44], was first pointed out by deGennes [45]. Here we refer to Dill's 'interphase' model by the term 'amorphous-crystalline hydrocarbon layer model' in order to differentiate it from the liquid-crystalline hydrocarbon layer model proposed by Martire and Boehm.

According to Dill, the properties of amorphous-crystalline phases are as follows. The interphase is comprised of chains which have one end anchored at the interface so that they are characterized by a gradient of disorder that joins regions of high order and of liquid-like disorder. The proportion of gauche

bonds varies along the length of a chain as it traverses the interphase layer. Thus, according to this model, bonded chains of RPC stationary phases will have greater orientational order near their anchored ends than near their free ends. This variation of properties with distance from the interface contrasts with bulk liquid phases, whose properties by definition are invariant.

According to the model, elute retention in RPC is governed by the partition mechanism when the bound elute is fully embedded within the amorphous-crystalline layer stationary phase. The mean field lattice approach developed for solutes in amorphous-crystalline phases [46] has been employed [11] to treat partitioning in RPC by adopting a lattice model similar to that shown in Fig. 4. Such lattice models have neglected the solvation of stationary phase ligates in the presence of organic modifier rich eluents.

In order to elucidate at the molecular level the structure and dynamics of the RPC stationary phase, Klatte and Beck [47,48] have carried out recently molecular dynamics simulations with a disordered alkane monolayer of intermediate density tethered to silica surfaces. They have examined the temperature, chain length, chain density and surface structure effects on the behavior of hydrocarbonaceous ligate chains and simulated the transfer of a simple nonpolar elute from a methanol–water mixture to the octadecylated silica at room temperature [49]. The calculated free energy was found to be similar in magnitude to that expected for hydrophobic transfer from water to oil-rich phases, but specific interfacial effects as described by Dill's model, drew into question bulk partitioning models.

4.4. Adsorptive hydrocarbon monolayer model

Dill considered adsorption as an alternative mechanism of retention in RPC [11]. A schematic of the elute retention in RPC according to the adsorption mechanism is illustrated in Fig. 5 (top). Retention in RPC is believed to be governed solely by the adsorption mechanism when the density of bonded nonpolar functions is high enough for the chains to interact laterally among themselves and to disallow penetration of elute molecules into the amorphous-crystalline hydrocarbon layer at the chromatographic surface. In this case, the tips of the alkyl-silica

bonded chains offer a hydrocarbonaceous surface for the adsorption of the sample components.

Lattice monolayer approximation [46,50–52] was employed [11] to treat adsorption in RPC. Assuming a cubic lattice as shown in Fig. 5 (bottom), the theory predicts that logarithmic retention factors in RPC depend linearly on the logarithmic equilibrium coefficients of the appropriate liquid–liquid partition system with a proportionality factor of $1/6$, since only one cubic face of the eluite surface is supposed to be in contact with the stationary phase. On the other hand, the lattice theory based on the amorphous-crystalline hydrocarbon layer model predicts a similar linear dependence but with a proportionality factor of 1. Upon analyzing the experimental RPC data obtained with hydrocarbonaceous eluents, Dill observed a value of unity for the proportionality

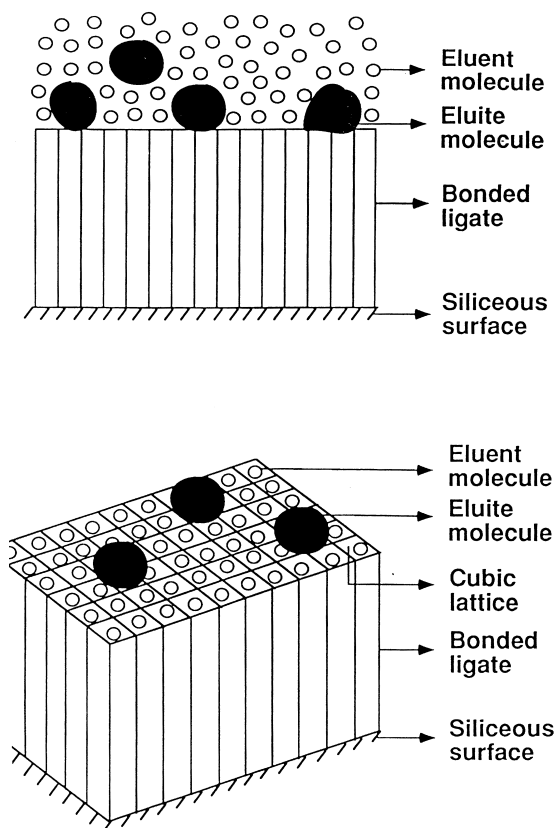


Fig. 5. Schematic illustration (top) and lattice model (bottom) of the retention in RPC according to the adsorption mechanism. The tips of the alkyl-silica bonded chains form a hydrocarbonaceous adsorptive monolayer serving as the chromatographic surface for eluite binding.

factor and concluded that partition is the primary mechanism of retention in RPC in a wide range of mobile phase conditions [11].

4.5. Isolated solvated hydrocarbon chains model

So far all models discussed to describe retention in RPC are based on lattice theories and evoke either a partition or an adsorption mechanism. In contrast, the solvophobic theory with the isolated solvated hydrocarbon chains model does not distinguish between adsorption or partition. Depending on the nature of the eluent, two stationary phase configurations are possible and they are depicted in Fig. 6. In organic modifier rich eluents, the bonded chains are extended and assume a fur-like configuration. Retention in RPC occurs by penetration of the eluite molecule within the interligate space and its interaction with the lateral surface of the ligates or its association with the tips of the bonded chains or both. In water-rich mobile phases, the bonded chains are in close contact with each other giving the appearance

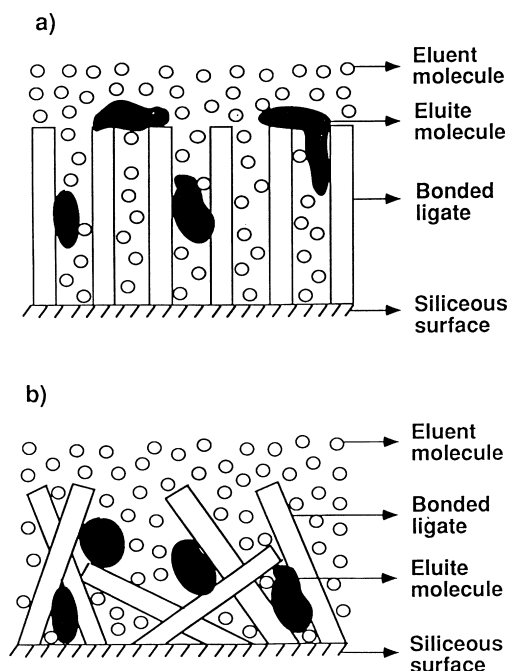


Fig. 6. Schematic illustration of (a) 'fur' and (b) 'stack' configuration of isolated solvated hydrocarbon chains stationary phase. Eluite retention in RPC is governed by the magnitude of contact area upon binding of the eluite with the alkyl-silica bonded chains.

of a collapsed structure that is termed the stack configuration. It is assumed that the ligate density is small enough so that the chains may be considered as isolated. In view of the various ways the eluite binds at the chromatographic surface, it is noted that retention in RPC is due to the statistical average of such binding configurations and the measured retention factors represent average values of the corresponding free energy of binding. The two stationary phase configurations illustrated in Fig. 6 reflect the belief that retention in RPC is governed by the magnitude of the contact area that is formed upon binding of the eluite molecule with the isolated solvated ligates of the stationary phase.

5. Solvophobic theory

The solvophobic theory was developed initially to describe the effect of solvent on the energetics of various molecular associations in solution [9]. Since its inception in the 1960s, the theory has successfully been adapted to many biological and chemical processes such as denaturation of DNA [54,55], reversed-phase [1,8,16,56,57] and hydrophobic interaction chromatography (HIC) [58,59], behavior of proteins in aqueous salt solutions [60,61], adsorption on activated carbon from dilute solution [29,62–64] as well as drug–biomolecule associations and denaturation of proteins [65].

The solvophobic theory is based on a rigorous thermodynamic cycle which decomposes an association reaction in solution into two processes: association in the gas phase and solvation of participating species. The solvation process is further broken down into several conceptual subprocesses for the purpose of calculating the change in free energy of molecular association from readily available physico-chemical properties of the solute and the solvent. Each subprocess represents a particular interaction term, such as solute–solvent van der Waals interactions, solvent–solvent van der Waals interactions or cavity formation, and electrostatic interactions. A number of theories are developed and/or adapted to describe each of these interactions and for this reason the solvophobic theory is a quite eclectic one.

In the following, a simplified version of the solvophobic theory [16] is adapted first to treat an oil–water and oil–hydroorganic liquid partition sys-

tem by invoking the conventional partition model. Then, retention in RPC is treated within the hermeneutics of the solvophobic theory by viewing the process as an association of the eluite with the isolated solvated hydrocarbon chains of the stationary phase. Finally, the theory is employed to describe adsorption on activated charcoal from aqueous solution by employing the adsorption model. The free energy change associated with the above processes will be expressed in terms of the nonpolar surface area of the molecules under investigation in order to facilitate the evaluation of free energy change per unit nonpolar surface area. We shall use for convenience the symbol a_g for the free energy change per unit nonpolar surface area. It is noted that a_g is proportional to the methylene group selectivity of the process so that the higher the value of a_g the larger is the selectivity of the system. Such normalized free energy quantities, called ‘group molecular parameters’ [66], have been found to characterize various processes driven by the hydrophobic effect and therefore shed light on their molecular aspects [67–72]. For instance, the observed invariance of group molecular parameters obtained in hydrophobic interaction chromatography and dissolution of aliphatic hydrocarbons in water reflects the thermodynamic identity of such disparate processes [59].

5.1. Oil–hydroorganic liquid and oil–water partitioning

Let us examine first the partitioning of a solute, A, between a hydroorganic liquid, S, such as methanol–water, and an immiscible liquid phase, O, e.g. octanol. The process may be decomposed into two steps: transfer of A from the hydroorganic liquid phase to the gas phase and its transfer from the gas phase to the organic phase. The free energy of oil–hydroorganic liquid partitioning, ΔG_p^0 , is simply the sum of the free energy changes associated with each of the two subprocesses as:

$$\Delta G_p^0 = \Delta G_{sg}^0 + \Delta G_{g0}^0 \quad (1)$$

where ΔG_{sg}^0 is the free energy change for the hydroorganic liquid-to-gas phase transfer process and ΔG_{g0}^0 is that for the gas-to-organic phase transfer process. Each subprocess entails solvent–solvent (cavity formation) and solute–solvent interactions as

well as the change in free volume. Expressing the change in free energy associated with cavity formation and solute–solvent interactions in terms of molecular surface area, ΔA , surface and interfacial tensions we obtain that:

$$\Delta G_{sg}^0 = (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS}) \Delta A - RT \ln \frac{RT}{V_S} \quad (2)$$

and

$$\Delta G_{go}^0 = (\kappa_{AO}^g \gamma_{AO} - \kappa_A^g \gamma_A) \Delta A + RT \ln \frac{RT}{V_O} \quad (3)$$

where γ_A, γ_{AO} and γ_{AS} are the solute surface tension, solute–oil and solute–hydroorganic liquid interfacial tensions and V_O and V_S are the molar volumes of oil and hydroorganic liquid. The κ 's convert the respective surface or interfacial tension to the microthermodynamic value applicable to molecular dimensions [73]. For hydrocarbonaceous solutes, $\Delta A = \Delta A_{np}$ where ΔA_{np} is the nonpolar surface area of the solute. Differentiating Eq. (2) with respect to ΔA_{np} yields the free energy change per unit nonpolar surface area for the transfer of nonpolar solutes from the hydroorganic liquid phase to the gas phase as:

$$\frac{\partial \Delta G_{sg}^0}{\partial \Delta A_{np}} = a_g(\text{transfer}) = (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS}) \quad (4)$$

A similar expression may be obtained for the gas-to-organic phase transfer process as well.

