Quantitative Host−Guest Complexation Studies Using Chemically Bonded Stationary Phases. A Comparison of HPLC and Solution Enthalpies

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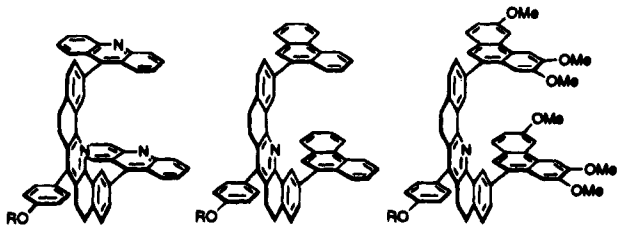
Quantifying binding interactions is an important part of many molecular recognition studies.[1] Currently, only a few methods are capable of simultaneously quantifying the stability of several complexes.[2] This feature will become increasingly important with the advent of methods for creating libraries of hosts and guests.[3] We recently described the synthesis of chemically bonded stationary phases **1** (CBSP-Acr) and **2** (CBSP-Phen),[4,5] which selectively retained nitrated polycyclic aromatic hydrocarbons (nitro-PAH), pervasive pollutants that are potent mutagens. In that work it was noted that the order of analyte (guest) elution paralleled the binding strengths in solution, suggesting the use of HPLC retention data for quantifying binding affinities.



1 **2** **3**

R = $(CH_2)_5SiMe_2Osilica$

4a,b **5a,b** **6a,b**

a : R = Me b : R = $(CH_2)_3CH{=}CH_2$

The basic expression describing the chromatographic process is $k' = \Phi K$, where k' is the capacity factor (corrected retention time), Φ is the phase ratio (volume stationary phase/volume mobile phase), and K is the partition coefficient.[6] This expression can be used to relate HPLC retention times to solution affinity constants if Φ is known, and if K is assumed to equal the association constant, K_{assoc}, in free solution.[7] At a minimum, the latter assumption requires the host−guest complexation in solution to be identical to that on the silica surface, with negligible nonspecific retention. The analysis is further complicated by the difficulty in determining phase ratios (Φ)[8] and the finding that Φ can change with both eluent and analyte.[9] The phase ratio problem can be avoided by measuring retention times as a function of temperature and calculating retention enthalpies ($\Delta H°$) with the expression $\ln k' = -\Delta H°/RT + \Delta S°/R + \ln(\Phi)$.[6] However, we are unaware of any comparisons between retention enthalpies determined in this way and the analogous complexation enthalpies measured in solution. Indeed, a potential difficulty was evident from a recent report by Pirkle that enthalpies for a retention process similar to that used by **1** and **2** can change by more than 3 kcal mol^{-1} as a function of CBSP loading.[10]

The ease with which HPLC retention enthalpies can be measured, and the key role that complexation enthalpies play in understanding molecular recognition processes,[11] led us to determine whether the enthalpies of retention of several guests on **1**−**3** would correlate with the corresponding complexation $\Delta H°$ measured in solution with **4**−**6**.[12] The interest in the new chemically bonded stationary phase, CBSP-tri-OMe-Phen (**3**), was in both having an additional bonded phase for comparison and increasing the range of $\Delta H°$ values.[13,14] To test the effect of loading, the bonding step in the synthesis of CBSPs **2** and **3** was carried out to two different degrees, giving "heavily" and "lightly" loaded phases. Combustion analysis showed these phases, designated **2**-h, **2**-l, **3**-h, and **3**-l, to contain, respectively, 0.145, 0.057, 0.025, and 0.003 mmol of host/g of 5 μM Spherisorb silica.

Each of the five bonded phases was slurry packed into 4.6 mm × 250 mm stainless-steel tubing, and the residual silanol groups were "capped" with trimethylsilane groups by treatment with hexamethyldisilazane. All five HPLC columns exhibited good peak shapes and showed significant retention and excellent separation of nitroaromatic analytes. A column prepared identically, but without the host, did not retain any of the nitroaromatic compounds used in this study. Furthermore, as found in our previous work,[4] the order of analyte elution paralleled the stability of the analogous host−guest complexes in solution. These results indicate that the mechanism of retention involved analyte complexation by the silica-bound host.

