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CALORIMETRIC TITRATION EXPERIMENT MODELLED BY EQUILIBRIUM OF 2:1 AND 1:1 COMPLEXES

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The analysis of the calorimetric continuous titration experiment is presented. The proposed method is basing on the collection of larger number of experimental data points than could be obtained from the classical isothermal titration calorimetry experiment. After the deconvolution procedure resulting in the correction for the calorimeter time response the pure power effect signal could be obtained. The collected data enable the detailed analysis of the closely populated 2:1 and 1:1 weak complexes.

Keywords: *calorimetry, complex stoichiometry, data processing*

Introduction

Nowadays, the design and synthesis of macrocyclic compounds (like crown ethers, calixarenes, etc.), which are able to form complexes with various species by non-covalent interactions, are objects of many studies [1]. It is especially interesting and useful in the field of biochemistry, in the case of reactions of macrocycles with substances of biological importance, like amino acids, peptides and biogenic amines, because studies of these interactions are helpful for understanding the molecular recognition processes. Since the calorimetric titration has been found as a convenient tool for determining the thermodynamic parameters of reactions, this technique is widely employed in the investigation of host-guest interactions in supramolecular chemistry as well as in interacting biomolecular systems. The advantage of this technique is that from the calorimetric signal obtained in only a single titration experiment the stability constant, K, the enthalpy change, ΔH, and binding stoichiometry can be determined simultaneously. Then free energy, ΔG and entropy, ΔS, can be evaluated using the classical relationship $\Delta G=-RT\ln K$, and $\Delta S=(\Delta H-\Delta G)/T$. However, evaluation of these thermodynamic data needs the assumption of the physical model of the reaction studied. Respective numerical methods have been elaborated [2, 3]. The appropriate algorithms are, sometimes, provided as standard software of some commercial calorimeters [4]. Unfortunately the analysis of the heterogeneous complexation phenomena is almost the state of art. As it was found in early 90's in the 2:1 complex formation studies, the precise analysis of the equilibrium of 2:1 and 1:1 and free species could be described theoretically, and the optimization procedure of four-parameter model could be done when the stability constants of both complexes differ [5], but usually fail when the concentrations of 2:1 and 1:1 complexes are comparable [6]. The usual method of the resolving the arising problem are either the independent determination of binding constant using alternative experimental approach (NMR, UV, potentiometric titration) or performing the calorimetric measurements in the reagent concentration range for which one of the complexation equilibrium is negligible.

In the manuscript we present the application of the numerical model to the investigation of 2:1 and 1:1 complex formation between of *D*-mannonaphto-crown-6 ether and phenylalanine hydrochloride using heat conduction calorimeter equipped with continuous injection system.

Experimental

Materials

D-mannonaphto-crown-6 ether of the purity better than 95% (as determined by NMR) was home synthesized by Pietraszkiewicz. The amino acid *L*-phenylalanine·HCl was prepared from *L*-phenylalanine (Fluka, analytical grade).

Measurements

Complexation of the ether with the amino acid was investigated in aqueous solution at temperature of 25°C. Calorimetric measurements were carried out in a home-made heat conduction microcalorimeter of sensitivity equal to 103 μV mW^{-1} [7]. 0.2 mL of 0.1 M

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amino acid solution was injected to 2 mL of 0.01 M ether solution placed in the vessel. Thereby, at the end of injection the 1:1 molar ratio of ether to amino acid was attained. The injection during 75 s, at a constant rate was performed by means of a dosimetric device. During the whole experiment the stirring (60 rpm) was applied. The calorimetric signal was recorded until it approached its initial value (baseline). In the same manner analogical measurement concerning the dilution of amino acid was also performed. The above procedure of introducing the agent to the reaction mixture can be treated as a continuous titration under condition that the reaction takes place rapidly and the time constant of calorimeter is small enough [8]. The fulfilment of the above two requirements was validated by additional measurements of two types. In the first, the calorimetric measurements of both thermal effects (complexation and dilution) were repeated with a sole difference: the time of amino acid injections (to ether or water) was reduced to 5 s. The experiment of second type was target to the determination of the time constant of the calorimeter, and its dependence on the volume of the liquid sample in the vessel, being changed during injection from 2.0 to 2.2 mL.