Combining Eqs. (2) and (3) yields, for the free energy change of partitioning between the hydroorganic liquid and the organic phase, that:

$$\Delta G_P^0 = (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS} + \kappa_{AO}^g \gamma_{AO} - \kappa_A^g \gamma_A) \Delta A + RT \ln \frac{V_S}{V_O} \quad (5)$$

Eq. (5) predicts that the free energy of partitioning between two phases is linearly dependent on the surface area of the solute provided the correction factors do not vary significantly with the surface area. Indeed, several studies on octanol–water partitioning [74,75] have confirmed this linear dependence on the surface area of the molecule. For hydrocarbonaceous solutes, the free energy change per unit nonpolar surface area for partitioning between two phases is obtained from Eq. (5) as:

$$\begin{aligned} \frac{\partial \Delta G_P^0}{\partial \Delta A_{np}} &= a_g(\text{partition}) \\ &= (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS}) + (\kappa_{AO}^g \gamma_{AO} - \kappa_A^g \gamma_A) \quad (6) \end{aligned}$$

According to Eq. (6), the free energy of partitioning normalized with respect to the nonpolar surface area may be evaluated from the slopes of linear plots of ΔG_P^0 against ΔA_{np} . The corresponding expressions for oil–water partitioning are easily obtained from Eqs. (5) and (6) by replacing the subscript S by W for water.

5.2. Retention in reversed-phase chromatography

An approach to describing retention in RPC, without assuming either a partition or an adsorption mechanism, is provided by adopting the isolated solvated hydrocarbon chains model for the stationary phase in RPC and viewing the retention process as an association of the eluite with the accessible surface of the free ligates, usually bonded aliphatic hydrocarbon chains.

According to the solvophobic theory, the free energy of retention of an eluite A is expressed as:

$$\Delta G_R^0 = \Delta G_{cav} + \Delta G_{int} + \Delta G_{gas} + \Delta G_{red} - RT \ln \frac{RT}{V_S} \quad (7)$$

where ΔG_{cav} is the change in free energy of cavity formation (eluent–eluent hydrogen bonding and van der Waals interactions), ΔG_{int} is that of eluite–eluent interactions (coulombic and van der Waals interactions), ΔG_{gas} is that of eluite–stationary phase or eluite–ligate interactions (coulombic and van der Waals interactions), ΔG_{red} is the reduction of ΔG_{gas} in the presence of the solvent and $RT \ln (RT/V_S)$ is the change in the free volume. In the last term, V_S is the molar volume of the solvent at atmospheric pressure. For the retention of hydrocarbonaceous eluites in RPC, the coulombic interactions are negligible and ΔG_{red} is about 25% of ΔG_{gas} upon contact with hydroorganic eluents. The free energy of cavity formation and eluite–eluent interactions have been previously expressed in terms of the nonpolar surface area of the eluite, surface and interfacial tensions [16]. With these enhancements Eq. (7) can be rewritten as:

$$\Delta G_R^0 = -\kappa_S^g \gamma_S \Delta A_{np} - (\kappa_{AS}^g \gamma_{AS} - \kappa_A^g \gamma_A - \kappa_S^g \gamma_S) \Delta A_{np} + \Delta G_{gas} - 0.25 \Delta G_{gas} - RT \ln \frac{RT}{V_S}$$

(cavity) (eluite – eluent interactions) (eluite – (reduction) (free volume) ligate interactions)

(8)

where γ_S is the surface tension of the solvent, γ_A is the surface tension of the hydrocarbonaceous eluite and γ_{AS} is the eluite–solvent interfacial tension. The correction factors, κ_S^g , κ_A^g and κ_{AS}^g , convert the respective surface or interfacial tension to the microthermodynamic value applicable to molecular dimensions.

The retention free energy change normalized with respect to the nonpolar surface area is given by:

$$\frac{\partial \Delta G_R^0}{\partial \Delta A_{np}} = a_g(\text{retention})$$

$$= a_g(\text{cav}) + a_g(\text{int}) + a_g(\text{gas}) + a_g(\text{red}) \quad (9)$$

where $a_g(\text{cav})$, $a_g(\text{int})$, $a_g(\text{gas})$ and $a_g(\text{red})$ are the normalized free energies of cavity formation, eluite–eluent interactions, eluite–stationary phase interactions and their reduction in the presence of the eluent, respectively. Differentiating Eq. (8) with respect to ΔA_{np} and assuming that the correction factors are independent of the surface area, we obtain for $a_g(\text{retention})$ that:

$$a_g(\text{retention}) = (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS}) + 0.75 a_g(\text{gas})$$

(mobile phase effect)	(stationary phase effect)
-----------------------	---------------------------

(10)

In Eq. (10), the first term on the right hand side, comprising cavity formation and eluite–eluent interactions, represents the mobile phase effect in RPC. According to Eq. (4), this is simply the change in free energy per unit nonpolar surface area for the transfer of hydrocarbonaceous solutes from the hydroorganic liquid phase to the gas phase, i.e., $a_g(\text{transfer})$. The second term in Eq. (10) entails eluite–stationary phase interactions and their reduction in the presence of the eluent. Although the reduction term is actually a mobile phase effect, for convenience we have lumped it with the eluite–ligate interactions term which represents the stationary

phase effect. Eq. (10) facilitates the evaluation of normalized retention free energy change if the terms on the right hand side are known or can be obtained from the literature. Experimentally, the normalized retention free energy changes may be evaluated from slopes of linear plots of ΔG_R^0 against ΔA_{np} .

It is noted that if in the above treatment we had adopted a partition model for describing the RPC retention process instead of the isolated solvated hydrocarbon chains model, we would have obtained the same expression for the retention free energy change as that for the free energy change of oil–hydroorganic liquid partition process vide Eq. (6).

5.3. Adsorption on activated charcoal from dilute aqueous solution

The thermodynamics of adsorption on activated charcoal from dilute aqueous solution has been extensively treated in the past by adapting the solvophobic theory [62]. According to the theory, adsorption is viewed as an association in solution of the adsorbate with the surface of the activated charcoal. The theory predicts the adsorbability to depend linearly on the nonpolar surface area of the adsorbate and this has strong experimental support [30].

Using the simplifications of the solvophobic theory [16], we obtain for the free energy of adsorption of nonpolar substances on activated charcoal from dilute aqueous solution that:

$$\Delta G_A^0 = (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS}) \Delta A_{np} + 0.75 \Delta G_{gas}$$

$$- RT \ln \frac{RT}{V_S} \quad (11)$$

and for the normalized free energy that:

$$\frac{\partial \Delta G_A^0}{\partial \Delta A_{np}} = a_g(\text{adsorption})$$

$$= (\kappa_A^g \gamma_A - \kappa_{AS}^g \gamma_{AS}) + 0.75 a_g(\text{gas}) \quad (12)$$

where ΔG_{gas} is the adsorption on activated charcoal from the gas phase and represents adsorbate–adsorbent interactions. Although the adsorption model is quite different from the isolated solvated hydrocarbon chains model in the physical sense, it is seen that Eqs. (11) and (12) are essentially the same as Eqs. (8) and (10) derived in the previous section for retention in RPC.

It should be noted that by adopting the adsorption model instead of the isolated solvated hydrocarbon chains model for describing the retention in RPC in Section 5.2, we would still obtain essentially the same expression for the free energy changes of RPC retention and adsorption processes and this may lead one to believe that the isolated solvated hydrocarbon chains model is basically an adsorption model. At this point we would like to remind the reader that neither the adsorption nor the partition mechanism is clearly defined in RPC retention. Consequently, the isolated solvated hydrocarbon chains model would be a combination of both adsorption and partition according to Dill's definition in the sense that the elutes penetrate the interligate space (partition) and then interact laterally with the ligates (adsorption).

The solvophobic theory has often been criticized that it is based solely on an adsorption model. From the results presented here it should now become evident that the theory is applicable not only to molecular associations in solution, such as adsorption or retention processes, but also to simple transfer or partition processes.

6. Prediction of RPC retention data

In this section, we shall evaluate $a_{\text{g}}(\text{cav})$, $a_{\text{g}}(\text{int})$, $a_{\text{g}}(\text{gas})$ and $a_{\text{g}}(\text{red})$ using data measured by methods other than liquid chromatography in order to predict, according to Eq. (9), the selectivity of hydrocarbonaceous elutes in RPC employing hydroorganic mixtures as the mobile phase. The prediction by the solvophobic theory will be tested by comparing the calculated $a_{\text{g}}(\text{retention})$ values with those obtained from experimental RPC data.

According to Eq. (10), the normalized free energy change associated with the mobile phase effect in RPC is represented by that for the transfer of nonpolar solutes from the hydroorganic liquid phase

to the gas phase. This was confirmed by the analysis of methylene selectivity data for RPC and for the transfer from hydroorganic liquid phase to the gas phase as follows. In Fig. 7, we plot the logarithmic methylene selectivity obtained with hydrocarbonaceous elutes in RPC employing C_{18} bonded phases against the organic content of the eluent. For comparison, we also present in Fig. 7 selectivity data [19] obtained for the transfer of alkylbenzenes from the hydroorganic liquid phase to the gas phase by using head-space gas chromatography. As seen in Fig. 7a for the retention in RPC employing methanol–water mixtures as the eluent, logarithmic selectivity varies nearly linearly with the methanol concentration. For other eluent systems shown in Fig. 7b–d, however, the variation in logarithmic selectivity with organic modifier concentration is not linear. Nevertheless, RPC selectivity (solid circles) with the four hydroorganic systems and the selectivity for the transfer from the hydroorganic liquid phase of the same composition to the gas phase (open circles) show the same dependence on the organic modifier concentration. This confirms the prediction by the solvophobic theory that the mobile phase effect in RPC can be modelled with reasonable accuracy by the transfer of nonpolar solutes from the hydroorganic liquid phase to the gas phase as pointed out also in Ref. [19]. Furthermore, the results contradict the predictions of the mixed model involving partition and displacement to describe the selectivity in RPC [15]. From the data presented in Fig. 7, it is evident that the observed nonlinear dependence of logarithmic selectivity on the organic modifier concentration in RPC is a mobile phase effect rather than the result of excess sorption [78–80] of the organic modifier by the stationary phase. Furthermore, the difference between the logarithmic selectivities of the RPC retention and the transfer process, which is represented by the space between the two curves in Fig. 7, is found to be nearly constant in the entire range of the organic modifier concentration with a value of close to unity for all the eluent systems. The finding strongly indicates that sorption of organic modifier by the stationary phase does not significantly affect the RPC selectivities of nonpolar substances in the entire range of the organic modifier.

