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CALCULATION OF SITE ENTHALPY FOR BINDING OF LIGANDS AND PROTONS TO MACROMOLECULES

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ABSTRACT

The analysis of equilibria in solution by the partition function method has shown how the total chemical amounts $[T_M]$, $[T_A]$, and $[T_H]$ of the components M, A, and H respectively can be expressed and determined as functions of different powers of site affinity constants k_j and cooperativity functions $\gamma(i_j, b_j)$, whereas the cumulative formation constants β_{PQR} cannot be used as statistically independent parameters to be refined in least-squares processes. The same drawback holds for molar enthalpies, ΔH_{PQR}.

A special algorithm has been developed by which the heat evolved can be expressed as a function of specific site enthalpies Δh_j and specific cooperativity enthalpies Δh_{γ_j} for each class j of sites. The algorithm represents the mathematical analog of the interconnections between components in the types of complex macrospecies $M_PA_QH_R$, M_PA_Q, A_QH_R, M_PH_R, $M_P(A_QH_R)$, etc., of the chemical model assumed and their deconvolution into microspecies $M_pA_qH_r$. Each class j of sites with site constant k_j and cooperativity coefficient b_j is described by a polynomial $J_j = (1 + k_j\gamma_{j,i}[Y_j])^{i_t}$, where Y_j is any ligand M, A, or H and i_t is the number of sites in the class j. The concentrations of the microspecies are calculated as single terms of the polynomials J_j or by products of more terms, each of which belongs to a different J_j. Each term of the polynomial is labeled by its own index (p_j, or q_j, or r_j, or in general i_j), which is the exponent of the term and contains the statistical factor m_{i_j} calculated as the coefficient of the term of the polynomial. The product of more terms is labeled by the indices of the component terms. The relations are therefore represented by combinations of indices $[p_1, p_2, \ldots, q_1, q_2, \ldots, r_1, r_2, \ldots]$.

In order to perform the calculation of concentrations of the microspecies and macrospecies by a procedure suitable for computer programming, each polynomial J_j is associated with a vector $\mathbf{J}_j$ whose elements $[i_j]$ are the terms of J_j. The cooperativity factors are set in a diagonal matrix $\mathbf{\Gamma}_j$ whose elements are $\gamma'_{j,i} = \exp[b_j(i_j - 1)i_j]$ and then introduced into the non-cooperative polynomials $\mathbf{J}_j^\star$ by vector products $\mathbf{J}_j^\star\mathbf{\Gamma}_j = \mathbf{J}_j$. The product of terms giving for each microspecies the contribution to the total concentrations $[T_M]$, $[T_A]$, and $[T_H]$ is calculated as the element of a matrix $\mathbf{L}_{l(j_1,j_2,\ \ )}$, obtained as tensor product: $\mathbf{L}_{l(1,2)} = \mathbf{J}_1\mathbf{J}_2$ or $\mathbf{L}_{l(1,2,3)} = \mathbf{J}_1\mathbf{J}_2\mathbf{J}_3$, etc. Depending on the chemical model, there are additional different matrices $\mathbf{L}_l$. The combination of indices of each element of $\mathbf{L}_l$ is $\{p_1, p_2, \ldots, q_1, q_2, \ldots, r_1, r_2, \ldots\}$. The indices are said to define an index space {i.s.}, parallel to the affinity

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cooperativity space. The elements of the matrices $\mathbf{L}_l$ are also used to set a matrix $\Delta\mathbf{C}$, whose elements Δc_{pqr} are the changes of concentration of the microspecies during the thermochemical reaction. The i.s. is parallel to the concentration space also.

The enthalpy change at the nth experimental point for each microspecies $M_pA_qH_r$ is calculated from the quantities $\delta H_{n,pqr} = \Delta c_{pqr}\ \{p[\Delta h_{j,p} + (p-1)\,\Delta h_{\gamma j,p}] + q[\Delta h_{j,q} + (q-1)\,\Delta h_{\gamma j,q}] + r[\Delta h_{j,r} + (r-1)\,\Delta h_{\gamma j,r}]\}$ which are then summed for all the indices p,q,r. This relation can also be put in a matrix form: $\delta\mathbf{H}_n = \Delta\mathbf{C}^{\mathrm{T}}\{\mathbf{H} + \mathbf{H}_\gamma\}$. All these matrices define spaces which are parallel to {i.s.}. The observed heat at the nth experimental point is the scalar product $\delta Q_{\mathrm{n.obs}} = \{\mathbf{a}_{j'}\}^{\mathrm{T}}\mathbf{x}_{j'}$, where the elements of the vector $\mathbf{x}_{j'}$, with $1 \le j' \le 2j_{\max}$, are the whole set of j couples of variables Δh_j, $\Delta h_{\gamma j}$, and the elements of the vector $\mathbf{a}_{j'}$ are weighted experimental thermochemical values.

By repeating the calculations and summing successively the values for all the n experimental points, the system of normal equations $\mathbf{A}\mathbf{x}_{j'} = \Delta\mathbf{Q}$ is set up, where the elements of $\Delta\mathbf{Q}$ are sums of n weighted experimental heats. By solution of the system, the values of Δh_j and $\Delta h_{\gamma j}$ for all the j classes are obtained.