HPLC retention times were measured across a broad temperature range, with simultaneous determination of t_0 by coinjection with the nonretained 1,3,5-tri-*tert*-butylbenzene[15] (Table 1). Enthalpies of retention were determined from van't Hoff plots constructed by plotting $\ln k'$ against $1/T$. Although the $\Delta H°$ determinations were made individually, it was shown

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Table 1. Enthalpy of Retention Process for Various Analytes on CBSPs **1**–**3** and Solution Enthalpies of Complexation

	solution complexation							HPLC			
entry	host	guest[a]	solvent	temp range	K_{assoc}[b]	no. of runs	$-\Delta H°$[c]	CBSP[d]	k'[e]	temp range, °C	$-\Delta H°_{ads}$[f]
1	**4b**	TNF	$CHCl_3$	0–55	150	4	4.2	**1**	5.2	0–85	4.10
2	**4b**	TENF	$CHCl_3$	0–55	660	4	4.1	**1**	9.9	0–85	3.84 ± 0.06
3	**5a**	mDNB	$CHCl_3$	10–50	9	6	2.5 ± 1.0	**2**-h	1.1	0–85	2.70
4	**5b**	TNB	EtOAc	5–65	25	4	2.9	**2**-h	4.2	0–85	3.48
5								**2**-l	1.1	0–85	4.03
6	**5a,b**	TNB	$CHCl_3$	5–55	110	6	4.9 ± 0.7	**2**-h	18.2	0–85	5.08
7								**2**-l	4.4	0–85	5.04
8	**5a,b**	TNF	$CHCl_3$	5–55	640	3	5.7	**2**-h	62.7	25–85	5.71
9								**2**-l	16.6	0–85	5.58
10	**5a**	TCNQ	$CHCl_3$	5–55	450	4	7.5	**2**-h	35.5	15–85	6.71
11								**2**-l	8.6	0–85	6.65
12	**5a,b**	TENF	$CHCl_3$	5–55	3000	7	7.0 ± 0.8	**2**-l	72.0	25–85	6.70
13	**5a,b**	TCNQ	CCl_4	5–55	3550	5	9.8 ± 2.0	**2**-l	210	25–85	10.3
14	**6a**	mDNB	$CHCl_3$	5–55	15	3	2.8	**3**-h	0.19	0–85	2.13
15	**6a**	TNB	$CHCl_3$	5–55	690	4	4.6 ± 0.6	**3**-h	6.0	0–85	4.94
16	**6a**	TCNQ	$CHCl_3$	5–55	1040	3	5.7	**3**-h	12.1	25–85	5.82
17	**6a**	TNF	$CHCl_3$	5–55	9010	4	6.0 ± 0.4	**3**-h	54.0	0–85	6.28
18								**3**-l	3.9	0–85	6.12
19	**6a**	TCNQ	CCl_4	15–55	50 200	10	11.4 ± 1.6	**3**-h	332	35–85	9.68
20								**3**-l	24.7	0–85	9.66
21	**6a**	TENF	EtOAc	20–60	71 400	9	7.3 ± 1.7	**3**-h	405	35–85	7.49
22								**3**-l	28.0	0–85	7.41

[a] TNF, TENF: 2,4,5-trinitro, 2,4,5,7-tetranitrofluorenone. mDNB, TNB: *m*-dinitro-, 1,3,5-trinitrobenzene. TCNQ: 7,7,8,8-tetracyanoquinodimethane. [b] Liters/mole, 298 K. [c] Kilocalories/mole, for values lacking standard deviations, triplicate runs were within 20%. [d] Same solvent as for solution studies. [e] Capacity factor, 298 K. See ref 6a. [f] All triplicate runs were within 12%, with an average variation of 4%.