Thus, the experimental data for the transfer of

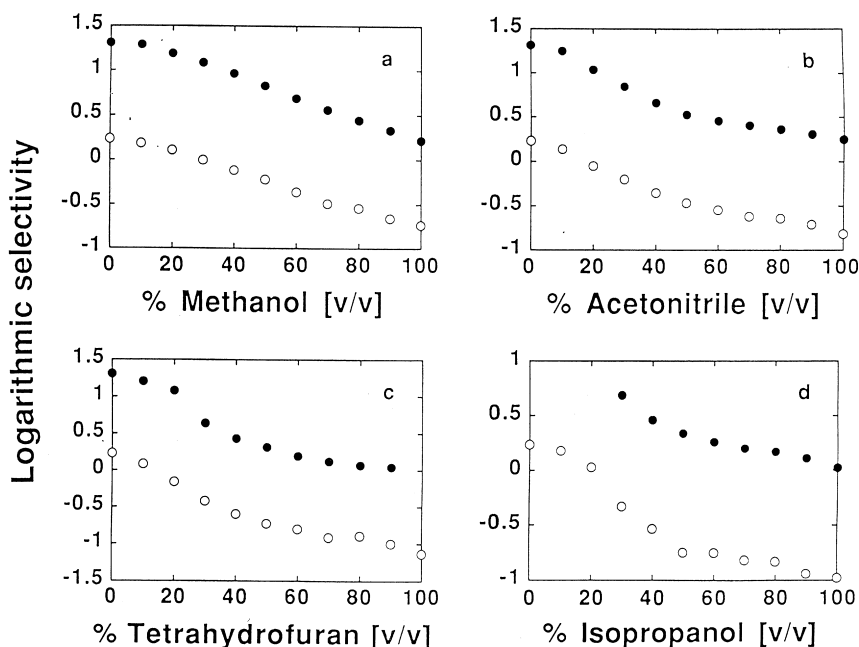


Fig. 7. Dependence of logarithmic methylene selectivity on concentration of (a) methanol, (b) acetonitrile, (c) tetrahydrofuran and (d) isopropanol in water for (●) the retention of hydrocarbonaceous eluities in RPC employing C_{18} bonded phases and (○) the transfer of alkylbenzenes from the hydroorganic liquid phase to the gas phase at 25°C. RPC data in (a)–(c) is taken from Ref. [76] and represents the average arithmetic selectivity values obtained with alkanes, alkanols, methylalkanoates, alkylchlorides and alkylbenzenes on LiChrosorb RP18 and Hypersil ODS columns. RPC data in (d) is taken from Ref. [77] and represents selectivity values obtained with alkylbenzenes on Hypersil ODS column. Selectivity data for the transfer process obtained with alkylbenzenes is from Ref. [19].

nonpolar solutes from the hydroorganic liquid to the gas phase may be used to evaluate a_g associated with the mobile phase effect in RPC. Since the transfer process entails both solvent–solvent (cavity formation) and solute–solvent (van der Waals interactions) terms, it is possible to evaluate from the transfer data both $a_g(\text{cav})$ and $a_g(\text{int})$ associated with retention in RPC, provided one of the two quantities is known or can be evaluated. Previously, we have presented a method based on pair-wise intermolecular potentials for calculating $a_g(\text{int})$ in RPC from readily available physicochemical properties of the eluities and eluent, such as molecular volumes, acentric factors, refractive indices and ionization potentials [16]. According to this method, the free energy of eluite–eluent interaction by van der Waals forces is calculated for each eluite using the following equation:

$$\Delta G_{\text{int}} = \frac{27 \times 0.564}{8\pi} I D_A D_E (Q' + Q'') \quad (13)$$

where I depends on the ionization potentials of both

the eluent and the eluite, D_A and D_E are the Clausius–Mosotti functions of the eluite and the eluent, and Q' and Q'' are dimensionless functions that can be obtained by integrating the effective pair potential between the eluite and eluent molecules over the total volume. The calculated ΔG_{int} values vary linearly with the nonpolar surface area of the eluities, evaluated using Hermann's method [81], so that $a_g(\text{int})$ values can be readily evaluated from the slopes of linear ΔG_{int} versus ΔA_{np} plots. The above method was employed to calculate $a_g(\text{int})$ in the entire concentration range of methanol by using physicochemical data from the literature [30,82] as well as appropriate mixing rules [83]. We then evaluated $a_g(\text{cav})$ from the experimental data [19] available for the transfer from the hydroorganic liquid to the gas phase by using the following equation:

$$a_g(\text{cav}) = a_g(\text{transfer}) + a_g(\text{int}) \quad (14)$$

In order to calculate the $a_g(\text{gas})$ and $a_g(\text{red})$ terms in

Table 2

Free energy changes at 25°C associated with the retention on C₁₈ bonded phase in gas chromatography [57] along with the surface areas of the substances [81,84]

Substances	ΔG_{gas}^0 (kJ mol ⁻¹)	ΔA_{np} (Å ²)
Benzene	-15.1	255.7
Toluene	-18.4	286.5
Ethylbenzene	-20.6	315.6
Pentane	-11.6	286.9
Hexane	-14.3	318.7
Heptane	-17.2	350.5
Octane	-20.2	382.3

Eq. (9), we employed the retention data [57] listed in Table 2 obtained on C₁₈ bonded phase in gas chromatography. The respective surface areas of the benzene and methane molecules were taken from Refs. [81,84] and the surface area increment of 31.8 Å² was assumed for each methylene group [80] to calculate the surface areas of benzene and methane homologues. Plots of ΔG_{gas}^0 against molecular surface area of the species were prepared for alkyl-benzenes and alkanes, respectively. Linear plots were obtained and from the slopes the respective a_{g} (gas) values of -92 and -91.1 J mol⁻¹ Å⁻² were evaluated. The a_{g} (red) value was taken as -0.25 a_{g} (gas) or 22.9 J mol⁻¹ Å⁻².

We calculated a_{g} (retention) values from experimental RPC data as follows. The surface area values of nonpolar eluities under consideration were obtained from the literature [81,84]. The retention free energy change was then plotted against the nonpolar surface area of the eluities. Such plots were found to be linear according to Eq. (8) so that we could evaluate a_{g} (retention) values from the slopes. Alternatively, we could also directly obtain a_{g} (retention) values from the RPC selectivity values listed in the literature.

The normalized free energies, a_{g} , associated with cavity formation, eluite–eluent van der Waals interactions, eluite–stationary phase van der Waals interactions and reduction are plotted against methanol concentration in water and illustrated by dotted lines in Fig. 8. Also shown by symbols are the a_{g} (retention) values obtained from experimental RPC data on C₁₈ bonded phases using methanol–water mixtures as eluents. The solid curve in Fig. 8 is the sum of eluite–eluent interactions, cavity formation,

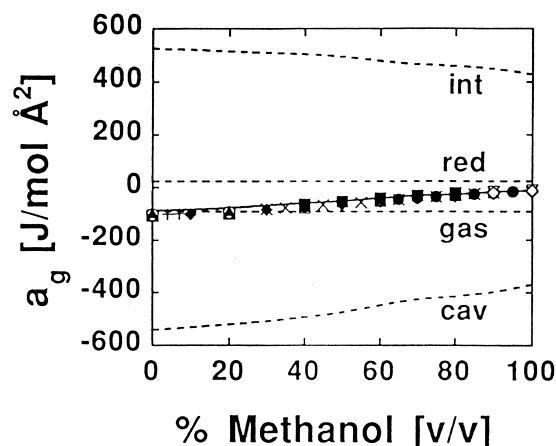


Fig. 8. Dependence of a_{g} associated with solute–solvent interactions (int), solvent–solvent interactions (cav), eluite–stationary phase interactions (gas) and reduction of eluite–stationary phase interactions in the presence of solvent (red) on methanol concentration in water. Symbols represent experimental RPC data from Refs. [76,78,85–94] obtained on C₁₈ bonded phases with hydroorganic eluities. The solid line shows the dependence of a_{g} on methanol content in the eluent for the RPC retention on C₁₈ bonded phases at 25°C, estimated by using Eq. (9) according to the solvophobic theory.

eluite–stationary phase interactions and reduction terms according to Eq. (9) and represents a_{g} (retention) estimated by the solvophobic theory for the retention in RPC using C₁₈ bonded phases and methanol–water mixtures. As seen, the solvophobic theory predicts with reasonable accuracy the values of a_{g} (retention) in the entire range of the organic modifier concentration from data obtained by measurements other than liquid chromatography. Similar results have been obtained with other eluent systems [16]. In view of the consistent results obtained upon analyzing a large body of RPC retention data and employing a wide provenance of physicochemical data, the solvophobic theory offers a quite satisfactory framework for the analysis and prediction of retention behavior in RPC. Data presented in Fig. 8 further indicates that the magnitude of both eluite–eluent interactions and cavity formation terms is much greater than that of eluite–stationary phase interactions and reduction terms, thus confirming the dominant role of eluite–eluent and eluent–eluent interactions in governing the retention behavior in RPC.

7. Thermodynamic similarity of RPC retention, partitioning and adsorption

In Section 5, we presented a unified framework of the solvophobic theory for the treatment of various processes driven by the hydrophobic effect. We shall now employ this framework to analyze experimental data obtained with hydrocarbonaceous substances in RPC as well as in partition and adsorption processes.

7.1. Retention in RPC and partitioning between hydroorganic liquid and hexadecane phases

So far we have examined RPC retention data in

the light of the solvophobic theory. Here we analyze both retention and partition data in order to elucidate the energetic similarities of the two processes. Table 3 lists the conditions and sources of RPC retention data obtained on various bonded phases with nonpolar homologues and hydroorganic eluents. As mentioned before, for all RPC data listed in Table 3 the retention free energy change depends linearly on the nonpolar surface area of the eluents so that $a_g(\text{retention})$ can be readily evaluated from the slopes according to Eq. (8). Similarly, the free energy changes associated with the partitioning of alkylbenzenes between hydroorganic liquid phase and bulk hexadecane taken from Ref. [19] also yields

Table 3
Summary of RPC literature data with various bonded phases examined in the present investigation