INTRODUCTION

In preceding papers, we have developed a partition function algorithm [1–7] to calculate the binding of ligands and/or protons to macromolecules as a function of site affinity constants k_j and class cooperativity functions $\Gamma_j(i_j, b_j)$ where b_j is the class cooperativity coefficient. This procedure overcomes the difficulties arising from the use of the cumulative or Adair constants $\beta_{PQR} = [M_PA_QH_R][M]^{-P}[A]^{-Q}[H]^{-R}$ for each complex $M_PA_QH_R$.

The partition functions in terms of cumulative formation constants are written as

$$Z_{\mathrm{M}} = \sum_{1}^{P}\sum_{0}^{Q}\sum_{0}^{R}\beta_{PQR}[\mathrm{M}]^{P-1}[\mathrm{A}]^{Q}[\mathrm{H}]^{R} \tag{1}$$

$$Z_{\mathrm{A}} = \sum_{0}^{P}\sum_{1}^{Q}\sum_{0}^{R}\beta_{PQR}[\mathrm{M}]^{P}[\mathrm{A}]^{(Q-1)}[\mathrm{H}]^{R} \tag{2}$$

$$Z_{\mathrm{H}} = \sum_{0}^{P}\sum_{0}^{Q}\sum_{1}^{R}\beta_{PQR}[\mathrm{M}]^{P}[\mathrm{A}]^{Q}[\mathrm{H}]^{(R-1)} \tag{3}$$

and the mass balance equations giving the total analytical concentrations of each component are

$$[\mathrm{T_M}] = \sum_{1}^{P}\sum_{0}^{Q}\sum_{0}^{R}P\beta_{PQR}[\mathrm{M}]^{P}[\mathrm{A}]^{Q}[\mathrm{H}]^{R} \tag{4}$$

$$[\mathrm{T_A}] = \sum_{0}^{P}\sum_{1}^{Q}\sum_{0}^{R}Q\beta_{PQR}[\mathrm{M}]^{P}[\mathrm{A}]^{Q}[\mathrm{H}]^{R} \tag{5}$$

$$[\mathrm{T_H}] = \sum_{0}^{P}\sum_{0}^{Q}\sum_{1}^{R}R\beta_{PQR}[\mathrm{M}]^{P}[\mathrm{A}]^{Q}[\mathrm{H}]^{R} \tag{6}$$

The constants β_{PQR} are in general calculated by optimizing the values of the constants themselves which fit the experimental data, e.g. a potentiometric curve fit by means of a least-squares process [8,9]. If, however, the constants within one class j are correlated with one another by the same site affinity constants k_j and by the same cooperativity functions $\Gamma_j(i_j, b_j)$, the constants β_{PQR} cannot be considered as independent variables in a least-squares process. These difficulties have sometimes been overcome by introducing imaginary roots [10,11].

We have proposed [6,7] instead to write the partition functions as functions of site affinity constants k_j and cooperativity factors γ_{j,i_j} which are the values of the cooperativity function $\Gamma_j(i_j, b_j)$ at the step i_j. The explicit form of the function $\Gamma_j(i_j, b_j)$ will be given below. For example, in a system with complexes MA_Q, having several classes j of sites

$$Z_M = 1 + \sum_{Q=1}^{Q_t} \prod_{q_j=1}^{q_{t,j}} m_{q_j} \left(\gamma_{j,q} k_j [A]\right)^{q_j} \tag{7}$$

$$Z_A = 1 + \sum_{Q=0}^{Q_t} \prod_{q_j=1}^{q_{t,j}} m_{q_j} \left(\gamma_{j,q} k_j [A]\right)^{q_j} [M][A]^{-1} \tag{8}$$

for binding between M and A, where the symbols q_j refer to indices of single sites for each class j, $Q = \Sigma\, q_j$ is the index of macrospecies, and Q_t is the index of the saturated complex. The mass balance equations are calculated as sums of single terms; for the above-mentioned example,

$$[T_M] = [M] + [M] \sum_{Q=1}^{Q_t} \prod_{q_j=1}^{q_{t,j}} m_{q_j} \left(\gamma_{j,q} k_j [A]\right)^{q_j} \tag{9}$$

$$[T_A] = [A] + [M] \sum_{Q=0}^{Q_t} \prod_{q_j=1}^{q_{t,j}} \left(\sum q_j\right) m_{q_j} \left(\gamma_{j,q} k_j [A]\right)^{q_j} \tag{10}$$

By this method, values of site affinity constants k_j and coefficients b_j of class cooperativity functions are used as the variables and refined by least-squares methods. Reasonable physicochemical interpretations of the site constants and the cooperativity coefficients can be given [4].

The adoption of site affinity constants and cooperativity functions to describe the binding of ligands and protons to macromolecules, and of self-association of macromolecules as well, requires that an analogous method be adopted in the calculation of binding enthalpies when obtained by calorimetric methods.

MICRO- AND MACROSPECIES

The introduction of the subdivision of the cumulative constants into contributions due to different powers of single site affinity constants implies

that each species of stoichiometry $M_P A_Q H_R$ is the sum of several microspecies corresponding to different combinations of indices p_j, q_j, r_j:

$$P = \sum p_j \qquad Q = \sum q_j \qquad R = \sum r_j \tag{11}$$

The relationship between the indices p_j, q_j, and r_j of terms and indices P, Q, and R can be conveniently represented by using so-called affinity–cooperativity space. This space is a formal mathematical representation which facilitates the calculation and manipulation of the factors composing the products of eqn. (7) and represents the mathematical analog of the interconnections between components in the types of complexes $M_P A_Q H_R$, $M_P A_Q$, $A_Q H_R$, $M_P H_R$, $M_P(AH_R)_Q$, etc. of the chemical model. The distinction between macrospecies $M_P A_Q H_R$ and microspecies $M_p A_q H_r$ can also be made on this basis.