Computational

The experimentally measured thermal response effect was converted into power signal using locally implemented algorithm of heat signal deconvolution [9].

Model of the equilibrium of 1:1 and 2:1 complexes

The Host Guest system, in which either one or two host molecules are involved in the binding of single guest molecule, could be described by the following two dissociation equilibria

$$[HG] = K_1[H][G] \qquad [HGH] = K_2[HG][H] \tag{1}$$

where [*G*], [*H*], [*HG*], [*HGH*] are the concentrations of free guest, free host, 1:1 and 2:1 complexes, respectively. The balance of the total concentrations of the guest (G_0) and host (H_0) molecules leads to the equations:

$$[H] + [HG] + 2[HGH] = H_0 \qquad [G] + [HG] + [HGH] = G_0 \tag{2}$$

The combination of Eqs (1) and (2) leads to the third order equation in the form

$$\{K_1K_2\}[H]^3 + \{K_1 + K_1K_2(2G_0 - H_0)\}[H]^2 + \{1 + K_1(G_0 - H_0)\}[H] = H_0 \tag{3}$$

Equation (3) could be analytically resolved using Cardano's formulae [10]. The concentration of the free guest is then expressed as

$$[G] = \frac{G_0}{1 + K_1[H] + K_1K_2[H]^2} \tag{4}$$

and the concentrations of the two complexes are described by Eq. (1).

Analysis of the heat effect of the host–guest complexation

Let's assume that 1:1 complex is characterised by the dissociation constant K_1, and heat of formation Q_1, and analoguously the 2:1 complex by K_2 and Q_2. The total heat effect of mixing the molecules in the concentrations of G_0 and H_0 is expressed as the sum of the heat effects of building 1:1 and 2:1 complexes, respectively:

$$Q(G_0,H_0) = Q_1[HG] + (Q_1 + Q_2)[HGH] \tag{5}$$

The concentrations of [*HG*] and [*HGH*] molecules could be determined from Eqs (1)–(4). Exact values of K_1, K_2, Q_1 and Q_2 must be optimized to obtain the agreement between the theoretical function $Q(G_0,H_0)$ and the experimentally measured data $Q^{exp}(G_0,H_0)$. The optimization procedure was based on the Marquardt–Levenberg algorithm [11] implemented in Gnuplot program [12].

Results and discussion

Validation of the experimental approach

Comparison of normalised responses of the calorimeter to either the complexation or the dilution experiments, both performed upon 5 s continuous injection is presented in Fig. 1. The two heat responses curves are very close one to the other, clearly indicating that the reaction of complexation takes place so rapidly that the kinetic aspects of the binding phenomena could be neglected. On the other hand, the time constant of the calorimeter when the vessel contained 2 mL of water was estimated to 145 s so, it may be considered as relatively small. The two above results justify the application of the proposed mathematical treatment for the determination of K and ΔH from the continuous titration experimental data. Taking into account, that the characteristic time constant of the calorimeter was found a linear function of the volume of the solution placed in the vessel, as it is presented in Fig. 2, the deconvolution algorithm [9] was enhanced for the correction according to the variable calorimeter time constant, which exact value is a linear function of the actual solvent volume in the calorimeter vessel. As it is

