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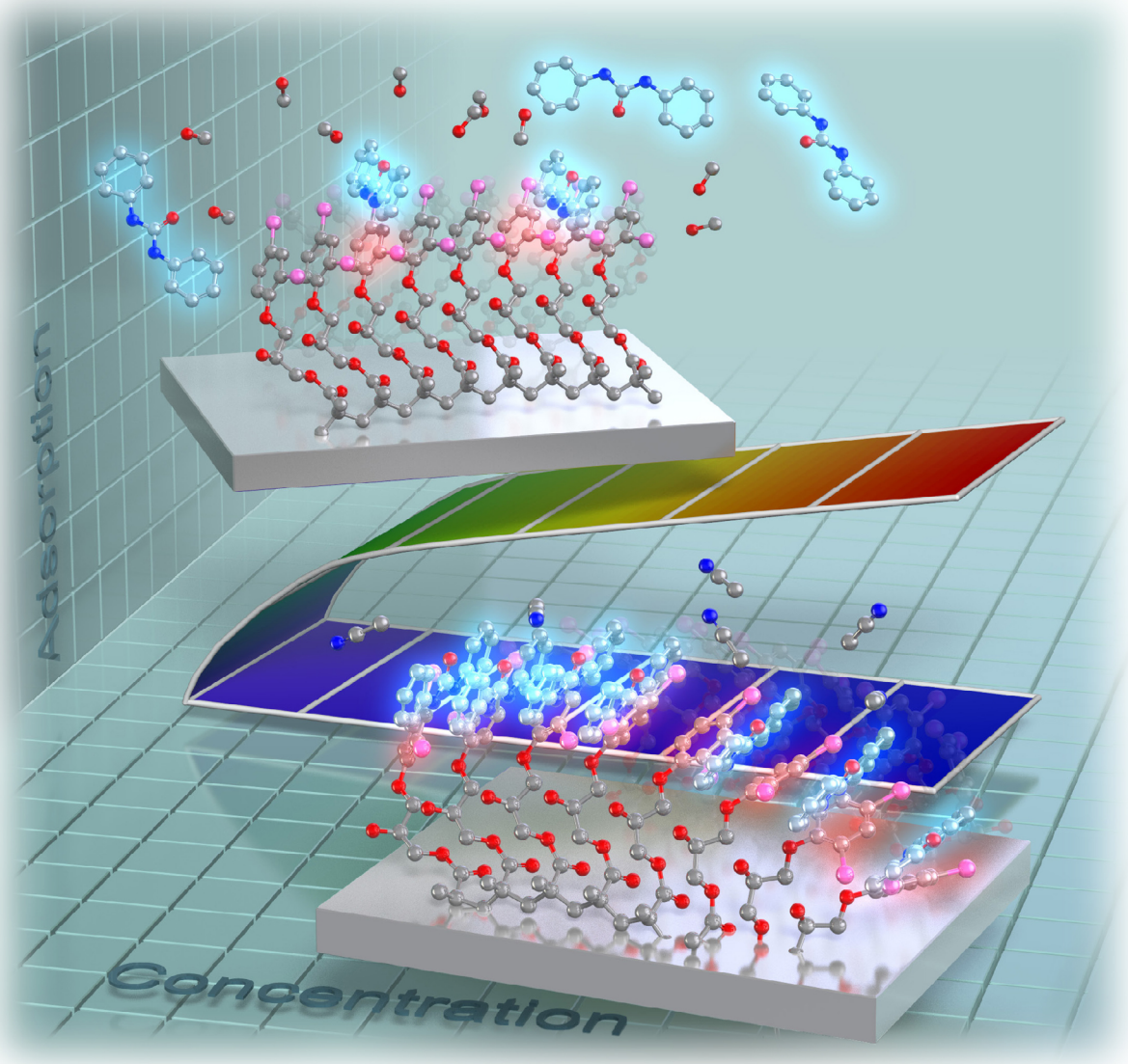