Each class j of sites with site constant k_j and cooperativity coefficient b_j is described by a polynomial, called the binary generating function:

$$J_j = \left(1 + k_j \gamma_{j,l} [Y_j]\right)^{i_t} \tag{12}$$

where Y_j is any ligand M, A or H, $\gamma_{j,l}$ is the cooperativity factor given by the value of the function $\Gamma_j(i_j, b_j)$ at point i_j, and i_t is the total number of sites in the class j. In eqn. (12) it is not specifically indicated which component is the receptor X_j = M, A, or H, although this information must be specified and used for the chemical model. The concentrations of the individual microspecies which contain only one class of sites are calculated as single terms of the polynomials J_j. When more than one class of sites is present in a microspecies, the concentration is the product of appropriate terms from different J_j polynomials. Each term of the polynomial contains a statistical factor from Fermi–Dirac statistics, calculated thus:

$$m_{ij} = i_{tj}!/i_j!(i_{tj} - i_j)! \tag{13}$$

Each term is labeled by its own index (p_j, q_j, r_j, or in general i_j), which is also the exponent of the term. The products of terms are labeled by the indices of the component terms from which they are constituted. These products can therefore be represented by combinations of indices $\{p_1, p_2, \ldots, q_1, q_2, \ldots, r_1, r_2, \ldots\}$ and the summations in eqns. (7)–(10) are limited by the combinations of lower-case indices compatible with the chemical model adopted.

VECTORS AND TENSORS

In order to set up the calculation of the concentrations of both micro- and macrospecies in a procedure suitable for computer programming, each

polynomial J_j is represented by a column vector $\{\mathbf{J}_j\}$ whose elements are the terms of J_j, i.e.

$$\{i_j\} = m_{i,j}\left(\gamma_{j,i}k_j[\mathrm{Y}_j]\right)^{i,j} = i_{i,j}!/i_j!(i_{i,j}-i_j)!\left\{\exp\left[b_j(i_j-1)\right]k_j[\mathrm{Y}_j]\right\}^{i,j} \qquad (14)$$

or by row vectors $[\mathbf{J}_j]$, with the same elements. The number of elements of each $\mathbf{J}_j$ is $i_{i,j}+1$, with $0 \le i_j \le i_{i,j}$. For the sake of simplicity, we may separate, whenever necessary, the binding factors, $k_j[\mathrm{Y}_j]$, from the cooperativity factors, $\gamma_{j,i}$, by representing the latter in a separate diagonal matrix Γ_j, whose elements are

$$\gamma'_{j,i} = \exp\left[b_j(i_j-1)i_j\right] \qquad (15)$$

These can be introduced into the polynomials by performing the vector multiplication

$$\mathbf{J}_j = \mathbf{J}_j^{\star}\Gamma_j \qquad (16)$$

where the vector $\mathbf{J}_j^{\star}$ represents the polynomial without the cooperativity component (i.e. $\gamma_{j,i}=1$). Products of terms belonging to different polynomials are calculated to obtain the contribution to the total amounts $[\mathrm{T_M}]$, $[\mathrm{T_A}]$ and $[\mathrm{T_H}]$. These products constitute the elements of a matrix $\mathbf{L}_{l(j_1,j_2,\ldots)}$, where l is an index number which identifies a reaction consistent with the chemical model and j_1, j_2, ... are the indices of the component vectors. This matrix is obtained from tensor products

$$\mathbf{L}_{l(1,2)} = \{\mathbf{J}_1\}[\mathbf{J}_2] \qquad (17)$$

or

$$\mathbf{L}_{l(1,2,3)} = \{\{\mathbf{J}_1\}[\mathbf{J}_2]\}[\mathbf{J}_3] \qquad (18)$$

etc. There will be additional different matrices L_l depending on the chemical model chosen.

INDEX SPACE

The elements of the matrix (tensor) $\mathbf{L}_l$ can be considered as points of a space (affinity and cooperativity space) (Fig. 1), where the coordinates along the axes are the elements of the vectors $\mathbf{J}_j$ used to calculate the tensor product. The combination of indices of each element of $\mathbf{L}_l$ is $\{p_1, p_2, \ldots, p_j, \ldots, q_1, q_2, \ldots, q_j, \ldots, r_1, r_2, \ldots, r_j, \ldots\}$. The indices define an index space {i.s.}, parallel to the affinity–cooperativity space. Every operation with the elements of $\mathbf{L}_l$ can be most simply represented by a combination of indices.