Column no.	Column	<i>T</i> (°C)	Organic modifier	Organic modifier range (%v/v)	Homologous series	Symbols in Figs. 9–11	Refs.
1.	Laboratory-made C ₁₈	27.5	Methanol	20–80	Alkanols	□	[85]
2.	Nucleosil 10 RP-18	25	Methanol	60–100	Alkylbenzenes	▽	[87]
3.	LiChrosorb RP-18	25	Methanol	30–90	Alkylbenzenes	×	[88]
4.	LiChrosorb RP-18	25	Methanol	0–80	Alkanols	▼	[89]
5.	LiChrosorb RP-18	25	Methanol	0–100	5 series ^a	◆	[76]
6.	Hypersil ODS	25	Methanol	0–100	5 series ^a	◆	[76]
7.	C ₁₈ SIL-X-5	23	Methanol	60–95	Alkylbenzenes	●	[91]
8.	Davisil C ₁₈	30	Methanol	0–80	Alkylbenzenes	+	[94]
9.	Laboratory-made C ₁₄	27.5	Methanol	0–80	Alkanols	△	[85]
10.	Laboratory-made C ₁₀	27.5	Methanol	0–80	Alkanols	△	[85]
11.	Chromegabond C ₁₀	25	Methanol	40–70	Alkylbenzenes	○	[95]
12.	Chromegabond C ₁₀	25	Methanol	40–100	Alkylphenones	◇	[95]
13.	LiChrosorb RP-8	25	Methanol	0–60	Alkanols	▲	[96]
14.	LiChrosorb RP-8	30	Methanol	40–80	Alkylbenzenes	■	[97]
1.	Nucleosil 10 RP-18	25	Acetonitrile	30–100	Alkylbenzenes	×	[87]
2.	LiChrosorb RP-18	25	Acetonitrile	0–80	Alkanols	△	[89]
3.	LiChrosorb RP-18	25	Acetonitrile	0–100	5 series ^a	◇	[76]
4.	Hypersil ODS	25	Acetonitrile	0–100	5 series ^a	◇	[76]
5.	Develosil ODS	30	Acetonitrile	60–95	Alkylbenzenes	▲	[98]
6.	Shim-pack ODS	30	Acetonitrile	40–80	Alkylbenzenes	●	[97]
7.	Laboratory-made C ₈	30	Acetonitrile	40–70	Ethylalkanoates	◆	[78]
8.	LiChrosorb RP-8	30	Acetonitrile	40–80	Alkylbenzenes	▼	[97]
1.	Nucleosil 10 RP-18	25	Tetrahydrofuran	30–90	Alkylbenzenes	▽	[87]
2.	LiChrosorb RP-18	25	Tetrahydrofuran	0–80	Alkanols	△	[89]
3.	LiChrosorb RP-18	25	Tetrahydrofuran	0–100	5 series ^a	+	[76]
4.	Hypersil ODS	25	Tetrahydrofuran	0–100	5 series ^a	+	[76]
5.	YMC ODS	40	Tetrahydrofuran	60–70	Alkylbenzenes	×	[99]
6.	Hypersil ODS	25	Tetrahydrofuran	30–90	Alkylbenzenes	◆	[77]
1.	Hypersil ODS	25	Isopropanol	30–100	Alkylbenzenes	●	[77]

^a Average arithmetic selectivity values on LiChrosorb RP-18 and Hypersil ODS columns using alkanes, alkanols, methylalkanoates, alkylchlorides and alkylbenzenes.

linear plots against the nonpolar surface area, thus facilitating the evaluation of a_g (partition) from the slopes according to Eq. (5). Fig. 9 illustrates the variation of a_g (retention) and a_g (partition) with organic modifier concentration obtained from the analysis of retention data listed in Table 3. At low organic modifier concentration, the RPC and partition data compare very well. At higher concentrations, a_g for RPC retention obtained on C_{18} bonded phases is slightly greater than that for partitioning, but not more than $5\text{--}10 \text{ J mol}^{-1} \text{ \AA}^{-2}$. Furthermore, data obtained with C_8 bonded phases at higher organic modifier concentrations exhibit slightly greater a_g values than those obtained with C_{18} bonded phases. In general, the good agreement between RPC retention and partition data in Fig. 9 strongly suggests the mechanistic similarity of the two apparently disparate processes. This has led to the wide belief that retention in RPC is governed by

the partition mechanism in most practical situations at least for nonpolar eluities [19,23,100].

7.2. Data analysis based on lattice theory

We surveyed the chromatographic literature for criteria and methods to distinguish between partition and adsorption mechanism in RPC. We found only one analytical test [11] which is based on the lattice theory to identify adsorption or partition as the mechanism underlying the retention in RPC. This test employs the amorphous-crystalline hydrocarbon layer and the adsorptive hydrocarbon monolayer models to represent RPC bonded phases, respectively. According to the lattice theory, the change in free energy of adsorption is much smaller than that of partitioning since in the adsorption process only a fraction of the total solute surface area participates.

Assuming a cubic lattice, the theory predicts that

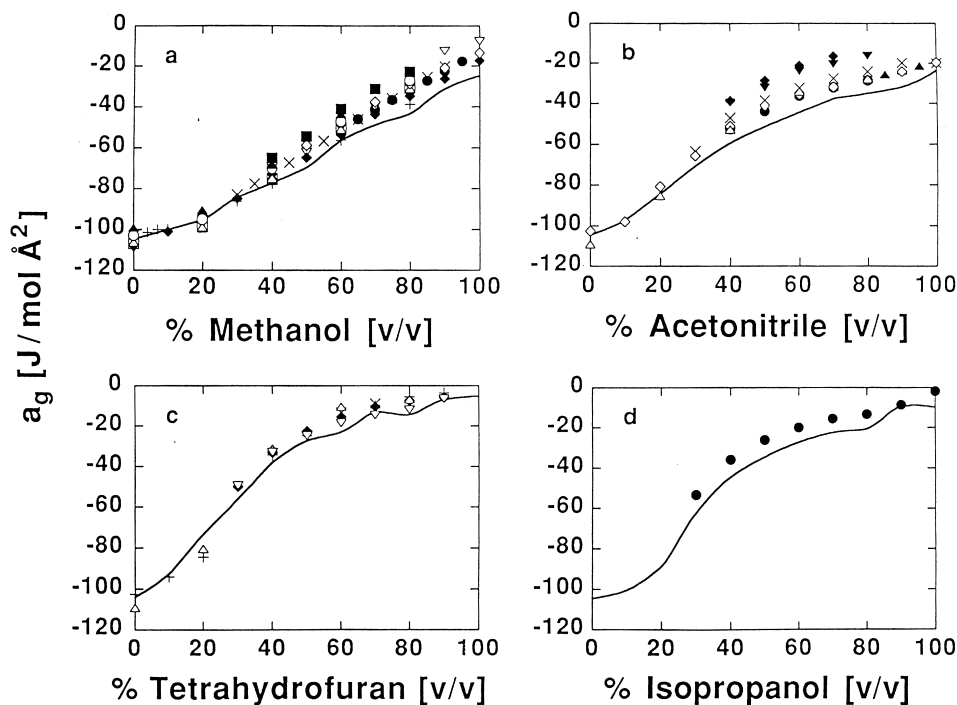


Fig. 9. Dependence of a_g on concentration of (a) methanol, (b) acetonitrile, (c) tetrahydrofuran and (d) isopropanol in water for the retention of hydrocarbonaceous eluities in RPC employing C_8 to C_{18} bonded phases and the transfer of alkylbenzenes from hydroorganic liquid phase to bulk hexadecane phase at 25°C . Data for the transfer process is taken from Ref. [19] and shown by solid lines while symbols represent experimental RPC data taken from references cited in Table 3.

the change in free energy of partitioning is six times the change in free energy of adsorption [11]. This may be expressed in terms of normalized free energy changes for nonpolar substances as:

$$a_g(\text{partition}) = 6a_g(\text{adsorption}) \quad (15)$$

It is noted that the factor six comes from the assumption of a cubic lattice model. On the other hand, a flat plate model could be developed for hydrocarbonaceous substances within the framework of the lattice theory that may yield a factor of two.

Based on Eq. (15), a parameter was introduced to distinguish between adsorption and partitioning in RPC [19,100], which is given by the ratio of the change in free energy of partitioning to the change in retention free energy in RPC, both per unit methylene group, and may be written in terms of normalized free energy changes as:

$$F = \frac{a_g(\text{partition})}{a_g(\text{retention})} \quad (16)$$

According to this approach, if $F=1$, i.e., $a_g(\text{partition})=a_g(\text{retention})$ then RPC retention is governed by the partition mechanism and if $F=6$, i.e., $a_g(\text{partition})$ is six fold higher than $a_g(\text{retention})$ then RPC retention is governed by the adsorption mechanism. Thus, the parameter F measures the extent of deviation of RPC retention data from partition data.

We analyzed RPC retention data presented in Fig. 9 and evaluated F by using Eq. (16). The F values are plotted in Fig. 10 against the organic modifier concentration. It is seen that for all four eluent systems F is quite close to 1 in a wide range of organic modifier concentration. At very high organic modifier concentration slightly greater F values are observed. Furthermore, data obtained with C_8 bonded phases yield F values greater than those for C_{18} bonded phases. In general, retention data obtained with higher organic modifier concentration and short alkyl-silica bonded chains yields F values between 1.25 and 2.5. Only at 100% organic modifier concentration does retention data presented in

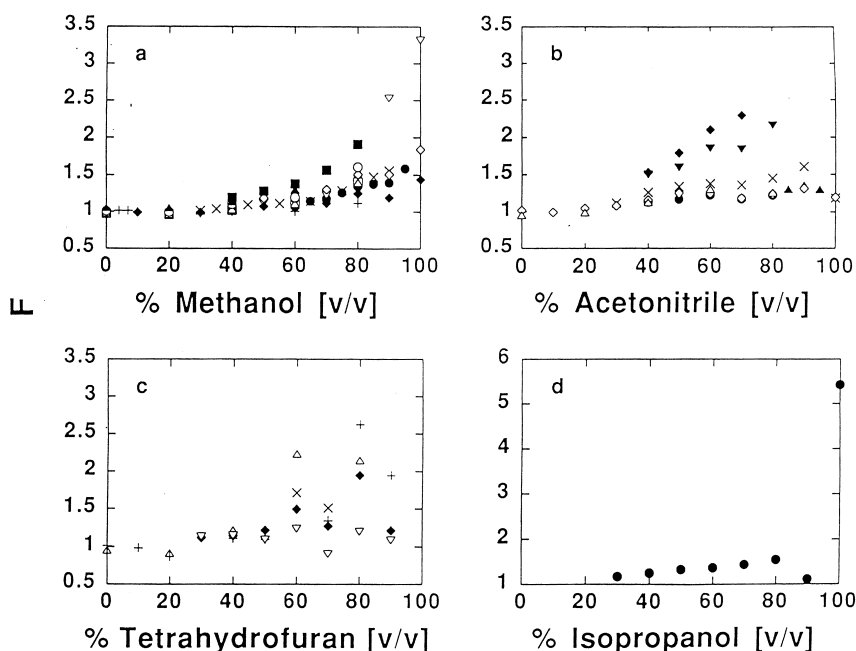


Fig. 10. Dependence of parameter F on concentration of (a) methanol, (b) acetonitrile, (c) tetrahydrofuran and (d) isopropanol in water for the retention of hydrocarbonaceous eluents in RPC and the transfer of alkylbenzenes from hydroorganic liquid phase to bulk hexadecane phase. Data and symbols from Table 3.

Fig. 10 exhibit F values greater than 3. Then, according to the analysis based on parameter F , retention in RPC employing C_{18} bonded phases is driven by the partition mechanism in a wide range of organic modifier concentration, whereas adsorption mechanism governs the retention behavior at very high organic modifier concentrations or when the silica bonded alkyl chains are short. This conclusion was drawn by Carr and co-workers [19] who employed the lattice theory to distinguish between adsorption and partition mechanism in RPC.