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Probing the Entropic Effect in Molecular Noncovalent Interactions between Resin-Bound Polybrominated Arenes and Small Substrates

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The associative interaction between resin-bound polybrominated arenes and small molecules was analyzed by using various spectroscopic techniques as well as a synthetic molecular model to establish the thermodynamics. The binding in acetonitrile was three orders of magnitude stronger than that in methanol, partly owing to the tertiary conformational gating of the resin that controls the entropic terms. By using the entropic superiority, the associative binding of up to $3\times10^4\ M^{-1}$ is achieved with the non-biological system. A modified Hill plot for the quantitative analysis of bindings was also devised, which enabled the interactions at the molecular level to be elucidated.

Controlling and understanding the interactions at solid–liquid interfaces is of particular importance in the broad fields of chemistry[1–8] and biology.[9–12] In particular, medicines and pesticides are made from organic chemicals, and their selective and specific detection and separation are important in the field of analytical chemistry. Various chromatographic techniques, including liquid chromatography (LC), have been extensively studied for the purpose,[1,13–16] and these established techniques are very useful in many areas, from materials science to medicinal science. However, the full understanding of the interactions at solid–liquid interfaces has yet to be achieved because of the interactions at the molecular level being elusive. Indeed, intermolecular interactions have been well understood by such diverse bonding modes as hydrogen bonding, CH–π interactions, and π–π interactions. Instead, we demonstrate another factor of dynamics using a model system in combination with various analytical techniques.

We previously reported substituted phenoxyl-modified polymers for the stationary phase of LC, in which phenols were anchored to glycidyl groups on the surfaces of resins, and among a series of the resins, poly**1** with a 2,4-dibromophenoxyl (DBP) unit (Scheme S1 in the Supporting Information) showed a good performance in terms of the solid-phase extraction for some chemicals;[16] therefore, we chose poly**1** as a model material for this study (for details, see the Supporting Information). The visualization of the interactions was achieved by using adsorption isotherms for the association between the solid-bound functional groups and substrates, dynamic light scattering (DLS) of resins, pyrolytic gas chromatography (GC), absorption analysis in the ultraviolet/visible (UV/Vis) region of homogeneous model systems, nuclear magnetic resonance (NMR) spectroscopy of the homogeneous model, and theoretical calculations using density functional theory (DFT) with a semiempirical local functional.[17] We also devised a modified Hill plot to evaluate the binding to understand the association at the molecular level.

First, poly**1** was synthesized according to the previously reported procedure,[16] and well characterized by infrared (IR) absorption, elemental analysis, DLS, cross-sectional elemental mapping using time-of-flight secondary ion mass spectrometry (TOF-SIMS), and adsorption isotherms (Figure 1a). Compound poly**1** showed an IR absorption characteristic for the C–H bending and ring breath modes of the anisole unit at 1474, 1458, 1048, 862, and 804 cm^{-1}, whereas the corresponding unfunctionalized resin showed no absorption (Figure S1; for details, see the Supporting Information). The introduction of the DBP unit into poly**1** was corroborated by the peripheral distribution of Br atoms in poly**1** as confirmed by TOF-SIMS (Figure S2) and elemental analysis. The particle diameters of poly**1** under dry conditions were about 60 μm (Figure S2), whereas the DLS and microscopic analysis showed much larger diameters, which indicated efficient solvation of the macromolecule (see below). The adsorption isotherms of poly**1** were then investigated using 1,3-diphenylurea, which is a potent cytokinin, as a standard adsorbent. When poly**1** was dispersed into a so-