The affinity space is useful to show how the cumulative constants β_{PQR} are biased when used as variables in a least-squares procedure [6]. The cumulative constants, taking for simplicity $\beta_{1Q0} \equiv \beta_Q$, are obtained by sum-

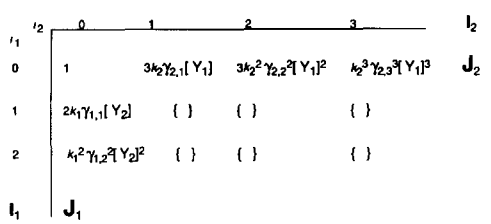

Fig. 1. Affinity/cooperativity space (a.c.s.) and index space (i.s.). Each element of the matrix is $\{i_1, i_2\} = m_{i1}(\gamma_{1,i}k_1[Y_1])^{i_1} m_{i2}(\gamma_{2,i}k_2[Y_2])^{i_2}$. For the sake of simplicity, two-index elements are indicated by { }. They are calculated as the product of the first element of the row multiplied by the first element of the column.

ming the terms along the diagonal lines in the matrix $\mathbf{L}_I$. This transformation (cf. Fig. 2 of ref. 6) is equivalent to a rotation of the axes $\mathbf{J}_1$ and $\mathbf{J}_2$ onto the bisector β_Q which is the new reference axis. The coordinates along the axis β_Q are biased because they are a mixture of different powers of different k_j and γ_j values, whereas the coordinates along the axes $\mathbf{J}_1$ and $\mathbf{J}_2$ separate the effects because they are uncorrelated (orthogonal).

POWER OPERATORS

In order to take into account some features of the chemical model, special operators $\mathbf{O}_i$ with unprimed index or $\mathbf{O}_{i'}$ with primed index are introduced. These operators are particularly important when competitive binding for the same sites occurs, when self-association of the receptor takes place, or when a ligand binds to a receptor which is in turn the ligand for another receptor. They define which ligands or groups of ligands are involved in the cooperativity effects [6,7].

One example which shows the difference between primed $\mathbf{O}_{i'}$ and unprimed $\mathbf{O}_i$ operators is a system with two components, M and H, with self-association of M. For the sake of simplicity, we assume that there is only one class $j = 3$ of $r_t = 3$ sites on M for H. The self-association polynomial is

$$J_1 = \left(1 + k_1\gamma_{1,p}[\mathrm{M}]\right)^{p_t - 1} \tag{19}$$

It is assumed that there is no self-association of H and that J_2 reduces to 1 since $k_2 = 0$. For the binding of H to M, the polynomial is

$$J_3 = \left(1 + k_3\gamma_{3,r}[\mathrm{H}]\right)^{r_t} \tag{20}$$

If we assume that $p_t = 2$ and $r_t = 3$, the saturated complex is $\mathrm{M_2H_6}$. We can choose a model where both statistics and cooperativity among H are

extended up to a maximum of six sites ($r_t' = 6$) with polynomials

$$J_{3'} = (1 + k_3\gamma_{3,r'}[\mathrm{H}])^{r_t'} \tag{21}$$

of variable degree $r_t' = p \times r_t$, changing with the index p controlling the self-association. The corresponding new vectors $\mathbf{J}_{3'}$ are obtained by the operator $\mathbf{O}_{p'}$, which modifies $\mathbf{J}_3$. In tensor notation, we can write

$$\mathbf{L}_{I(1,3)} = \{\mathbf{J}_1\}[\mathbf{J}_{3'}] = \{\mathbf{J}_1\}[\mathbf{O}_{p'}\mathbf{J}_3] = \{J_1\}[(\mathbf{O}_{p'}\mathbf{J}_3^{\star})(\mathbf{O}_{p'}\Gamma_3)] \tag{22}$$

Note that $\mathbf{O}_{p'}$ modifies both $\mathbf{J}_3^{\star}$ and Γ_3, prior to tensor multiplication, giving rise to a triangular matrix $\mathbf{L}_{I(1,3)}$.

In contrast to the above chemical model, we could choose a model where both statistics and cooperativity are restricted within the set of protons bound to the same M. In this case we have a product of p polynomials J_3 which is different from the pth power of J_3 in the $\gamma_{3,r}$ term. Thus

$$(J_3)^p \neq \prod_p (J_3) \tag{23}$$

because in the operation on the left-hand side γ is a function of p as well as r, whereas on the right-hand side γ is a function of r only:

$$\gamma_{3,2}^2 \neq \gamma_{3,1}\gamma_{3,1} \tag{24}$$

In tensor notation, we can write

$$\mathbf{L}_{I(1,3)} = \{\mathbf{J}_1\}[\mathbf{O}_p\mathbf{J}_3] = \{\mathbf{J}_1\}[\mathbf{O}_p(\mathbf{J}_3^{\star}\Gamma_3)] \tag{25}$$

where the operator $\mathbf{O}_p$ indicates that we have to calculate the pth tensor power of $\mathbf{J}_3$, without modifying Γ_3. This keeps the cooperativity effect restricted to those protons which are bound to the same M. The resulting matrix $\mathbf{L}_{I(1,3)}$ is a matrix of matrices. In this example, by increasing the number of H beyond $r_t = 3$ for $p > 1$, the cooperativity effect, and the statistics as well, start to be counted again from $r = 1$ on addition of the fourth proton up to the sixth.

COMPUTER INPUT INFORMATION

The following information is needed for input into a computer program based on the above concepts.

(1) The chemical model (Table 1), with complex macrospecies $\mathrm{M}_P\mathrm{A}_Q\mathrm{H}_R$, $\mathrm{M}_P\mathrm{H}_R$, $\mathrm{M}_P\mathrm{A}_Q$, $\mathrm{M}_P(\mathrm{AH}_R)_Q$, etc. and classes j with number of sites. An example is given in Table 1 for a macromolecule M which may bind a total of three ligands A or H.