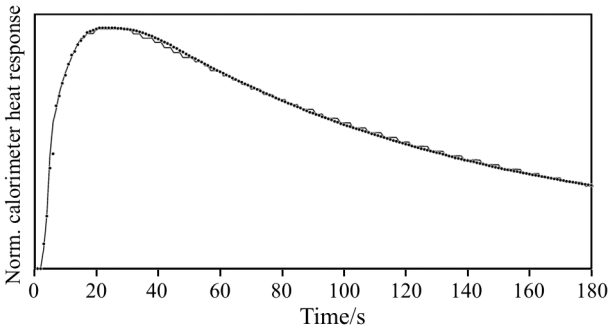

Fig. 1 Normalised calorimeter heat response of the phenylalanine·HCl — – complexation and • – dilution upon injection of 0.2 mL of 100 mM amino acid solution in 5 s. The curves were arbitrary rescaled to enable overlaying

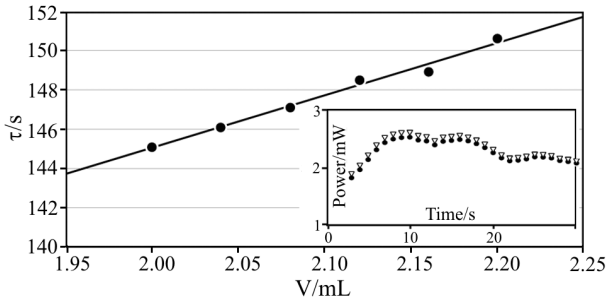

Fig. 2 Experimentally determined dependence of the calorimeter time constant on the solution volume placed in the vessel. In the inset comparison of the power signal in first 30 s of titration experiment estimated by the deconvolution under assumption of either ▽ – constant or • – variable calorimeter time constant, τ

presented in the inset of Fig. 2 the power signals estimated under assumption of either fixed (145 s) or variable calorimeter time constant differ exhibiting the evident, although small, variations between the power curves. The latter forced us to introduce the variable calorimeter time constant to the deconvolution procedure.

Determination of the thermal power

The power signal, $W(t)$, calculated from the raw calorimetric response and related to complex formation is presented in Fig. 3. This indicates that the binding phenomena is an exothermic process, and the total heat generated in the time of the reaction is equal to 5.07 kJ mol^{-1}. The run of the curve seems really interesting, as it reflects not only the dynamics, but also the progress of the reaction [13, 14]. Two observations are significant for further analysis of the results, and of course the modelling of the mechanism of the reaction. Firstly, the existence of two well distinguishable maxima indicates a complicated reaction mechanism, pointing towards at least two coupled binding equilibria. Secondly, the position of

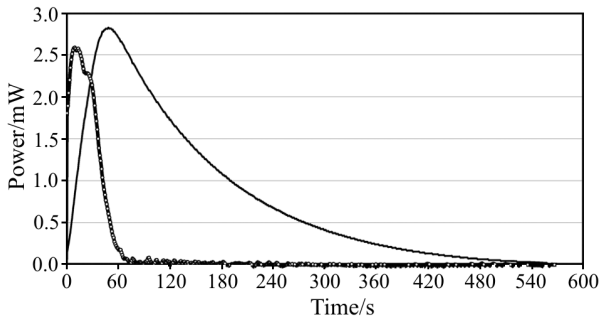

Fig. 3 The raw response of — – calorimeter and estimated power arising from injection of amino acid to the crown ether solution. After ~75 s no heat effect is observed, which agrees with the time order of injection procedure

the first maximum of $W(t)$ curve is located at early time of the injection procedure, indicating that at this stage of the complexation process at least two ether molecules must be involved in single amino acid molecule binding.

Modelling the binding equilibrium

The power signal, $W(t)$, was integrated to obtain the heat of the reaction on the time of experiment. The experiment time is the measure of the reactant concentration. Thereby, the results presented in Fig. 4 could be treated as obtained by commonly used simple titration experiment. As an advantage of the usage the continuous injection approach, the enlarged set of data points useful in the analysis could be obtained, as compared to the standard ITC titration calorimeter. The heat response after 75 s is constant, clearly indicating that no further reaction between amino acid and ether undergoes.