The employment of parameter F to distinguish between adsorption and partition in RPC on the basis of the lattice theory, however, appears to be associated with some inconsistencies. Firstly, F is a ratio of two quantities so that when the denominator, i.e., $a_g(\text{retention})$, approaches zero, then F becomes statistically meaningless. In such cases, F may not be a good measure of the deviation of retention in RPC from partition and therefore it hardly can be used to distinguish between partition and adsorption mechanisms in RPC. Indeed, a closer examination of data presented in Fig. 9 shows that at very high organic modifier concentrations $a_g(\text{retention})$ values approach zero and this introduces a considerable amount of uncertainty in the evaluation of F . For instance, the retention of alkylbenzenes on C_{18} bonded phase employing 90% and 100% methanol (symbol ∇ in Figs. 9a and 10a) as well as 100% isopropanol (symbol \bullet in Figs. 9d and 10d) yields very large values of F as seen in Fig. 10a and d even though the magnitude of the corresponding deviation from partition data in Fig. 9a and d is quite similar to that at other organic modifier concentrations, such as at 40, 60 and 80%. From this it follows that the large F values at high organic modifier concentrations is a consequence of the $a_g(\text{retention})$ values being close to zero rather than the deviation being large. This would then cast a shadow of doubt on the results of studies that employ F analysis to conclude that the retention in RPC with organic modifier rich eluents might be driven by the adsorption mechanism. In our opinion, the difference between $a_g(\text{partition})$ and $a_g(\text{retention})$ denoted by the symbol Δa_g gives a better measure of the extent of deviation of the RPC retention from the partition data than the parameter F . From data in Fig. 9 we evaluated Δa_g in the entire range of the organic modifier concentration and the

results are presented in Fig. 11. As seen, the difference in a_g for the two processes is close to zero for low organic modifier concentrations and attains a slightly higher value at other concentrations. Furthermore, the average Δa_g values appear to remain constant in the organic modifier concentration range from 40%–100%. For C_{18} bonded phases, the largest difference in a_g is not more than $-15 \text{ J mol}^{-1} \text{ \AA}^{-2}$. The small deviations measured by Δa_g values do not prove that there is a shift in retention mechanism from partition to adsorption.

Secondly and more importantly, it must be noted that the above analysis for distinguishing between adsorption and partitioning in RPC is solely based on the prediction by the lattice theory that $a_g(\text{partition})$ is six fold higher than $a_g(\text{adsorption})$, according to Eq. (15). Surprisingly, it appears that so far no one has tested this prediction by analyzing adsorption data. In the following section, we compare experimental data obtained with RPC retention, partition and adsorption processes in order to investigate the validity of the above approach and to shed light on the role of adsorption and partition in governing retention in RPC.

7.3. Data analysis by group molecular parameters

In order to distinguish between adsorption and partition in RPC, thermodynamic data obtained with partition, adsorption and RPC retention processes listed in Table 4 were analyzed. Fig. 12 illustrates plots of the logarithmic retention factors of alkanols and alkylbenzenes obtained in RPC using neat water as the eluent and the logarithmic equilibrium constants for the transfer of alkanes and alkylbenzenes from water to hexadecane against the molecular surface areas of the substances. Also shown are such plots for the logarithmic equilibrium constants associated with the transfer of alkanes and alkanones from water to octanol and the logarithmic adsorption coefficients of alkanols and alkanones on activated charcoal from dilute aqueous solution. It is seen that the plots are linear with correlation coefficients of 0.95 or higher in accordance with Eqs. (5), (8), (11), and have similar slopes. The group molecular parameters, a_g , associated with each process were evaluated from the slopes of the linear plots in Fig. 12 and

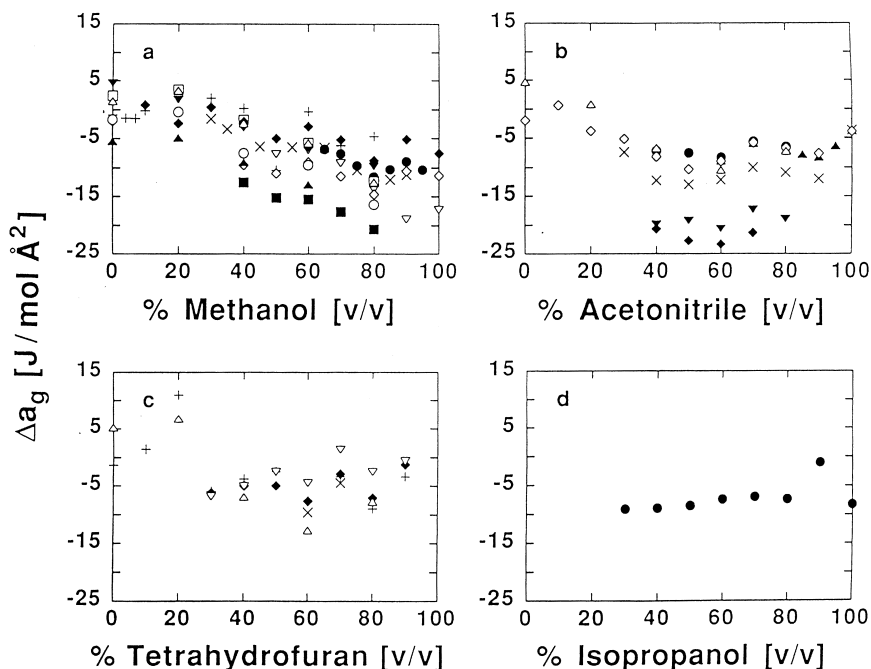


Fig. 11. Plots of difference between a_g for partitioning between hydroorganic liquid phase and bulk hexadecane and that for the retention in RPC against concentration of (a) methanol, (b) acetonitrile, (c) tetrahydrofuran and (d) isopropanol in water. Data and symbols from Table 3.

are listed in Table 4 along with the normalized free energy ratios. The $a_g(\text{retention})$, $a_g(\text{partition})$ and $a_g(\text{adsorption})$ values are found to be essentially the same with an average value of $-103 \text{ J mol}^{-1} \text{ \AA}^{-2}$ so that the normalized free energy ratios in Table 4 are close to unity. From the invariance of group molecular parameters listed in Table 4 it follows that RPC retention, partition and adsorption processes involving nonpolar substances in aqueous solutions are thermodynamically nearly alike. Consequently, adsorption and partition mechanisms in RPC cannot be distinguished purely on the basis of thermodynamic analysis.

In order to further examine the thermodynamic differences between adsorption, partition and RPC retention, data obtained under different mobile phase conditions on porous carbons of various provenance representing a wide range of properties was compared with partition and RPC retention data. Specifically, data [103,86] with graphitized carbon packing materials possessing rigid planar surfaces, such as HypercarbTM [104] and CarbonexTM [103], as well as carbon-black hardened by pyrocarbon or

pyrocarbon modified silica gel [86], was analyzed in a chromatographic system with methanol–water mixtures used as the eluent. Fig. 13 illustrates the dependence of the free energy change per unit nonpolar surface area against the methanol concentration in the eluent for the retention of alkanols and alkylbenzenes on carbonaceous adsorbents like HypercarbTM, CarbonexTM and pyrocarbon, the retention of nonpolar substances on octadecylated silica and the partitioning of alkylbenzenes between a hydroorganic liquid mixture and hexadecane. It is seen that the values of $a_g(\text{adsorption})$ for the porous carbons are slightly higher than those of $a_g(\text{retention})$ for C₁₈ bonded phases and $a_g(\text{partition})$ for transfer into hexadecane from hydroorganic liquid phase. Furthermore, data presented in Fig. 13 shows that $a_g(\text{adsorption})$ is greater than $a_g(\text{partition})$ in magnitude by about $15 \text{ J mol}^{-1} \text{ \AA}^{-2}$ over the entire range of methanol concentration. The higher selectivities observed with graphitized carbonaceous adsorbents have been attributed to strong π – π interactions of planar solute molecules with the graphite surface due to dispersion forces [103]. The above results are in

Table 4

Experimental and calculated values of free energy change per unit nonpolar surface area, a_g , associated with RPC retention, partitioning and adsorption processes at 25°C

Process	a_g^i J mol ⁻¹ Å ⁻² Experimental	a_g^j J mol ⁻¹ Å ⁻² Calculated	Normalized free energy ratio ^k	Refs.
RPC retention with C ₁₈ bonded phase and neat water of				
alkanols ^a	-99.77	-90.9	1	[85]
alkylbenzenes ^b	-102.21	-91.6	1	[94]
Transfer from water to hexadecane of				
alkanes ^c	-113.77	-116.9	1.13	[27]
alkylbenzenes ^d	-100.66	-104.4	1.00	[27]
Transfer from water to octanol of				
alkanes ^e	-108.92	-114.2	1.08	[25,26]
alkanones ^f	-102.65	-100.9	1.02	[25]
Adsorption from aqueous solution on activated charcoal of				
alkanols ^g	-98.17	-125.9	0.97	[30]
alkanones ^h	-98.15	-125.6	0.97	[30]

^a Ethanol, propanol and butanol.

^b Benzene and toluene.

^c Methane, ethane, propane, butane, 2-methylpropane, pentane, hexane, heptane, octane, cyclopropane, cyclopentane, cyclohexane, dimethylpropane and diethylpentane.

^d Benzene, toluene, ethylbenzene, propylbenzene, butylbenzene, pentylbenzene and hexylbenzene.

^e Methane, ethane, hexane, heptane and octane.

^f Propanone, 2-butanone, 3-pentanone and 2-hexanone.

^g Butanol, 2-methylbutanol, pentanol, 2,3-dimethyl-2-butanol, 3,3-dimethylbutanol, 2-ethylbutanol, 2-methyl-3-pentanol, 4-methylpentanol, hexanol, 2,4-dimethyl 3-pentanol, 3 ethyl-3-pentanol and heptanol.

^h Propanone, butanone, pentanone, 3-pentanone, 3-methyl-2-butanone, hexanone, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 3,3-dimethyl-2-butanone, heptanone, 3-heptanone, 4-heptanone, 4-methyl-2-hexanone, 5-methyl-2-hexanone, 2,4-dimethyl-3-pentanone, octanone, 3-methyl-2-heptanone, 5-methyl-2-heptanone and 2,6-dimethyl-4-heptanone.

ⁱ Evaluated from slopes of linear plots in Fig. 12.

^j Calculated for RPC retention, partitioning and adsorption processes from Eqs. (10), (6), (12), respectively.

^k Normalized free energy ratio, $a_g(\text{process})/a_g(\text{retention})$, was calculated from experimental data by taking the average value of 101 J mol⁻¹ Å⁻² for $a_g(\text{retention})$.

stark conflict with the predictions by Eq. (15), which has been proposed on the basis of the lattice theory [11], that $a_g(\text{partition})$ is six times greater than $a_g(\text{adsorption})$. This casts doubt on the validity of using parameter F as a philosophers' stone to distinguish between adsorption and partition and thus identify one as the genuine retention mechanism in RPC.