(2) The list of binary generating functions J_j corresponding to vectors $\mathbf{J}_j$ necessary to construct the chemical model, with initial estimates of k_j and b_j

TABLE 1

Macromolecule M, ligand A, proton H. Competitive binding for the same sites. Complexes: MA_Q, MH_R, MA_QH_R with $Q + R \leqslant Q_t = R_t = 3$

$P = 1$			
$0 \leqslant Q \leqslant 3$	MA_Q	Class 4	3 sites
$1 \leqslant R \leqslant 3$	MH_R	Class 5	3 sites
$1 \leqslant Q + R \leqslant 3$	MA_QH_R	Class 4	3 sites
		Class 5	3 sites
Saturated Complexes		MA_3, MA_2H, MAH_2, MH_3	

TABLE 2

Generating functions (vectors $\mathbf{J}_j$)

j	Receptor $[X_j]$	Ligand $[Y_l]$	k_j	b_j	i_t			Γ_j
					p	q	r	
1	[M]	[M]	0	0	1			$(q-1)(q-2)$
2	[A]	[A]	0	0		1		$(q-1)(q-2)$
3	[H]	[H]	0	0			1	$(r-1)(r-2)$
4	[M]	[A]	10^7	-0.2		3		$q(q-1)$
5	[M]	[H]	10^6	-0.2			3	$r(r-1)$

Numerical values of k_j and b_j are hypothetical.

TABLE 3

Tensor matrices $\mathbf{L}_l$

Products	
l	
1	$\mathbf{J}_1$
2	$\mathbf{J}_2$
3	$\mathbf{J}_3$
4	$\{\mathbf{J}_1\}\{\mathbf{J}_4(\mathbf{O}_{r_t-q}\mathbf{J}_5)\}$ Each $\mathbf{J}_j$ is intended as multiplied by its appropriate Γ_j
Elements	
l	
1	$p-1=0$
2	$q-1=0$
3	$r-1=0$
4	$\{(p-1)_0\}, \{q_4(O_{r_t-q}r_6)\}$

TABLE 4

Tensor matrix $\mathbf{L}_4$: expanded elements

Minimum						
				P	Q	R
$p-1$	0_1			1		
Intermediate (examples)						
				P	Q	R
$p-1$	0_1			1		
q		3_4			3	
r						0
				P	Q	R
$p-1$	0_1			1		
q		2_4			2	
r			1_5			1
				P	Q	R
$p-1$	0_1			1		
q		1_4			1	
r			2_5			2
Affinity–cooperativity space						
Compact indices				Π_{qr}		
$0_1 3_4 0_5$				$(k_4\gamma_4(3)[\mathrm{A}])^3$		
$01_1 2_4 1_5$				$2(k_4\gamma_4(2)[\mathrm{A}])^2 k_5[\mathrm{H}]$		
$0_1 1_4 2_5$				$k_4\gamma_4(1)[\mathrm{A}]^2(k_5\gamma_5(2)[\mathrm{H}])^2$		

For a complete matrix $\mathbf{L}_4$ see appendix A.

and maximum numbers of sites p_t, q_t, and r_t. The example is carried forward in Table 2.

(3) The possible multiplication rules (Table 3), obtained by inspection of the chemical model, of vectors $\mathbf{J}_j$ and power operators, $\mathbf{O}_t$ or $\mathbf{O}_{t'}$, giving rise to the matrices $\mathbf{L}_l$, from which microspecies $\mathbf{M}_p\mathbf{A}_q\mathbf{H}_r$ are derived.

Some examples of elements of $\mathbf{L}_l$ both for index and for affinity–cooperativity space are given in Table 4.

ENTHALPY SPACE

By taking the logarithm of each element of $\mathbf{L}_l$, we pass from affinity space to free energy space. This principle holds for all the factors which make up each element, as obtained from eqn. (14), $[L_{j1,j3}]$ of $\mathbf{L}_l$:

$$\Delta G^{\ominus}_{ij}/RT = \ln\left[m_{ij}\left(\gamma_{j,i}k_j\right)^{ij}\right] = \ln\, m_{ij} + i_j\, \ln\, \gamma_{j,i} + i_j\, \ln\, k_j \qquad (26)$$

In this equation, the statistical contribution $\ln\, m_{ij} = \Delta S_{ij(\mathrm{st})}/R$ is purely entropic. Each term of eqn. (26) can be factored into enthalpic and entropic

components expressed for a class j of sites in terms of specific site enthalpy, Δh_j, specific cooperativity enthalpy, $\Delta h_{\gamma j}$, specific site entropy, Δs_j, and specific cooperativity entropy, $\Delta s_{\gamma j}$. Thus we obtain

$$\Delta G_{ij}^{\ominus} = i_j\,\Delta h_j + i_j(i_j-1)\,\Delta h_{\gamma j} + T\left[i_j\,\Delta s_j + i_j(i_j-1)\,\Delta s_{\gamma j}\right] + T\,\Delta S_{ij(\mathrm{st})} \tag{27}$$

from which we can derive the partial contribution to enthalpy $\Delta H_{ij}^{\ominus}$ due to the formation of one of the microspecies:

$$\Delta H_{ij}^{\ominus} = i_j\,\Delta h_j + i_j(i_j-1)\,\Delta h_{\gamma j} \tag{28}$$

By summing the enthalpy changes for a whole class j, one obtains the class enthalpy, ΔH_j:

$$\Delta H_j = \sum_{i_j=0}^{i_{t,j}} \left[i_j\,\Delta h_j + i_j(i_j-1)\,\Delta h_{\gamma j}\right] \tag{29}$$