Preliminary analysis of the obtained data, made according to the method originally proposed by Job [15], demonstrated the dominance of 2:1 stoichiometry complex in the analysed system, indicating that two ether molecules are involved in complexation of single phenylalanine·HCl, as it is presented in Fig. 5.

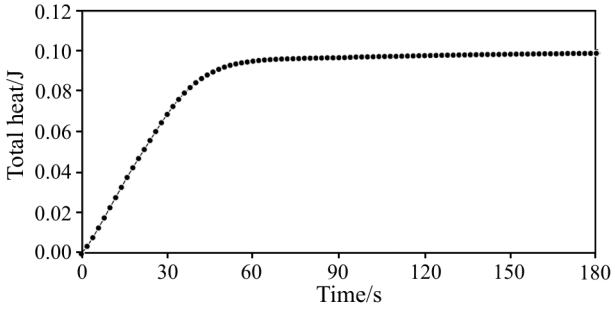

Fig. 4 The integrated total heat response of the analyzed process. After ~75 s no heat flow is observed, what strictly corresponds to the 75 s injection time of phenylalanine·HCl solution to the calorimeter vessel

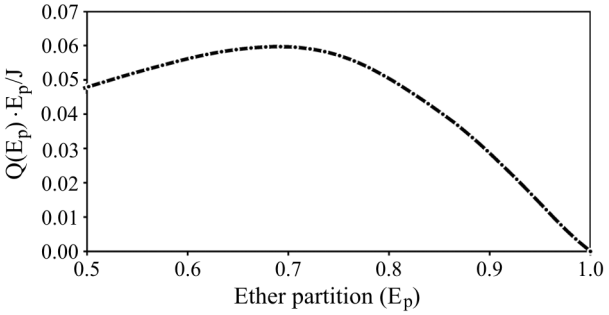

Fig. 5 Job plot determined for the integrated heat response in titration experiment. The maximum localised at ~0.7 ether partition clearly indicates the dominant population of the 2:1 (ether:amino acid) complex

Unfortunately the procedure optimising 2:1 model failed. Generally, depending on the initial parameter values, the procedure bifurcated to the two alternative parameter sets, as presented in Fig. 6. The observed significant deviations clearly indicates, thus besides the 2:1 one at last one additional complexation equilibrium undergoes. The most probable is the existence of 1:1 complex in the solution. The proposed model, assuming equilibrium of 2:1 and 1:1 complexes was then optimised, exhibiting very good agreement with the experimental data in whole concentration range (Fig. 6).

The estimated model parameters K_1= 12(4)·10^3 M^{-1}, Q_1=6.8(0.3) kJ mol^{-1}; K_2=9(3)·10^3 M^{-1}, Q_2=2.2(0.1) kJ mol^{-1} enable to follow all species concentrations during the titration experiment (Fig. 7). At the very beginning, when only a small number of amino acid molecules are injected to the solution, most of the phenylalanine·HCl species are interacting with two ether molecules. In low amino acid concentration solution the 2:1 complex dominates, but when the amino

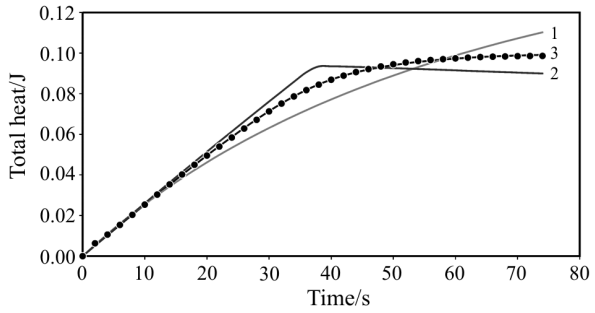

Fig. 6 Optimization of the titration model. Curves 1 and 2 represent the two numerically stable results of the optimization procedure based on simple 2:1 stoichiometry model. The estimated second order binding constants (K_2) is either 1 – 2.2·10^3 M^{-2} or 2 – 7·10^9 M^{-2}, respectively. Both curves exhibit significant deviation from • – experimental data. Only the final model, 3 – assuming the coexistence of 2:1 and 1:1 complexes, reproduce the experimental data