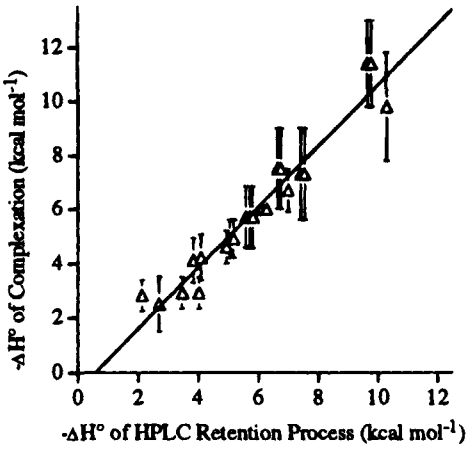

Figure 1. Plot of $\Delta H°$ for retention of various guests on CBSPs **1**–**3** versus corresponding complexation enthalpies using hosts **4**–**6**.

on CBSP-Acr that the values determined simultaneously for 2-nitro-, 2,7-dinitro-, 2,4,7-trinitro-, and 2,4,5,7-tetranitro-9-fluorenone were identical to those measured individually. Enthalpies for complexes of **4**–**6** in solution were determined from van't Hoff plots constructed using K_{assoc} values obtained from full binding titrations[4,16] at five temperatures (Table 1).

A plot comparing solution and HPLC enthalpies is shown in Figure 1. Strikingly, the data fit a line that is within experimental error of a one-to-one correlation (slope = 1.1 ± 0.3, intercept = −0.7 ± 1.9). Several other points are worth noting. First, the $\Delta H°$ values span an 8 kcal mol^{-1} range, with K_{assoc} from ca. 10 to 10^5 M^{-1}. Second, the loading negligibly affected the retention $\Delta H°$. Thus, in contrast to the Pirkle report,[10] the $\Delta H°$ does not change with the loading of CBSPs **2** and **3**. This contrasting dependence on loading could result from the different host–guest systems involved; however, we believe textural differences between the bonded phases to be responsible. The Pirkle phase[10] was prepared with a trifunctional silane that can produce a polymeric phase with close proximity of ligands.[17] CBSPs **1**–**3** were made from monofunctional silanes that form "brush phases", consisting of clusters of ligands in a liquid-like environment.[17]

Although the comparison of $\Delta H°$ values was the primary objective of this study, the HPLC method can also provide complexation free energies. This was accomplished by eluting multiple guests, one of whose $\Delta G°$ values (solution) is known, and calculating the $\Delta G°$ of the other guests using the relationship $\Delta\Delta G°$ (HPLC) $= -RT \ln(k_2'/k_1')$.[6b] Using the known $\Delta G°$ for the complex between TNF and **5** in chloroform, the $\Delta G°$ values calculated from HPLC ($CHCl_3$) on **2**-h and determined experimentally in solution are respectively as follows (kcal mol^{-1}): mDNB, 1.4, 1.3; TNB, 3.0, 2.8; TCNQ, 3.5, 3.6; TENF, 4.7; 4.7.

The advantages of this HPLC method[18] over the traditional techniques are that (1) 0.1–1.0 g of host is sufficient to make a column that can be used indefinitely; (2) multiple $\Delta H°$ and $\Delta G°$ values can be measured simultaneously; (3) $\Delta H°$ can be determined from van't Hoff plots that span broad temperature ranges (≥80 °C), providing higher accuracy; (4) small quantities of impure guests are sufficient; and (5) solutions of host and guest at known concentrations are not required. Our current efforts are directed toward determining the generality of this method.

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Supplementary Material Available: Synthetic scheme and details for the preparation of **3** and **6** (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(18) This method is conceptually similar to affinity chromatography (see refs 12), but does not require elutions with varying concentrations of competing receptor or ligand, thus allowing the simultaneous determination of binding parameters for multiple guests with a single injection.