The class enthalpy can be associated with row vectors $[\mathbf{h}_j]$ and $[\mathbf{h}_{\gamma j}]$ and with column vectors $\{\mathbf{h}_j\}$ and $\{\mathbf{h}_{\gamma j}\}$:

$$\left[\mathbf{h}_j\right] = \left[0\ 1\Delta h_j\ 2\Delta h_j\ \ldots\ i_{t,j}\,\Delta h_j\right] \tag{30}$$

$$\left[\mathbf{h}_{\gamma j}\right] = \left[0\ 1(1-1)\Delta h_{\gamma j}\ 2(2-1)\Delta h_{\gamma j}\ \ldots\ i_{t,j}(i_{t,j}-1)\Delta h_{\gamma j}\right] \tag{31}$$

which can be identified with parallel index vectors $\mathbf{i}_j$ and $\mathbf{i}_{\gamma j}$ respectively:

$$\left[\mathbf{h}_j\right] = \left[\mathbf{i}_j\right]\,\Delta h_j \tag{32}$$

$$\left[\mathbf{h}_{\gamma j}\right] = \left[\mathbf{i}_{\gamma j}\right]\,\Delta h_{\gamma j} \tag{33}$$

Note that every column vector is the transpose of a corresponding row vector: $\{\mathbf{h}_j\} = [\mathbf{h}_j]^{\mathrm{T}}$.

Equations (32) and (33) show that vectors $\mathbf{h}_j$ and $\mathbf{i}_j$ define spaces parallel to one another as do $\mathbf{h}_{\gamma j}$ and $\mathbf{i}_{\gamma j}$. The indices can be used to define both the affinity space and the enthalpy space. The vectors $\mathbf{i}_{\gamma j}$ are likewise parallel to the i.s. because its elements can be derived easily from those of $\mathbf{i}_j$. The index set is a very potent tool to handle problems of affinity, enthalpy, and cooperativity.

From the term enthalpy of eqn. (27), we can express the enthalpy ΔH_{pqr} of each microspecies:

$$\Delta H_{pqr} = q_j\left[\Delta h_j + (q_j-1)\,\Delta h_{\gamma j}\right] + r_j\left[\Delta h_j + (r_j-1)\,\Delta h_{\gamma j}\right] \tag{34}$$

The above equation applies to a reaction scheme where there is one class of binding A to M and one class of binding H to M. The summation can be accomplished in a more general way by referring to vectors $\mathbf{h}_j$ and $\mathbf{h}_{\gamma j}$. These vectors can be combined following the identical combination rules used to generate the elements of the matrices $\mathbf{L}_l$ of the affinity space. For enthalpy, however, tensor sums are substituted for tensor products. Both

affinity and enthalpy spaces are ruled by the same combination of indices {i.s.} which is parallel to both.

From the vectors $\mathbf{h}_{j=1}$ and $\mathbf{h}_{j=2}$, we can obtain by tensor sum, indicated by the symbol $\oplus$, the enthalpy space represented by a matrix

$$\mathbf{H}_{l(1,2)} = \{\mathbf{h}_1\} \oplus [\mathbf{h}_2] \tag{35}$$

whose elements are labeled by indices $\{i_1, i_2\}$ as the elements of a matrix $\mathbf{L}_{l(1,2)}$, and from vectors $\mathbf{h}_{\gamma j=1}$ and $\mathbf{h}_{\gamma j=2}$. By tensor sum we obtain the elements of the γ–enthalpy space, represented by a matrix

$$\mathbf{H}_{\gamma l(1,2)} = \{\mathbf{h}_{\gamma 1}\} \oplus [\mathbf{h}_{\gamma 2}] \tag{36}$$

whose elements have the same indices as $\mathbf{H}_{l(1,2)}$. Both are parallel to {i.s.}. By summing term by term two matrices $\mathbf{H}_l$ and $\mathbf{H}_{\gamma l}$, we obtain the total enthalpy matrix $\mathbf{H}_{T,l}$, i.e.

$$\mathbf{H}_{Tl} = \mathbf{H}_l + \mathbf{H}_{\gamma l} \tag{37}$$

representing the enthalpy + cooperativity space.

CONCENTRATIONS

In calorimetric experiments, the heat evolved is produced by those reactions that take place within the time interval of measurements. Therefore the changes in concentration of microspecies that participate in the reaction need to be identified. This can be done by calculating the equilibrium concentrations of each microspecies $[M_pA_qH_r]$, [M], [A], [H], etc., before and after each addition, using the elements of matrices $\mathbf{L}_l$ of the affinity space. The calculation of concentrations can be performed using site constants k_j and cooperativity coefficients b_j obtained, by successive approximations, from the mass balance equations which give the total amounts $[T_M]$, $[T_A]$, and $[T_H]$ expressed as sums of the appropriate elements of matrices $\mathbf{L}_l$.

The concentrations of microspecies correspond to the terms of the partition functions Z_M, Z_A, and Z_H. Each partition function is the sum of all the microspecies containing M, or A, or H respectively contributing to the osmotic pressure.