7.4. Evaluation of group molecular parameters by the solvophobic theory

In the following, we shall employ the solvophobic theory and calculate the group molecular parameters

associated with various distribution processes, i.e., RPC retention, partition and adsorption, in order to compare them to the experimental values in Table 4. According to Eqs. (6), (10), (12) derived in Section 5 for partition, RPC retention and adsorption, the free energy change per unit nonpolar surface area associated with these processes may be divided into two main contributions as shown in Table 5. The first contribution is common to all the above-mentioned processes and represents the free energy change per unit nonpolar surface area for the transfer of nonpolar solutes from water to the gas phase, $a_g(\text{transfer})$. The second contribution to $a_g(\text{partition})$ is due to the transfer of nonpolar solutes from the gas

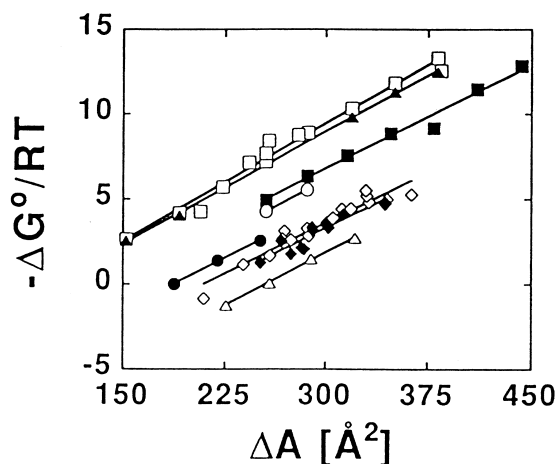


Fig. 12. Plots of reduced free energy change against the molecular surface area of the substances at 25°C for the RPC retention on C_{18} bonded phase of (●) alkanols and (○) alkylbenzenes using neat water as the eluent, partitioning of (□) alkanes and (■) alkylbenzenes between water and hexadecane, partitioning of (▲) alkanes and (△) alkanones between water and octanol and adsorption of (◆) alkanols and (◇) alkanones on activated charcoal from dilute aqueous solution. Experimental data was obtained from references cited in Table 4. The molecular surface areas were taken from Refs. [30], [81,84].

phase to the organic phase. On the other hand, for RPC retention and adsorption processes the second contribution, i.e., $0.75a_g(\text{gas})$, represents the elute–stationary phase and adsorbate–adsorbent interactions, respectively, as well as their reduction in the presence of the solvent.

For the evaluation of the a_g value by using Eq. (4), *n*-heptane is used as the nonpolar sample since its κ and γ values are well documented in the literature. The surface tension of *n*-heptane (γ_A) as well as the *n*-heptane/water interfacial tension (γ_{AW}) are taken as 118.4 [105] and 306 [106] $\text{J mol}^{-1} \text{Å}^{-2}$, respectively. The corresponding κ_A^g and κ_{AW}^g values are 0.869 and 0.41 [59,73]. The $a_g(\text{transfer})$ value of $-22.6 \text{ J mol}^{-1} \text{Å}^{-2}$ was calculated and this compares reasonably well with the value of $-18.4 \text{ J mol}^{-1} \text{Å}^{-2}$ obtained from experimental data [19].

In order to calculate the second contribution to a_g associated with the above processes, we examined retention on C_{18} bonded phases in gas chromatography, transfer of nonpolar solutes from the gas phase to bulk hexadecane or octanol and adsorption on graphitized thermal carbon black from the gas

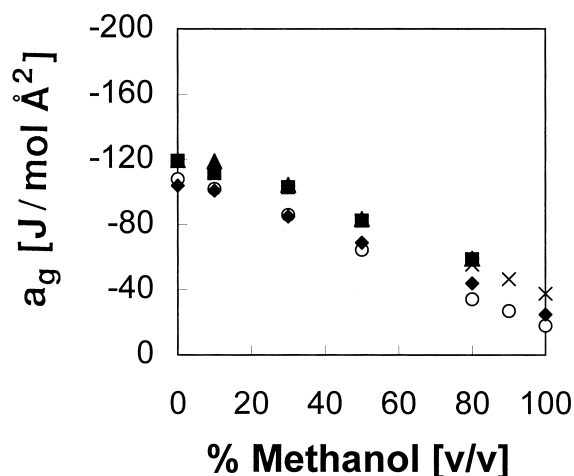


Fig. 13. Plots of the group molecular parameter, a_g , against the methanol concentration in the eluent for the retention of nonpolar substances on C_{18} bonded phases (○), of alkylbenzenes on Hypercarb adsorbent (■), of alkylbenzenes and alkanols on Carbonex adsorbent (▲), of alkylbenzenes on pyrocarbon adsorbent (×) and for the partitioning of alkylbenzenes between hydroorganic liquid phase and hexadecane (◆). RPC retention data is taken from Ref. [76], adsorption data on Hypercarb and Carbonex from Ref. [103], adsorption data on pyrocarbon from Ref. [86] and partition data from Ref. [19]. All the data was obtained at room temperature.

phase. The latter process was investigated instead of adsorption on activated charcoal from the gas phase owing to paucity of data. In Fig. 14, the logarithmic equilibrium constants associated with these processes at 25°C are plotted against the molecular surface area of the substances. Linear plots are obtained and from the slopes the free energy per unit molecular surface area is evaluated for the three processes and listed in Table 6. Data presented in Table 6 for the retention on C_{18} bonded phase in gas chromatography and for the transfer from the gas phase to bulk hexadecane or octanol compare very well. The a_g values obtained with adsorption on graphitized thermal carbon black from the gas phase are slightly higher. The results indicate that nonspecific van der Waals interactions between the elute and the bonded phase in gas chromatography, between the solute and molecules of hexadecane or octanol and between the adsorbate and graphitized thermal carbon black surface are of similar nature.

With the data listed in Table 6 and $a_g(\text{transfer})$ value calculated above, $a_g(\text{partition})$, $a_g(\text{retention})$

Table 5

Contributions to free energy change per unit nonpolar surface area, a_g , associated with RPC retention, oil–water partitioning and adsorption on activated charcoal from dilute aqueous solution according to Eqs. (10), (6), (12), respectively

Process	Contributions to normalized free energy change, a_g	
	I	II
Retention in RPC employing neat water as the mobile phase	$(\kappa_A^g \gamma_A - \kappa_{AW}^g \gamma_{AW})$	$0.75a_g(\text{gas})$
Partitioning between oil (octanol or hexadecane) and water	$(\kappa_A^g \gamma_A - \kappa_{AW}^g \gamma_{AW})$	$(\kappa_{AO}^g \gamma_{AO} - \kappa_A^g \gamma_A)$
Adsorption on activated charcoal from dilute aqueous solution	$(\kappa_A^g \gamma_A - \kappa_{AW}^g \gamma_{AW})$	$0.75a_g(\text{gas})$

and a_g (adsorption) were evaluated by using Eqs. (6), (10), (12) and the results are listed in Table 4 along with the corresponding values obtained from experimental data. As seen, the calculated values are in good agreement with the experimental data. The results confirm the capability of the solvophobic theory to accurately predict the selectivities of nonpolar substances in processes driven by the hydrophobic effect.

The strength of the solvophobic theory lies in its ability to calculate the individual contributions to the free energy change of various interactions (elute–

water, water–water and elute–stationary phase or adsorbate–adsorbent or solute–organic phase van der Waals interactions) occurring in processes dominated by hydrophobic interactions, such as RPC retention employing neat aqueous solution, adsorption on activated charcoal from aqueous solution and octanol–water partitioning. The first two types of interactions are the same for all the three above mentioned processes whereas the third type of

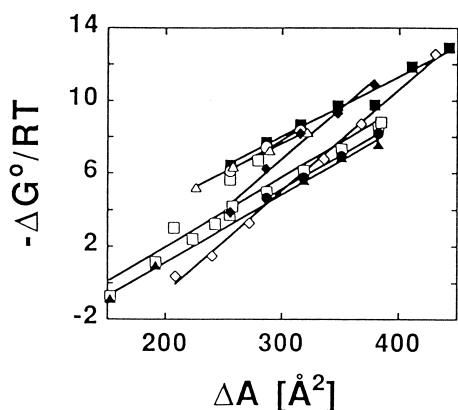


Fig. 14. Plots of reduced free energy change against the molecular surface area of the substances at 25°C for the retention on C_{18} bonded phase of (●) alkylbenzenes and (○) alkanes in gas chromatography, transfer of (□) alkanes and (■) alkylbenzenes from the gas phase to hexadecane, transfer of (▲) alkanes and (△) alkanones from the gas phase to octanol and adsorption of (◆) alkanols and (◇) alkanones on graphitized thermal carbon black from the gas phase. Experimental data was obtained from references cited in Table 6. The molecular surface areas were taken from Refs. [30,81,84].

Table 6

Free energy change per unit nonpolar surface area, a_g , associated with processes involving the gas phase at 25°C evaluated from slopes of linear plots in Fig. 14

Process	a_g $\text{J mol}^{-1} \text{Å}^{-2}$	Reference
Retention on C_{18} bonded phase in gas chromatography of		
alkanes ^a	-91.1	[57]
alkylbenzenes ^b	-92.0	[57]
Transfer into hexadecane of gaseous		
alkanes ^c	-94.3	[27]
alkylbenzenes ^c	-81.8	[27]
Transfer into octanol of gaseous		
alkanes ^d	-91.6	[25,101]
alkanones ^d	-78.3	[25,101]
Adsorption on graphitized thermal carbon black of gaseous		
alkanols ^e	-137.8	[102]
alkylbenzenes ^f	-137.4	[102]

^a Pentane, hexane, heptane and octane.

^b Benzene, toluene and ethylbenzene.

^c Compounds are the same as in Table 4.

^d Compounds are the same as in Table 4.

^e Ethanol, propanol, butanol, hexanol, heptanol and nonanol.

^f Benzene, toluene, ethylbenzene, propylbenzene and butylbenzene.

interactions may be different. However, since the stationary phase in RPC, the adsorbent in adsorption process and the organic phase in partitioning process are hydrocarbonaceous in nature and exhibit similar favorable interactions with nonpolar substances, it is not surprising that such processes are fundamentally similar and characterized by quite similar values of free energy change per unit nonpolar surface area. In other words, the methylene groups are expected to interact in the same manner whether the nonaqueous phase is a solid carbonaceous adsorbent, a hydrocarbonaceous stationary phase or an organic liquid phase. Slight differences in such values may be expected with stationary phases or adsorbents of different carbon loading, but the fundamental principles underlying these processes remain and yield very similar values for the free energy change per unit nonpolar surface area.

7.5. Chain length of the alkyl ligates in RPC

The effect of chain length of the alkyl ligate on a_g (retention) as well as that of alkane on a_g (partition) was studied by analyzing data [23] for RPC retention obtained with various alkyl bonded stationary phases of different chain lengths and methanol–water (1:1, v/v) as the mobile phase and that for partitioning between methanol–water (1:1, v/v) and alkanes of various alkyl chain lengths. The results are shown in Fig. 15. While a_g (partition) is not significantly affected by the chain length of the alkane, RPC retention data exhibits a decrease in the magnitude of a_g (retention) with the decrease in the chain length of the alkyl ligate. For stationary phases with short alkyl bonded chains, such as pentyl-, hexyl-, heptyl- and octyl-bonded phases, a_g (retention) values are smaller than a_g (partition) values obtained with corresponding alkanes. With increasing length of the bonded chains, a_g (retention) approaches a_g (partition) and for dodecyl bonded phase the a_g values in RPC and liquid–liquid partition system are essentially identical. Further increase in the chain length produces only a very small increase in selectivity, so that the plot of a_g (retention) versus the chain length of the alkyl ligate levels off after the dodecyl bonded stationary phase.