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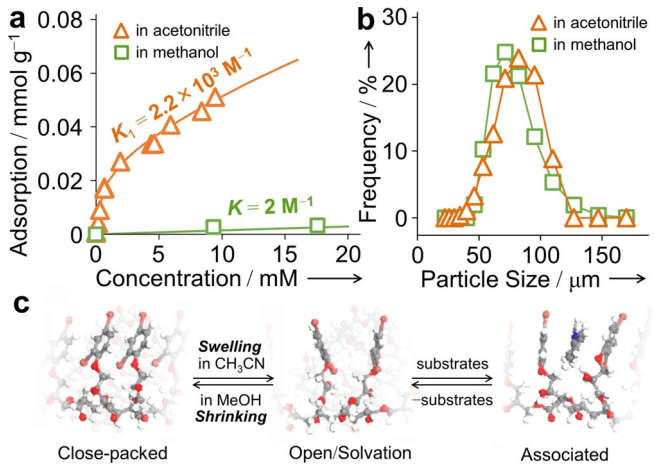

Figure 1. (a) Adsorption isotherms using diphenylurea as a substrate and poly**1** as a stationary phase in acetonitrile (triangle) and methanol (square) at 293 K. The former gave a bi-Langmuir plot with $K_1 = 2.2 \times 10^3$ M^{-1} and $K_2 = 25$ M^{-1}. For details, see Figure S3 in the Supporting Information. (b) DLS plot of poly**1** in acetonitrile (triangle) and methanol (square) at 293 K ($n = 3$). (c) The reaction scheme for the association between poly**1** and substrates. White: hydrogen; gray: carbon; blue: nitrogen; red: oxygen; brown: bromine. The solvent was omitted for clarity.

lution of diphenylurea in acetonitrile, the absorbance of the solution at 255 nm decreased to a level close to zero. This accounts for the fact that the complexation between poly**1** and diphenylurea proceeded spontaneously. On the contrary, when methanol was used as a solvent, almost no change in the absorbance was observed. From the adsorption isotherms at 293 K, the association constants for a poly**1**/diphenylurea pair were determined to be 2.2×10^3 M^{-1} in acetonitrile and 2 M^{-1} in methanol (Figure 1a). LC analysis using the stationary phase showed that diphenylurea was well retained with acetonitrile as a mobile phase, whereas it was soon eluted with methanol. Thus, the results of the adsorption isotherms agreed well with the LC results, and it was concluded that the retention of diphenylurea in acetonitrile and the elution with methanol using the stationary phase were thermodynamically achieved, and the association constants were controlled over three orders of magnitudes by only changing the solvent. Now, the adsorption isotherms were based on the following equilibrium: A + B⇌(AB), in which A is the guest, B is the host, and (AB) is the associated form. The only information required for the adsorption isotherm is the concentration of the guest molecule (A) at equilibrium; therefore, we can trace the guest at equilibrium, but there is no direct information regarding the host and the associated form from the adsorption isotherm. This motivated us to further investigate the event.

The DLS data of poly**1** revealed an average diameter of (65.4 ± 3.7) μm in methanol; the value changed to (71.5 ± 10.1) μm in acetonitrile (Figure 1b). As the analogous polymer without bromo groups or unfunctionalized polymer showed no difference in the average diameters with different solvents (Figure S4, see the Supporting Information), such a swelling of poly**1** indicated that the tertiary structure changes as the solvents are varied from methanol to acetonitrile. Because the changes were repeated by evaporating the solvents, the swelling/shrinking process should be a reversible solvation process. With the adsorption isotherms and the DLS data in mind, we proposed a plausible mechanism of the association/dissociation process (Figure 1c): When methanol is used as a solvent, the two adjacent DBP units may associate strongly, which leads to 1000 times less efficient association towards the substrate compared with that in acetonitrile (Figure 1a). For the analogue poly**2**, in which five Br atoms are introduced to the phenoxyl unit, such a significant difference of the association in acetonitrile and methanol is not observed, and the scale of the equilibrium constant is compressed tenfold for the poly**2** + diphenylurea pair (Figure S5); this demonstrates the noticeable solvent dependence of the association reactions for the poly**1** + diphenylurea pair.