If $[c_M]$, $[c_A]$, and $[c_H]$ are defined as thermodynamic concentrations of species containing M, A, and H respectively, we have

$$[c_M] = [M] Z_M \tag{38}$$

$$[c_A] = [A] Z_A \tag{39}$$

$$[c_H] = [H] Z_H \tag{40}$$

Note that $[c_M]$, $[c_A]$, and $[c_H]$ can be different from $[T_M]$, $[T_A]$, and $[T_H]$. In fact, only those concentrations must be calculated that contribute to the osmotic pressure and to other thermodynamic properties as enthalpy. Therefore the terms shared by eqns. (38)–(40) have to be taken only once, and hence

$$[c_{pqr}] = [c_{M,pqr}], \qquad [c_{0qr}] = [c_{A,0qr}] \qquad [c_{H,00r}] = [c_{H,00r}] \tag{41}$$

The triple indices p, q, r are used for simplicity. Actually the indexing of the concentrations is the same as for the elements of $\mathbf{L}_l$. The concentrations can also be organized in a matrix $\mathbf{C}$. The data necessary are the concentration values before and after each addition. Therefore a matrix $\Delta\mathbf{C}$ can be formed.

HEATS

At the nth experimental point in a calorimetric titration, the enthalpy change due to formation of each microspecies is calculated by multiplying eqn. (34) by the corresponding concentration change:

$$\delta H_{n,pqr} = \Delta C_{n,pqr}\left\{ p_j\left[\Delta h_j + (p_j - 1)\,\Delta h_{\gamma j}\right] + q_j\left[\Delta h_j + (q_j - 1)\,\Delta h_{\gamma j}\right] + r_j\left[\Delta h_j + (r_j - 1)\,\Delta h_{\gamma j}\right]\right\} \tag{42}$$

These terms are summed to give the total calculated enthalpy change at the nth point:

$$\delta H_n = \sum_{j=1}^{j_{\max}} \sum_{p_j=1}^{p_{t,j}} \sum_{q_j=1}^{q_{t,j}} \sum_{r_j=1}^{r_{t,j}} \delta H_{n,pqr} \tag{43}$$

Equation (43) can be calculated also as the sum of the elements of a matrix

$$\delta\mathbf{H}_n = \Delta\mathbf{c}^{\mathrm{T}}(\mathbf{i}\,\Delta h_j + \mathbf{i}_{\gamma j}\,\Delta h_{\gamma j}) \tag{44}$$

In the concentration–cooperativity–enthalpy space of eqn. (44) (Fig. 2), the relationship between the axes $\Delta\mathbf{h}_j$, $\Delta\mathbf{h}_{\gamma j}$ and the axis $\mathbf{H}_Q$ of the cumulative enthalpy for macrospecies MA_Q shows again how the cumulative values are biased, whereas the axes based on site-specific thermodynamic parameters are orthogonal to each other (compare Fig. 2 in ref. 6).

We may group together the contributions to δH_n from the same class j of sites (eqns. (43) and (44)) and set this equal to the negative value of the experimental heat $-\delta Q_{n,\mathrm{obs}}$:

$$-\delta Q_{n,\mathrm{obs}} = \sum_{j=1}^{j_{\max}} \left[\sum_{i_j} (\Delta c_{pqr} i_j)\right] \Delta h_j + \sum_{j=1}^{j_{\max}} \left[\sum_{i_j} \left(\Delta c_{pqr} j_j (i_j - 1)\right)\right] \Delta h_{\gamma j}$$

$$= \sum_{j=1}^{j_{\max}} a'_{n,j}\,\Delta h_j + \sum_{j=1}^{j_{\max}} b'_{n,j}\,\Delta h_{\gamma j} \tag{45}$$

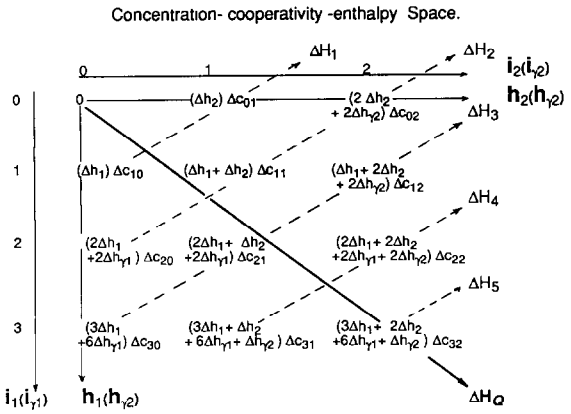

Fig. 2. Transformation from site enthalpy Δh_1, Δh_2 to cumulative enthalpy ΔH_Q. $\mathbf{h}_1(\mathbf{h}_{\gamma 1})$ and $\mathbf{h}_2(\mathbf{h}_{\gamma 2})$ are the original axes, $\Delta \mathbf{H}_Q$ the transformed axis. The heat evolved at each experimental point, δQ_n is calculated by summing the values of all the elements of the matrix.

where $i_j = p_j$ or q_j or r_j and the coefficients $a'_{n,j}$ and $b'_{n,j}$ are calculated from the matrix (44). The summation Σ_{i_j} is extended to all the microspecies in the matrix $\Delta \mathbf{c}$.