We further evaluated according to the solvophobic

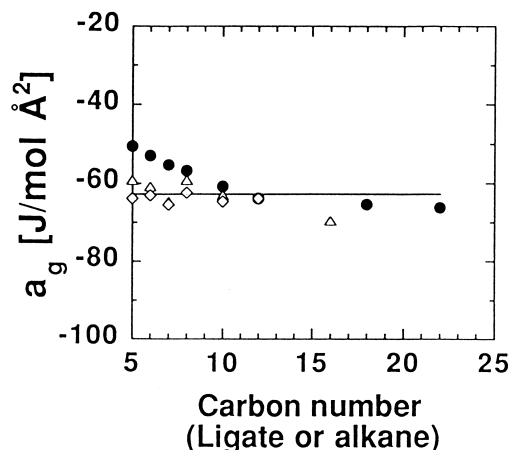


Fig. 15. Dependence of a_g at 25°C for the partitioning of nonpolar solutes between methanol–water (1:1, v/v) and alkane determined by (Δ) the traditional shake flask method or by (\diamond) entrained alkanes on the corresponding RPC column, and for (\bullet) the retention of hydrocarbonaceous elutes in RPC employing C_{18} bonded phase methanol–water (1:1, v/v) on the carbon number of the alkane in partitioning or of the bonded alkyl chains in RPC. Data taken from Ref. [23]. The solid line represents data for the hypothetical process of adsorption on activated charcoal from methanol–water (1:1, v/v) evaluated by using Eq. (12).

theory the a_g value associated with the hypothetical adsorption on activated charcoal from methanol–water (1:1, v/v) and the result is presented in Fig. 15 as a horizontal line. It is seen that both adsorption and partition data compare very well. In our opinion, the small deviation of RPC retention data from partition data with short chains of the bonded phases shown in Fig. 15 or at high organic modifier concentrations in Fig. 9 may be attributed to the presence of silanophilic interactions, the changes in the geometric configuration of the stationary phase ligates due to changes in the mobile phase composition, steric effects on eluite retention caused by changes in stationary phase configuration and/or to the sorption of organic modifier in the stationary phase, but not to the shift in mechanism from partition to adsorption as proposed earlier [19,23,100].

The retention data presented in Fig. 15 further illustrates the effect of stationary phase chain length on the selectivity of nonpolar substances in RPC employing methanol–water (1:1, v/v) as the eluent. It is seen that the magnitude of a_g (retention) in-

creases by $15.6 \text{ J mol}^{-1} \text{ \AA}^{-2}$ from $-50.7 \text{ J mol}^{-1} \text{ \AA}^{-2}$ for C_5 to $-66.3 \text{ J mol}^{-1} \text{ \AA}^{-2}$ for C_{22} bonded stationary phase. On the other hand, as seen from Fig. 8 a_g (retention) for RPC employing C_{18} bonded phases varies from $-103 \text{ J mol}^{-1} \text{ \AA}^{-2}$ in neat water to $-17 \text{ J mol}^{-1} \text{ \AA}^{-2}$ in neat methanol. Thus, the 100% variation in methanol concentration of the eluent brings about more than a five fold change in RPC selectivity compared to the variation of stationary phase chain length from C_5 to C_{22} . This strongly indicates the dominant role of the mobile phase, represented by elute–eluent and eluent–eluent interactions, in determining the selectivity changes of nonpolar elutes in RPC.

8. Conclusions

In this study, the solvophobic theory was employed to present a unified treatment of the following three processes based on the hydrophobic effect: retention in RPC, oil–water partitioning and adsorption on activated charcoal from dilute aqueous solution. Analysis of experimental data obtained with substances having substantial hydrocarbonaceous moieties was carried out within the unified framework of the solvophobic theory. The free energy change per unit nonpolar surface area for each of these processes was found to be similar in agreement with the prediction by the solvophobic theory. This lends strong support to this theoretical approach that it can accurately describe RPC retention, partition and adsorption processes. Furthermore, the results show that the physicochemical principles underlying such apparently disparate processes are fundamentally identical. In the light of the solvophobic theory, the quite similar selectivities exhibited by the above separation processes for nonpolar substances is attributed to the similar magnitudes of van der Waals forces governing these processes, such as solvent–solvent, solute–solvent and solute–stationary phase interactions. However, selectivities toward polar substances are expected to differ due to the considerable energetic differences between the partitioning of the polar moieties in organic phases, i.e., octanol and hexadecane, and between their interactions with bonded stationary phases in RPC and with activated

charcoal in adsorption. In addition, silanophilic interactions at the chromatographic surface in RPC may also play an important role in determining the different selectivities observed in RPC. Understanding the polar selectivities of such processes would be facilitated by incorporating additional terms in the solvophobic framework that quantify electrostatic interactions between polar functions.

Several studies based on thermodynamic principles have attempted to shed light on the relative significance of partition and adsorption in RPC [12,17,19,22,100]. These investigations employed an analytical method based on the prediction by the lattice theory that the free energy change for partitioning is six times higher than the free energy change for adsorption, and in light of the excellent agreement between partitioning and RPC retention data in most practical situations it was concluded that retention in RPC is driven by the partition mechanism. This led to a criticism of the solvophobic theory for being based on an incorrect solution phase model by treating RPC retention in terms of the association of two molecules, an elute molecule and a bound ligate molecule, in a single solvent (mobile phase) rather than on the transfer of an elute from one solvent (mobile phase) to another (stationary phase). The finding of the present study that the free energy changes per unit nonpolar surface area for oil–water partitioning and for adsorption on activated charcoal from dilute aqueous solution are quite similar in magnitude is in conflict with the prediction of the lattice theory and exposes its limitation in providing a consistent framework for the treatment of both partition and adsorption processes. It further raises the question whether this approach is suitable to distinguish between partition and adsorption mechanisms in RPC. From the results presented here, we conclude that a good agreement between RPC retention and partition data does not necessarily imply that retention in RPC is governed by the partition mechanism. As shown in this study, RPC retention data compares just as well with the adsorption data. On these grounds, the bulk liquid, the liquid-crystalline or the amorphous-crystalline hydrocarbon layer models adopted by the lattice theory may fall short of accurately describing retention in RPC. Since these models are a priori based on partition mechanism, they automatically force us to

take side with the partition mechanism in RPC retention. In contradistinction, the solvophobic theory employing the isolated solvated hydrocarbon chains model to represent the stationary phase configuration in RPC does not presume that retention in RPC occurs by a partition or an adsorption mechanism, thus providing an unbiased theoretical framework for the analysis of the retention data.

This brings us back to the issue we hoped to resolve at the outset, i.e., whether the retention mechanism in RPC is driven by adsorption or partition. The results based on the thermodynamic analysis of partition, adsorption and RPC retention data obtained with nonpolar substances reveal only very slight differences as far as the energetics of the processes are concerned. This suggests that even though the processes are different in practice, both the partition and the adsorption process involving aqueous solutions exhibit similar thermodynamic behavior owing to the fundamental similarity of the physicochemical phenomena underlying such processes. Therefore, a clear distinction between partition and adsorption in RPC of nonpolar eluents is not apparent from thermodynamic analysis so that other means have to be employed to resolve the issue. But with no apparent thermodynamic or practical significance as shown by the present study, this issue may not be worth any further investigation.

As mentioned earlier, partition and adsorption processes have substantial differences in the physical sense. In the beginning, adsorption was the main separation mechanism, however, due to the energetic heterogeneity of the adsorbent surface the process was rather unfavorable and this significantly arrested the further development and application of adsorption chromatography in the purification and analysis of a wide variety of substances. On the other hand, by replacing the solid surface by a layer of an immiscible liquid to retain the eluents much higher separation efficiencies could be obtained. This led to the development of partition chromatography by A.J.P. Martin [107] for which he received the Nobel prize. In RPC, the chromatographic surface is very different from that of an adsorbent, such as activated charcoal, and its surface density is much less than that of a bulk hydrocarbon liquid so that it is most likely that the mechanism of retention cannot be ascribed to either adsorption or partitioning alone in

the ideal sense. Furthermore, retention in RPC is a result of various plausible binding configurations of the elute with the stationary phase ligates so that the measured retention factors represent average values of the corresponding free energy of binding. In this light it is quite possible that RPC retention is driven by a combination of adsorption and partition mechanisms.

For over the past twenty years, reversed-phase chromatography has functioned as the leading analytical separation method for a vast variety of substances and the solvophobic theory has provided a rigorous framework for the understanding of the fundamental principles underlying the RPC retention process. The results of this study expand the scope of earlier investigation [59] in which the solvophobic theory was used to analyze data on the retention in HIC and dissolution of nonpolar gases in water. The results support the theory that has provided a framework for an accurate treatment of the energetics of RPC retention and of various other processes involving hydrophobic interactions as well. However, we believe that the lattice theory, if properly developed and handled, can also describe accurately the RPC retention, partition and adsorption processes and interpret the results with physically sensible molecular/macroscopic parameters. Indeed, the solvophobic and the lattice approach are two different ways of looking at the same chromatographic process. Both theories have their strengths and weaknesses, and it is important to realize that a unified theory may be developed that takes the best elements of both theories.

When the solvophobic theory was first applied to RPC retention in 1976 [8], it was assumed that the interligate space was accessible for the elute molecules to interact unhindered with the isolated ligates of the stationary phase. Indeed, the stationary phases employed in those times had low surface ligate density. The primary goal of that study was to elucidate the fundamental principles underlying the retention in RPC under experimental conditions employed and the results of the early treatment indicated the dominant role of the mobile phase in governing retention and selectivity changes in RPC. Later improvements in stationary phase properties in terms of ligate density affected the chromatographic data collected and used to shed light on the role of

the stationary phase density and ligate chain length on the retention factor and, as a result, some workers questioned the validity of the solvophobic theory in predicting stationary phase effects in RPC. Since the solvophobic theory is eclectic and draws from several well established concepts, it can be adapted easily to describe retention by hydrocarbonaceous stationary phases of high ligate density. For instance, certain important features of the lattice theory, such as the stationary phase effects, can be easily incorporated within the framework of the solvophobic theory to expand the scope of its application and thus to describe RPC retention under a wide range of experimental conditions.

Acknowledgements

This work was supported by Grant No. GM 20993 from the National Institutes of Health, US Public Health Service.