To further understand association/dissociation at the molecular level, we also carried out various spectroscopic analyses of a molecular model system, through which the interaction between the unit structure of poly**1** and the substrates can be considered. First, the homogeneous model (**1**, Figure 2) was

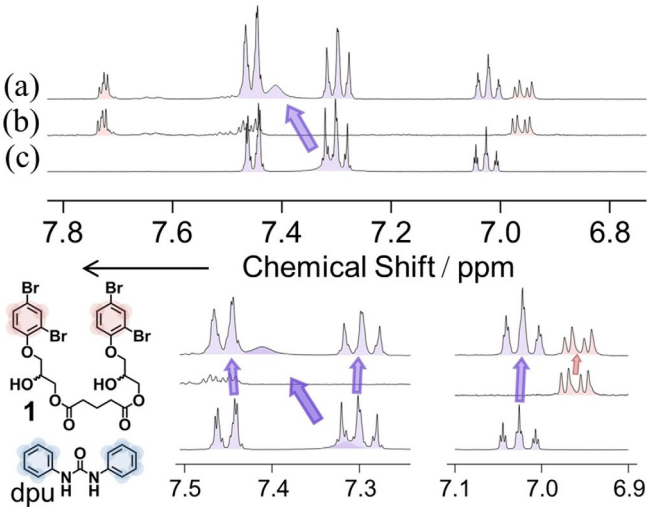

Figure 2. ^{1}H NMR spectra of (a) **1** + diphenylurea, (b) **1**, and (c) diphenylurea in [D$_3$]acetonitrile at 293 K.

synthesized by a conventional S_N2 reaction[18] from an epoxide precursor, characterized by mass spectrometry (Figures S6–S8), and UV/Vis (Figure S9), ^{1}H NMR (Figures S10 and S11), and IR (Figure S12) spectroscopy,[19] and its complexation with diphenylurea was then investigated. ^{1}H NMR spectra of **1** in acetonitrile showed that when mixed with diphenylurea the chemical shift of each signal changed and the signals of the protons became broader (Figure 2). The change in the chemical shifts and the shorter relaxation time of each proton supported the association of diphenylurea and **1** in [D$_3$]acetonitrile (for details, see the Supporting Information).

Because the dipolar coupling depends on the reciprocal of the distance to the power of six, r^6, the spectrum strongly corroborated that **1** and diphenylurea are in close proximity in solution in acetonitrile. Indeed, from the slope of a modified Hill plot (Figure 3a)[20] and mass spectrometry (Figure 3b), a 1:1 as-

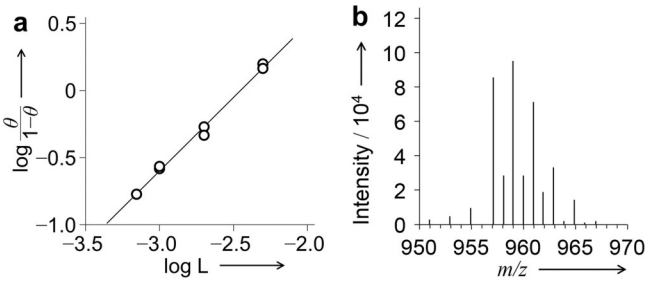

Figure 3. (a) A modified Hill plot for the **1** + diphenylurea pair in acetonitrile at 283 K showing the relationship between $\log[\theta/(1-\theta)]$ and the logarithm of the substrate concentration. For the derivation of the equations, see the Supporting Information. (b) Mass spectrum of the **1** + diphenylurea pair with a negative mode.

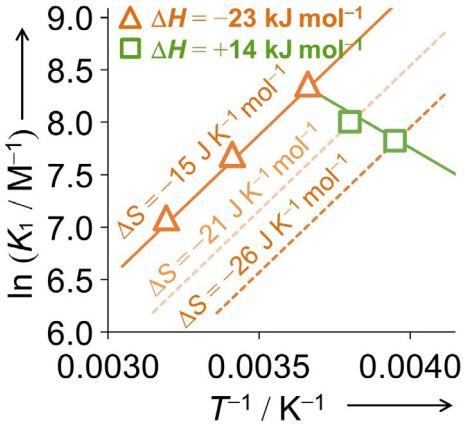

Figure 4. Arrhenius-type plot of $\ln K_1$ using poly**1** as the stationary phase and diphenylurea as the substrate in acetonitrile. The $\ln K_1$ values were determined by using a constant θ_{sat} of 27 μmol g^{-1}. The original data are shown in Figure S3 and Table S1 of the Supporting Information.

