Isothermal Titration Calorimetry in Supramolecular Chemistry

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On the corresponding day we will meet at 10:00 in NC5/129

Introduction

If we look onto an ensemble of different molecules in solution, every species interacts with each other in one way or another. This depends on the nature of the dissolved compounds in a solution, e.g. their functional groups and electronic structure, on the solvent, which can promote or inhibit an interaction between two molecules, and the concentration of all molecules. In supramolecular chemistry especially the strong interacting molecules are of interest and the overall goal is to understand those interactions and therefore be able to tune and use these for various applications.

In the simplest case the interaction between two molecules is investigated, which can be defined as host and guest. The host is a large molecule or aggregate such as an enzyme or synthetic cyclic compound possessing a sizeable, central hole, or cavity. The guest may be a monatomic cation, a simple inorganic anion, an ion pair, or a more sophisticated molecule such as a hormone, pheromone, or neurotransmitter. More formally, the host is defined as the molecular entity possessing convergent binding sites and the guest as one with divergent binding sites.

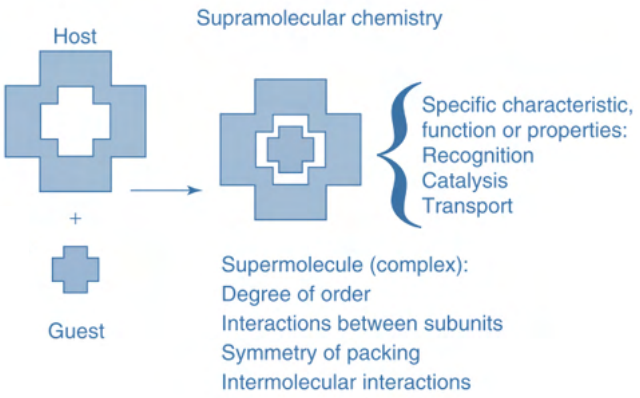

Figure 1: Defintion oft the traditional supramolecular "host-guest" chemistry according to Lehn.

Supramolecular compounds are formed by additive and coorperative non-covalent bonds, which can range from hundreds of kJ/mol with an ion-ion interaction to only a few kJ/mol in van der Waals-interactions. The most common noncovalent interactions, along with their approximate energies, are listed in Table 1[1].

Table 1: Common supramolecular interactions

Supramolecular Interactions	Directionality	Bond energies (kJ/mol)	Examples
Ion-ion	Nondirectional	100-350	NaCl
Van der Waals	Nondirectional	<5	Inclusion componds
Ion-dipole	Slightly directional	5-60	Na^+ crown ether complex
Dipole-dipole	Slightly directional	50-200	-CN Groups
Coordination bonds	Directional	100-300	M-pyridine
Hydrogen Bonds	Directional	4-120	Carboxilc acid dimer
Halogen bonds	Directional		Sulfur-iodine complex
π-π interaction	Directional	2-50	Benzene, DNA
Cation-π and anion-π interactions	Directional	5-80	$N^+(CH_3)_4$·(toluene)

Despite their strength in interaction, the main feature is that in solution non-covalent interactions readily break up due to thermal collisions, which leads to a rapid interchange of all host-guest interactions. As a consequence of this reversibility in bonding, a constant equilibrium is formed at some time, which can be manipulated by chemical (addition of compounds) and physical (temperature, pressure) means. These results in an energetic response, which can be measured and if the heat is measured, it is called calorimetry. In fact, the heat change determined is indeed a global response function of many processes occurring simultaneously. The direct mutual interaction of the binding partners as the process of prime interest is accompanied, and partly covered, by supplementary side reactions like proton transfers, counter-ion release, conformational rearrangements, and so on, that add an initially unknown share to the heat output. Also less specific contributions, like the heats of dilution and mixing, may intervene and must be taken into account. Naturally, the deconvolution of all intertwined processes is easier when there are fewer components participating in the specific process under study and less severe interference by non-specific side reactions. On both counts isothermal titration calorimetry (ITC) studies of artificial host–guest systems, which are more readily controlled than the biological pendants, should be particularly suited to find out about the general factors governing supramolecular interactions. They can also be readily extended to nonaqueous solvents and thus unfold the energetic basis for technical applications, such as extractions, gel formation, or assembly processes. Inspecting the binding energetics of the same host–guest system in various solvents may also shed light on the role played by solvation. Despite being the most fundamental, yet

unspecific supramolecular interaction empirically known to affect binding, there is little quantitative knowledge available that reaches beyond general polarity concepts to explain the influence of solvation on host–guest affinity, and is eventually suitable in assisting rational host design[2].