References

- [1] Cs. Horváth, W. Melander, *J. Chromatogr. Sci.* 15 (1977) 393.
- [2] R.E. Majors, *Anal. Chem.* 44 (1972) 1722.
- [3] W.R. Melander, Cs. Horváth, in: Cs. Horváth (Ed.), *High-performance Liquid Chromatography – Advances and Perspectives*, Academic Press, New York, 1980, p. 113.
- [4] C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley-Interscience, New York, 1980.
- [5] A. Ben-Naim, *Hydrophobic Interactions*, Plenum Press, New York, 1980.
- [6] W. Kauzmann, *Adv. Protein Chem.* 14 (1959) 1.
- [7] D. Haidacher, A. Vailaya, Cs. Horváth, *Proc. Natl. Acad. Sci. USA* 93 (1996) 2290.
- [8] Cs. Horváth, W.R. Melander, I. Molnár, *J. Chromatogr.* 125 (1976) 129.
- [9] O. Sinanoğlu, in: B. Pullman (Ed.), *Molecular Associations in Biology*, Academic Press, New York, 1968, p. 427.
- [10] D.E. Martire, R.E. Boehm, *J. Phys. Chem.* 87 (1983) 1045.
- [11] K.A. Dill, *J. Phys. Chem.* 91 (1987) 1980.
- [12] J.G. Dorsey, K.A. Dill, *Chem. Rev.* 89 (1989) 331.
- [13] M. Jaroniec, D.E. Martire, *J. Chromatogr.* 351 (1986) 1.
- [14] M. Jaroniec, D.E. Martire, *J. Chromatogr.* 387 (1987) 55.
- [15] M. Jaroniec, *J. Chromatogr. A* 656 (1993) 37.
- [16] A. Vailaya, Cs. Horváth, *J. Phys. Chem. B* 101 (1997) 5875.
- [17] L.C. Tan, P.W. Carr, *J. Chromatogr. A* 775 (1997) 1.
- [18] J.H. Park, J.E. Lee, M.D. Jang, J. Li, P.W. Carr, *J. Chromatogr.* 586 (1991) 1.
- [19] P.W. Carr, J. Li, A.J. Dallas, D.I. Eikens, L.C. Tan, *J. Chromatogr. A* 656 (1993) 113.
- [20] J.H. Park, M.D. Jang, J.J. Chae, H.C. Kim, J.K. Suh, *J. Chromatogr. A* 656 (1993) 69.
- [21] R. Tijssen, P.J. Schoenmakers, M.R. Böhmer, L.K. Koopal, H.A.H. Billiet, *J. Chromatogr. A* 656 (1993) 135.
- [22] J.H. Park, Y.K. Lee, Y.C. Weon, L.C. Tan, J. Li, L. Li, J.F. Evans, P.W. Carr, *J. Chromatogr. A* 767 (1997) 1.
- [23] C.H. Lochmüller, D.R. Wilder, *J. Chromatogr. Sci.* 17 (1979) 574.
- [24] A. Leo, C. Hansch, D. Elkins, *Chem. Rev.* 71 (1971) 525.
- [25] S. Cabani, G. Conti, V. Mollica, L. Bernazzani, *J. Chem. Soc. Faraday Trans. I* 87 (1991) 2433.
- [26] P. Berti, S. Cabani, G. Conti, V. Mollica, *J. Chem. Soc. Faraday Trans. I* 82 (1986) 2547.
- [27] M.H. Abraham, G.S. Whiting, R. Fuchs, E.J. Chambers, *J. Chem. Soc. Perkin Trans. 2* (1990) 291.
- [28] L.R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- [29] L.R. Snyder, H. Poppe, *J. Chromatogr.* 184 (1980) 363.
- [30] G. Belfort, G.L. Altshuler, K.K. Thallam, C.P.J. Feerick, K.L. Woodfield, *AIChE J.* 30 (1984) 197.
- [31] K. Karch, I. Sebastian, I. Halasz, *J. Chromatogr.* 122 (1976) 3.
- [32] H. Colin, G. Guiochon, *J. Chromatogr.* 141 (1977) 289.
- [33] G.E. Berendsen, K.A. Pikaart, L. de Galan, *J. Liq. Chromatogr.* 3 (1980) 1437.
- [34] E.A. DiMarzio, *J. Chem. Phys.* 35 (1961) 658.
- [35] M.A. Cotter, D.E. Martire, *Mol. Cryst. Liq. Cryst.* 7 (1969) 295.
- [36] H.T. Peterson, D.E. Martire, M.A. Cotter, *J. Chem. Phys.* 61 (1974) 3547.
- [37] F. Dowell, D.E. Martire, *J. Chem. Phys.* 69 (1978) 2332.
- [38] K. Motomura, R. Matuura, *J. Colloid Interface Sci.* 29 (1969) 617.
- [39] R.E. Boehm, D.E. Martire, *Mol. Phys.* 43 (1981) 351.
- [40] E.A. DiMarzio, C.M. Guttman, J.D. Hoffman, *Macromolecules* 13 (1980) 1194.
- [41] P.J. Flory, *J. Am. Chem. Soc.* 84 (1962) 2857.
- [42] K.A. Dill, P.J. Flory, *Proc. Natl. Acad. Sci. USA* 77 (1980) 3115.
- [43] K.A. Dill, P.J. Flory, *Proc. Natl. Acad. Sci. USA* 78 (1981) 676.
- [44] K.A. Dill, D.E. Koppel, R.S. Cantor, J.D. Dill, D. Benedouch, S.-H. Chen, *Nature* 309 (1984) 42.
- [45] P.G. deGennes, *Phys. Lett.* 47A (1974) 123.
- [46] J.A. Marqusee, K.A. Dill, *J. Chem. Phys.* 85 (1986) 434.
- [47] S.J. Klatte, T.L. Beck, *J. Phys. Chem.* 97 (1993) 5727.
- [48] S.J. Klatte, T.L. Beck, *J. Phys. Chem.* 99 (1995) 16024.
- [49] S.J. Klatte, T.L. Beck, *J. Phys. Chem.* 100 (1996) 5931.
- [50] T.L. Hill, *Introduction to Statistical Thermodynamics*, Addison-Wesley, Reading, MA, 1960.
- [51] D.H. Everett, in: R.H. Ottewill, C.H. Rochester, A.L. Smith (Ed.), *Adsorption from Solution*, Academic, New York, 1983, p. 1.

- [52] D.C. Locke, *J. Chromatogr. Sci.* 12 (1974) 433.
- [53] D.E. Martire, R.E. Boehm, *J. Phys. Chem.* 84 (1980) 3620.
- [54] O. Sinanoğlu, S. Abdulnur, *Photochem. Photobiol.* 3 (1964) 333.
- [55] O. Sinanoğlu, S. Abdulnur, *Fed. Proc.* 24 (1965) S12.
- [56] Cs. Horváth, W. Melander, I. Molnár, *Anal. Chem.* 49 (1977) 142.
- [57] K. Miyabe, M. Suzuki, *AIChE J.* 41 (1995) 536.
- [58] W.R. Melander, D. Corradini, Cs. Horváth, *J. Chromatogr.* 317 (1984) 67.
- [59] A. Vailaya, Cs. Horváth, *Biophys. Chem.* 62 (1996) 81.
- [60] W.R. Melander, Cs. Horváth, *Arch. Biochem. Biophys.* 183 (1977) 200.
- [61] W.R. Melander, Cs. Horváth, *J. Solid-Phase Biochem.* 2 (1977) 141.
- [62] G. Belfort, *Environ. Sci. Technol.* 13 (1979) 939.
- [63] S. Miller, *Environ. Sci. Technol.* 14 (1980) 1037.
- [64] W.R. Melander, Cs. Horváth, in: M.J. McGuire, I.H. Suffett (Ed.), *Activated Carbon Adsorption of Organics from the Aqueous Phase*, Ann Arbor Science, Ann Arbor, 1980, p. 65.
- [65] O. Sinanoğlu, A. Fernández, *Biophys. Chem.* 21 (1985) 157.
- [66] A. Vailaya, Cs. Horváth, *J. Phys. Chem.* 100 (1996) 2447.
- [67] R.S. Spolar, J.-H. Ha, M.T. Record Jr., *Proc. Natl. Acad. Sci. USA* 86 (1989) 8382.
- [68] J.-H. Ha, R.S. Spolar, M.T. Record Jr., *J. Mol. Biol.* 209 (1989) 801.
- [69] K.P. Murphy, S.J. Gill, *Thermochim. Acta* 172 (1990) 11.
- [70] K.P. Murphy, S.J. Gill, *J. Mol. Biol.* 222 (1991) 699.
- [71] B. Lee, *Proc. Natl. Acad. Sci. USA* 88 (1991) 5154.
- [72] A. Vailaya, Cs. Horváth, *Ind. Eng. Chem. Res.* 35 (1996) 2964.
- [73] O. Sinanoğlu, in: H. Ratajczak, W.J. Orville-Thomas (Eds.), *Molecular Interactions*, J. Wiley, New York, 1982, p. 283.
- [74] S.H. Yalkowsky, S.C. Valvani, *J. Med. Chem.* 19 (1976) 727.
- [75] W.J. Doucette, A.W. Andren, *Chemosphere* 17 (1988) 345.
- [76] H. Colin, G. Guiochon, Z. Yun, J.C. Diez-Masa, J. Jandera, *J. Chromatogr. Sci.* 21 (1983) 179.
- [77] W.J. Cheong, P.W. Carr, *J. Chromatogr.* 499 (1990) 373.
- [78] R.K. Gilpin, M. Jaroniec, S. Lin, *Anal. Chem.* 62 (1990) 2092.
- [79] R.K. Gilpin, M. Jaroniec, S. Lin, *Chromatographia* 30 (1990) 393.
- [80] M. Jaroniec, S. Lin, R.K. Gilpin, *Chromatographia* 32 (1991) 13.
- [81] R.B. Hermann, *J. Phys. Chem.* 76 (1972) 2754.
- [82] J. Timmermans, *Physicochemical Constants of Binary Systems in Concentrated Solutions*, Interscience, New York, 1960.
- [83] R.C. Reid, J.M. Prausnitz, B.E. Poling, *The Properties of Gases and Liquids*, McGraw Hill, New York, 1987.
- [84] G.L. Amidon, S.H. Yalkowsky, S. Leung, *J. Pharm. Sci.* 63 (1974) 1858.
- [85] G.E. Berendsen, L. de Galan, *J. Chromatogr.* 196 (1980) 21.
- [86] H. Colin, G. Guiochon, *J. Chromatogr. Sci.* 18 (1980) 54.
- [87] P.J. Schoenmakers, H.A.H. Billiet, L. de Galan, *J. Chromatogr.* 218 (1981) 261.
- [88] W.E. Hammers, G.J. Meurs, C.L. de Ligny, *J. Chromatogr.* 247 (1982) 1.
- [89] C.R. Yonker, T.A. Zwier, M.F. Burke, *J. Chromatogr.* 241 (1982) 269.
- [90] H. Colin, A.M. Krstulovic, M. Gonnord, G. Guiochon, Z. Yun, J. Jandera, *Chromatographia* 17 (1983) 9.
- [91] M. Harnisch, H.J. Mockel, G. Schulze, *J. Chromatogr.* 282 (1983) 315.
- [92] A. Tchaplal, H. Colin, G. Guiochon, *Anal. Chem.* 56 (1984) 621.
- [93] B.P. Johnson, M.G. Khaledi, J.G. Dorsey, *J. Chromatogr.* 384 (1987) 221.
- [94] M. Hsieh, J.G. Dorsey, *J. Chromatogr.* 631 (1993) 63.
- [95] P.C. Sadek, P.W. Carr, M.J. Ruggio, *Anal. Chem.* 59 (1987) 1032.
- [96] C.R. Yonker, T.A. Zwier, M.F. Burke, *J. Chromatogr.* 241 (1982) 257.
- [97] M.M. Sanagi, U.K. Ahmad, K. Hassan, G. Musa, *J. Chromatogr. A* 722 (1996) 59.
- [98] T. Hanai, J. Hubert, *J. Chromatogr.* 290 (1984) 197.
- [99] T. Hanai, J. Hubert, *J. Chromatogr.* 291 (1984) 81.
- [100] P.W. Carr, L.C. Tan, J.H. Park, *J. Chromatogr. A* 724 (1996) 1.
- [101] M.H. Abraham, *J. Am. Chem. Soc.* 104 (1982) 2085.
- [102] A.V. Kiselev, Y.I. Yashin, *Gas-adsorption Chromatography*, Plenum, New York, 1969.
- [103] N. Tanaka, T. Tanigawa, K. Kimata, T. Araki, *J. Chromatogr. A* 549 (1991) 29.
- [104] J.H. Knox, B. Kaur, G.R. Millward, *J. Chromatogr.* 352 (1986) 3.
- [105] D.R. Lide (Ed.), *CRC Handbook of Chemistry and Physics*, CRC Press, Boca Raton, 1995.
- [106] R. Aveyard, D.A. Haydon, *Trans. Faraday Soc.* 61 (1965) 2255.
- [107] A.J.P. Martin, *Biochem. Soc. Symp.* 3 (1949) 4.