sociation is suggested for a **1** + diphenylurea pair in acetonitrile, and the analysis afforded an association constant of 7.0×10^2 M^{-1} at 293 K. This value for the homogeneous model is in fairly good agreement with the value obtained in the adsorption isotherms for the heterogeneous poly**1** + diphenylurea pair (Figure 1a), which indicates that the mechanism of the host–guest interaction at the solid–liquid interfaces will be similar to the homogeneous 1:1 association. The hydrogen bonding between the hydroxyl unit of **1** and diphenylurea was also confirmed by IR spectroscopy (Figure S12). The IR spectrum of pristine diphenylurea showed the N–H bending vibration peak at 1605 cm^{-1} and the C=O stretching peak at 1648 cm^{-1}, whereas that of **1** showed a broad absorption at 3447 cm^{-1} that is characteristic of a hydroxyl group. On the contrary, when a 1:1 mixture of **1** and diphenylurea was prepared from a solution in acetonitrile, the IR spectrum showed no absorption for the hydroxyl group, and the C=O stretching peak of diphenylurea seemed to be shifted to a lower energy, which suggests that there was an association between the hydroxyl group of **1** and the urea unit of diphenylurea to a certain extent. This explanation matched well with the disappearance of the hydroxyl proton peak of **1** at 3.2 ppm (Figure S11) and the down-field shift of the amide signal (Figure 2) in the ^{1}H NMR spectra when **1** was mixed with diphenylurea. We then investigated the temperature dependence of the interactions between poly**1**/**1** and diphenylurea using acetonitrile as a solvent, and compared this with a theoretical calculation that is based on the 1:1 association.

Figure 4 shows the relationship between the logarithm of K_1 and the reciprocal of the temperature. The $\ln K_1$ values at temperatures higher than 273 K ($T^{-1} < 0.037$ K^{-1}) have a linear dependence on the reciprocal of temperature as expected by the van't Hoff equation [Eq. (1)] and the isotherm equation [Eq. (2)]:

$$d\ln K/dT = \Delta H/RT^2 \quad (1)$$

$$\ln K = -\Delta H/RT + \Delta S/R \quad (2)$$

in which ΔH is the enthalpy change in J mol^{-1}, ΔS is the entropy change of a reaction (herein the adsorption and desorption events, J K^{-1} mol^{-1}), T is the temperature in K, and R is the gas constant (8.314 J K^{-1} mol^{-1}). The experimental ΔH of −23 kJ mol^{-1} for poly**1** + diphenylurea (Figure 4) is in a good agreement with ΔH of −22 kJ mol^{-1} for the **1** + diphenylurea pair (Figure S14 and Table S2, see the Supporting Information). Although the interaction is fundamentally dynamic, theoretical calculations that are based on the sandwiching 2:1 association of the DBP unit and diphenylurea afforded the lowest-energy structure of association with ΔH of −16 kJ mol^{-1} (Tables S3–S6 and Figure S15). The agreement between the experimental values and the theoretical prediction also supports the idea that the association between poly**1** and diphenylurea at the solid–liquid interface will be mainly a pinching 2:1 interaction between the DBP unit and the substrate when acetonitrile is used as a solvent. However, there is a deviation at temperatures lower than 290 K, which causes biphasic behavior with apparent enthalpic anomalies (orange and green solid traces in Figure 4). The reaction between diphenylurea and poly**1** is a spontaneous adsorption event with a negative ΔS value, so it is less likely for the ΔH to be greater than 0. In fact, the theoretical calculation for the association between **1** and diphenylurea gave a constant ΔH irrespective of the temperature (Table S6), which indicates that a simple mode of association cannot explain the biphasic plot; therefore, the deviation from the Arrhenius-type plot in Figure 4 may be primarily brought about by temperature-dependent solvation,[21,22] which can cause a significant change in ΔS (dashed line in Figure 4). Indeed, the microscopic analysis showed that the mean diameter of poly**1** at higher temperatures is significantly larger than that at lower temperatures (Figure S16 and Table S7), and the swelling indicates solvation of the surfaces on poly**1**. The pyrolysis GC, which can quantify the amount of the solvated acetonitrile, also showed that poly**1** at 293 K was more solvated than that at 253 K (Figure S17). When the association for the poly**1** + diphenylurea pair takes place from the solvated state at higher temperatures, the solvents included onto the surface should be expelled as a substrate enters, leading to $\Delta\Delta S > 0$, which is favorable to the association. When the temperature is