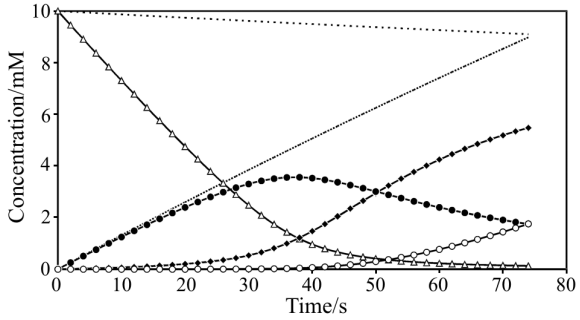

Fig. 7 The optimized model of titration. The estimated concentrations of all species observed in the solution are represented as the function of experiment time. Dotted lines represent total populations of crown ether (E – host) and amino acid (A – guest). Empty marks follow the populations of ○ – bulky amino acid and △ – ether. The solid marks describe the concentration of ✦ – 1:1 and ● – 2:1 complexes

acid concentration exceed 50% of the ether, the amino acid molecules start to compete for ether partners, and thus one of ether molecules dissociate from 2:1 complex to build its own 1:1 complex. Thus the decrease of 2:1 complex and increase of 1:1 complex concentrations is observed. Finally at 1:1 ether to amino acid ratio ~60% of molecules are engaged in 1:1 complex, while the rest of ~40% of amino acid population are either bulky or bound in 2:1 complex. Approximately all ether molecules are involved in amino acid binding.

Conclusions

Presented experimental approach based on the deconvolution of raw calorimetric response upon continuous injection titration experiment enables the analysis of the experimental data in the way similar to that usually applied for standard ITC calorimeters. The advantage of the presented method is the possibility to collect the significantly larger set of experimental data, which enables the analysis of complicated complexation equilibria. In the presented analysis the 2:1 and 1:1 binding equilibria characterised by close values of stability constants was successfully assigned and described for the phenylalanine·HCl complexed by crown ether in aqueous solution.

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References

1 H. J. Buschmann, L. Mutihac and K. Jansen, J. Inclusion Phenom. Macrocyclic Chem., 39 (2001) 1.
2 R. Karlson and L. Kullberg, Chemica Scripta, 9 (1976) 54.
3 T. Wiseman, S. Williston, J. F. Brands and L.-N. Lin, Anal. Biochem., 179 (1989) 131.
4 ITC Data Analysis in Origin, Microcal. Inc. Version 2.9.
5 A. A. Saboury, J. Therm. Anal. Cal., 77 (2004) 997.
6 H. J. Buschmann, E. Cleve and E. Schollmeyer, Thermochim. Acta, 207 (1992) 329.
7 E. Utzig, J. Therm. Anal. Cal., 54 (1998) 391.
8 J. J. Christensen, J. Ruckman, D. J. Eatough and R. M. Izatt, Thermochim. Acta, 3 (1972) 203.
9 W. Zielenkiewicz and E. Margas, Theory of Calorimetry, Kluwer Academic Publishers B. V., London 2002.
10 Cardano's derivation of the cubic formula, first time published: G. Cardano 'Ars magna' 1545.
11 D. W. Marquardt, J. Soc. Ind. Appl. Math., 11 (1963) 431.
12 T. Williams and C. Kelley, Gnuplot, Version 4.0.
13 W. Zielenkiewicz, Thermochim. Acta, 204 (1992) 1.
14 R. J. Wilson, A. E. Beezer, J. C. Mitchell and W. Loh, J. Phys. Chem., 99 (1995) 7108.
15 P. Job, Ann. Chim., 9 (1928) 113.

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