The Thermodynamic Background[2]

Calorimetry reports on ensembles averaged over time and the individual energies of their members. This sets the stage to employ the framework of thermodynamics for a full energetic characterization of the system under study. Heat, as the primary observable in calorimetry, is commonly measured at constant (atmospheric) pressure and thus represents an enthalpy change ΔH. If enthalpy is measured in response to a change in the total composition of the system, the output depends on the extent of interaction between the components permitting access to molecular affinity. Provided just one 1:1 stoichiometric binding process dominates the molecular bond rearrangement the corresponding constant of mass action K_a can be determined. The affinity constant K_a relates to the Gibbs enthalpy of association ΔG° according to Equation (1):

$$\Delta G^\circ = -RT \ln K_a \qquad (1)$$

$$\Delta S^\circ = \frac{\Delta H^\circ}{T} - \frac{\Delta G^\circ}{T} \qquad (2)$$

With enthalpy ΔH° and Gibbs enthalpy ΔG° at hand the change in standard entropy ΔS° is easily calculated from the Gibbs–Helmholtz equation (Equation 2). From a single calorimetric experiment at constant temperature the main state functions ΔH, ΔG, and ΔS of the binding process are accessible. Conducting such measurements over a range of temperatures yields the heat capacity ΔC_p:

$$\Delta C^\circ{}_p = \frac{d\Delta H^\circ}{dT} \qquad (3)$$

In principle, ΔC_p itself can be a function of temperature, but in the narrow span of temperatures of interest in supramolecular binding there is little risk in approximating ΔC_p by Equation (4) where T_1 and T_2 are two different absolute temperatures, providing the corresponding standard enthalpies.

$$\Delta C^\circ{}_p = \frac{\Delta H_2 - \Delta H_1}{T_2 - T_1} \qquad (4)$$

Experimental Setup[2]

The measurement of heat conveniently takes temperature as an indicator. In modern calorimeters, the instrumental design employs two principal approaches that are easily distinguished with respect to the effect on the temperature output: In the adiabatic mode, heat evolution or consumption by the chemical process under investigation leads to a permanent increase or decrease in temperature. The extent of the change depends on the heat capacity of the instrument, which must be calibrated in separate experiments. Alternatively, the heat effect may be dissipated to a heat sink, so that the measurement following an initial perturbation falls back on a constant temperature baseline (isothermal mode). The heat flow may be observed directly using a pile of thermocouples (heat conduction device) or may be actively regulated to maintain a fixed level (power compensation). Both instrumental designs reach sensitivities in the nanowatt regime by means of a differential measurement relative to an internal reference. The schematic blueprint of a power compensation titration calorimeter is shown below (fig. 2).

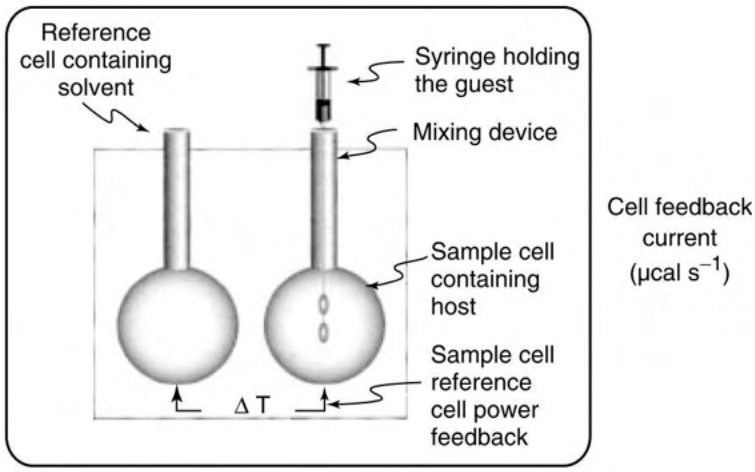

Figure 2: Schematic instrumental set-up of a power compensation calorimeter.

Two coin-shaped identical cells (each holding about 1.7 ml) are permanently seated in an insulated compartment typically regulated 5–10°C above the environmental temperature to allow a cooling heat flow. Both cells are completely filled, the reference cell containing pure solvent and the measurement cell the solution of one partner of the binding reaction to be studied. The other reactant, usually prepared in 10–20-fold higher concentration, is delivered from a syringe. The tip of the syringe is deformed to a paddle to allow rapid mixing of the cell contents when the syringe device is rotated. The reference cell is continuously heated to set a temperature. A similar electrical power heater is attached to the sample cell and is automatically regulated by a feedback mechanism to minimize the temperature difference ΔT between the cells. On injecting aliquots of several microliters from the syringe, the association of the binding partners produces a heat effect that raises or lowers the temperature in the sample cell. The deflection of temperature triggers the feedback regulator to adjust

the electrical power needed to maintain identical temperatures in both cells. The change in the respective feedback current is the primary signal observed and corresponds to a heat pulse (heat production over time). Integration with respect to time gives the energy that was traded on injecting the known amount of the reaction partner to the sample cell. If a series of injections is made, the compound in the cell is progressively converted to the molecular complex, leading to diminishing heat effects as the association approaches completion.