lower than 273 K ($T^{-1} > 0.037\ K^{-1}$), on the contrary, the association starts from a closed DBP pair with a fewer number of solvent molecules involved, and the entropy-change terms go in the negative direction as the guest is trapped by the host, causing $\Delta\Delta S < 0$. Thus, the biphasic behavior of the equilibrium constant in the temperature range can be explained by such an entropy change caused by the dynamics of the solid state as shown in Figure 1c, which is supported by the temperature dependence of the mean particle size (Figure S16) and the analysis of the amount of the solvated acetonitrile (Figure S17), as well as an enthalpy change.[23] Figure 4 also indicates that the associative interaction at 273 K will be the most efficient. The thermodynamic parameters are listed in Table 1.

Table 1. Thermodynamic parameters for the association/dissociation in acetonitrile and methanol (equilibrium constant K_1 in M^{-1}, enthalpy change ΔH in $kJ\,mol^{-1}$, and entropy change ΔS in $J\,K^{-1}\,mol^{-1}$) at 293 K.

	Acetonitrile			Methanol
	K_1 [M^{-1}]	ΔH [$kJ\,mol^{-1}$]	ΔS [$J\,K^{-1}\,mol^{-1}$]	K_1 [M^{-1}]
poly**1** + diphenylurea	2.2×10^3	$-23^{[a]}$	$-15^{[a]}$	2
poly**2** + diphenylurea	4.3×10^2	$-40^{[b]}$	$-84^{[b]}$	50
poly**3** + diphenylurea	12	–	–	8
1 + diphenylurea	7.0×10^2	$-22^{[c]}$ $(-16)^{[d]}$	$-27^{[c]}$	52

[a] These values were derived from the Arrhenius plot between 273 and 313 K as shown in Figure 4. [b] The plot is shown in Figure S18. [c] The limiting plot is shown in Figure S14. [d] The value in parentheses was theoretically calculated (Table S6).

If hydrogen bonding is a significant factor for the association of diphenylurea and poly**x**, it is plausible that poly**3** with no Br atoms (Scheme S1 in the Supporting Information) would also show a large equilibrium constant for the association. However, as shown in Table 1, the K_1 for the poly**3** + diphenylurea pair is too weak, which indicates that the introduction of Br atoms on the periphery of the anisole unit also plays a crucial role in the retention of the substrates at solid–liquid interfaces. Noncovalent interactions often must occur simultaneously to overcome the entropy loss of binding. In this study, these interactions will also be required at the same time for the associative binding to take place spontaneously, and the binding will become unfavorable if one of them is missing. Then, if π–π interactions and/or halogen bonding are important for the capture of the substrates, introduction of more Br atoms to the solid structure will result in much stronger capturing. However, as mentioned above, poly**2** with five Br atoms on the aromatic ring showed a weaker association constant of $4.3\times10^2\ M^{-1}$ at the same temperature. Noticeable here is the solvent and temperature dependence of the mean particle diameter on poly**2**; unlike poly**1**, the $PhBr_5$-modified material showed almost no changes when varying the solvent or temperature with a constant diameter (Figure S16 and Table S7). This result can corroborate the schematic understanding of the associative/dissociative interactions shown in Figure 1c; for poly**2** with five Br atoms, the inter-anisole interaction takes precedence over the solvation under these circumstances, so that a smaller number of solvent molecules is enclosed onto the surfaces (close-packed in Figure 1c). The association starts from the closed state, so it is unfavorable in terms of ΔS. For poly**1** with two Br atoms on the anisole unit, on the contrary, the inter-anisole interaction might be modest and susceptible to solvation at higher temperatures, and the association could start from the solvated state. Certainly, ΔS for the poly**2** + diphenylurea pair ($-80\ J\,K^{-1}\,mol^{-1}$) is much more negative than that for poly**1** + diphenylurea ($-15\ J\,K^{-1}\,mol^{-1}$), thereby demonstrating the entropic superiority of poly**1** over poly**2** by $20\ kJ\,mol^{-1}$ for $T\Delta S$ at 293 K.