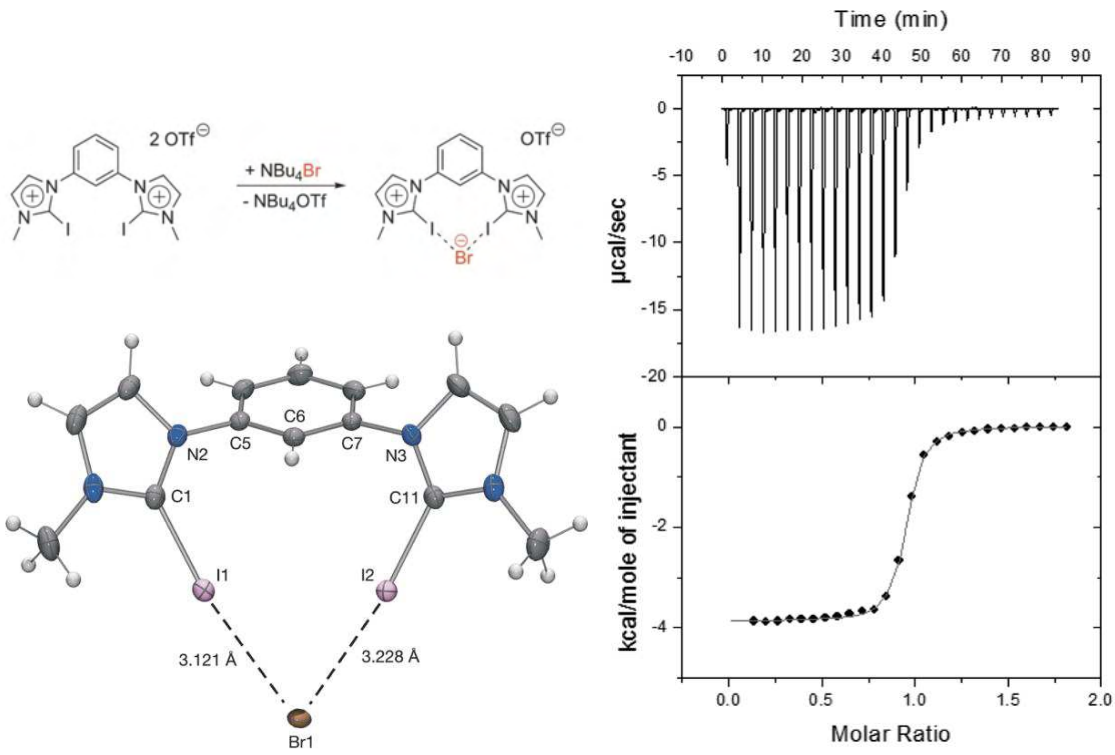

Figure 3: Illustrates the data output of an ITC experiment, consisting of heat pulses that decrease, if the host reaches saturation by the addition of the guest (right). The corresponding reaction of that particular ITC-experiment and the matching crystal structure (left)[3].

A typical output picture showing the exothermic binding of bromide by a bidentate halogen-bond donor in acetonitrile is shown in Figure 3. It shows downward directed pulses, indicating the decrease of the feedback current necessary to keep a zero temperature difference to the reference cell as the heat from the exothermic association reaction makes up for the rest. The integration of the heat pulses when plotted versus the nominal molar ratio of the injected compound over the one contained in the cell yields a titration curve that exhibits a characteristic s-shape. In this case shown the sigmoidal appearance reflects the adequate choice of absolute concentration relations to allow the determination of the molar enthalpy $\Delta H°$ from the extrapolated step height of the curve, the stoichiometry n of the binding process from the position of the inflection point along the molar ratio axis and the affinity constant K_a from the slope in the inflection point. Modern calorimeters offer the advantage of having these quantities determined by software routines that use nonlinear curve fitting

to find the most probable parameters describing the supramolecular association with regard to the specifics of the instrument (cell volume, volume displacement, etc.).

ITC is a versatile, sensitive, destruction-free, label-free, and quite rapid (1–3 h) instrumental method that requires typically less than micromolar amounts of material to learn about the thermodynamic parameters of reversible molecular associations. The accuracy and reliability of ITC for this purpose is unsurpassed, making it the gold standard in the characterization of intermolecular interactions in solution.

1. Steed, J. W.; Atwood, J. L.; Gale, P. A., *Supramolecular Chemistry: From Molecules to Nanomaterials*. 1st Edition ed.; John Wiley & Sons, Ltd.: 2012.
2. Schmidtchen, F. P., *Analytical Methods in Supramolecular Chemistry*. 1st ed.; Wiley-VCH: Weinheim, 2007; p 55-78.
3. Walter, S. M.; Kniep, F.; Rout, L.; Schmidtchen, F. P.; Herdtweck, E.; Huber, S. M., Isothermal Calorimetric Titrations on Charge-Assisted Halogen Bonds: Role of Entropy, Counterions, Solvent, and Temperature. *J. Am. Chem. Soc.* **2012,** *134* (20), 8507-8512.