The theoretically optimized structure of the associated form for the **1** + diphenylurea pair showed inter-π-plane interaction with an interplanar distance of approximately 3.0 Å and hydrogen bonding between the hydroxyl unit of **1** and the carbonyl group of diphenylurea with a distance of 1.9 Å (Figure S15). The parallel-displaced interaction gave a stronger coupling than a T-shaped interaction, as previously reported for natural enzymatic systems.[12] Thus, the factors for the intermolecular interaction are (1) π–π interactions and halogen bonding between the anisole units and substrates, (2) hydrogen bonding between **1**/poly**1** and the substrates, and (3) reversible, solvent- and temperature-dependent shrinking/swelling of poly**1**. These events should be achieved at the same time to compensate for the unfavorable entropic contribution to binding, and poly**1** is suitable for this purpose. With the entropic superiority as well as the conventional interactions, poly**1** selectively captured various chemicals containing backbones that are similar to diphenylurea with large equilibrium constants at 273 K ($K_1 \approx 10^4\ M^{-1}$, Figures S20 and S21). The significant differences in the saturation values of the adsorption (θ_{sat}) can be explained in terms of the differences in the numbers of the DBP units involved in the association toward the substrates: For example, chlorfluazuron as a larger guest will require more DBP units for its host–guest interactions toward poly**1** than smaller guests of forchlorfenuron, thidiazuron, and diphenylurea. As a consequence, the former led to a smaller θ_{sat} value than the latter. Owing to the large difference in the K values, an efficient capture of the substrates in acetonitrile and their release in methanol were also achieved for these chemicals.

In conclusion, we have developed a new strategy for understanding and controlling the interaction between a stationary host and small guest molecules to realize their separation and quantification. The pinching associative interaction between poly**1** and small molecules was thermodynamically achieved at 293 K with a free-energy change of $-19\ kJ\,mol^{-1}$ for diphenylurea, which was supported by the experimental analysis of a synthetic model (**1**)/diphenylurea association coupled with the application of a modified Hill plot as well as a theoretical calculation using a local density functional. The associative 2:1 interaction between the DBP unit and substrates was controlled by the solvent- and temperature-dependent change in the tertiary structure of the polymer caused by solvation, and this is of interest in relation to the controversial natural enzymatic dynamics[24–29] such as hydride transfer in alcohol dehydrogenases (ADH). The unusual temperature dependence made the association at 273 K most effective. The interaction in acetonitrile

was 1000-times stronger than that in methanol owing to the tertiary conformational gating, which was supported by the temperature dependence of the particle-size distribution of materials, the pyrolysis GC analysis for the quantification of the solvation of acetonitrile, and a deviation from the Arrhenius plot, and this enabled an efficient solid-phase extraction of various chemicals by only changing the solvent used. Such an approach together with an understanding of interfacial interactions at the molecular level, which is usually less feasible, will provide a rational way to designing general systems that achieve more precise control of the interactions at solid–liquid boundaries. A study of the development of new systems for the detection and efficient retention of various agents that are potent to vital effects in living forms, such as *Ochratoxin A*,[30] is now under investigation.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: adsorption · entropy · liquid chromatography · noncovalent interactions · solid-phase extraction

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