LEAST SQUARES

We can represent the equation (45) in matrix notation if we set up Δh_1, Δh_2 ..., Δh_j, ..., $\Delta h_{j_{max}}$, $\Delta h_{\gamma 1}$, $\Delta h_{\gamma 2}$, ..., $\Delta h_{\gamma j}$, ..., $\Delta h_{\gamma j_{max}}$ in an array with $j' = 2j_{max}$ elements, represented by a vector

$$\mathbf{x}_{j'} = \left[\Delta h_1\ \Delta h_2\ \ldots\ \Delta h_j\ \ldots\ \Delta h_{j_{max}}\ \Delta h_{\gamma 1}\ \Delta h_{\gamma 2} \ldots \Delta h_{\gamma j}\ \ldots\ \Delta h_{\gamma j_{max}}\right] \qquad (46)$$

with index $j' = 1, 2, \ldots, j_{max}, j_{max}+1, j_{max}+2, \ldots, 2j_{max}$. On the other hand, the coefficients a' and b' of eqn. (45) can be represented in a unique array forming a vector $\mathbf{a}_{n,j'}$ with the same indices and number of elements as $\mathbf{x}_{j'}$:

$$\mathbf{a}_{n,j'} = \left[a_{n,1}\ a_{n,2}\ \ldots\ a_{n,j}\ a_{n,j_{max}}\ b_{n,1}\ b_{n,2}\ \ldots\ b_{n,j}\ \ldots\ b_{n,j_{max}}\right] \qquad (47)$$

The observed heat $-\delta Q_{n,obs}$ can be considered equal to the scalar product

$$\mathbf{a}_{n,j'}\mathbf{x}_{j'} = -\delta Q_{n,obs} \qquad (48)$$

This equation is the basis for the least-squares calculations of the variables of the system, represented by the elements of $\mathbf{x}_{j'}$. To this end, we premultiply (tensor multiplication) both members of eqn. (48) $\mathbf{a}^{T}_{n,j'} = \{\mathbf{a}_{n,j'}\}$:

$$\{\mathbf{a}_{n,j'}\}\left[\mathbf{a}_{n,j'}\right]\{\mathbf{x}_{j'}\} = \{\mathbf{a}_{n,j'}\}(-\delta Q_{n,obs}) \qquad (49)$$

By defining $\mathbf{A}_n = \{\mathbf{a}_{n,j'}\}[\mathbf{a}_{n,j'}]$ and $\delta\mathbf{Q}_{n,a_{j'}}$ equal to the right-hand side of eqn. (49),

$$\mathbf{A}_n\mathbf{x}_{j'} = \delta\mathbf{Q}_{n,a_{j'}} \tag{50}$$

The elements of $\delta\mathbf{Q}_{n,a_{j'}}$ can be considered as weighted experimental values. The elements of $\mathbf{A}_n$ are $[A_n(j_1', j_2')]$ and the elements of $\mathbf{x}_{j'}$ are given by eqn. (46).

By repeating these calculations for n experimental points and summing the n equations (50), one obtains

$$\mathbf{A}\mathbf{x}_{j'} = \Delta\mathbf{Q}_{\text{obs},a_{j'}} \tag{51}$$

The elements j' of $\Delta\mathbf{Q}_{\text{obs},a_{j'}}$ are the result of the sum of corresponding j' elements of $\delta\mathbf{Q}_{n,a_{j'}}$. The elements $[A(j_1', j_2')]$ of $\mathbf{A}$ are obtained by summing elements with equal indices of the n matrices $\mathbf{A}_n$.

By solving the equation system (51), which is the set of normal equations for the linear least-squares method, the values of the unknown Δh_j and $\Delta h_{\gamma j}$ are calculated.

CONCLUSIONS

In the development of a computer program suitable for the calculation of site enthalpies, the following points have been considered.

(1) The cumulative constants β_{PQR} and the cumulative molar enthalpies ΔH_{PQR} of macrospecies $\mathrm{M}_P\mathrm{A}_Q\mathrm{H}_R$ cannot be calculated as independent variables in least-squares procedures, because they are interrelated. The real independent variables, orthogonal to one another, are the thermodynamic functions corresponding to microspecies $\mathrm{M}_p\mathrm{A}_q\mathrm{H}_r$, with lower-case indices.

(2) Affinity space and parallel index space are calculated from matrices $\mathbf{L}_l$, obtained by tensor products of generating function vectors $\mathbf{J}_j$. The elements of $\mathbf{L}_l$ are determined by multiplication tables derived from the chemical model. Partition functions Z_{M}, Z_{A}, and Z_{H}, and total amounts $[\mathrm{T_M}]$, $[\mathrm{T_A}]$, and $[\mathrm{T_H}]$ can be calculated by the same combination of indices of the elements of $\mathbf{L}_l$.

(3) The concentrations of microspecies $\mathrm{M}_p\mathrm{A}_q\mathrm{H}_r$ are identified by parallel index sets and calculated by elements of $\mathbf{L}_l$, provided that repetitions are avoided.

(4) The enthalpy change δH_n at the nth experimental point can be expressed as a function of concentration changes, ΔC_{pqr}, of specific class enthalpy, Δh_j, and specific class cooperativity enthalpy, $\Delta h_{\gamma j}$. The enthalpy and concentration of microspecies are controlled by the same index space as the affinity space. So is the product $\Delta\mathbf{c}^{\mathrm{T}}(\mathbf{i}\,\Delta h_j + \mathbf{i}_{\gamma j}\,\Delta h_{\gamma j})$, giving the enthalpy–concentration space.

(5) The values of couples of variables Δh_j, $\Delta h_{\gamma j}$, can be found by linear least-squares calculations from the experimental heats, $\delta Q_{n,\mathrm{obs}}$.

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APPENDIX A

The application of the method presented here is illustrated in the following by a worked example.

The model is the same as found in Table 1, with A and H competing for the same sites on the receptor M. The number of sites is $Q_t = R_t = 3$.

It is worth noting in advance that the same indices hold for every matrix from (A.3) to (A.6). Matrices (A.5) and (A.6) and eqns. (A.7) and (A.8) are reported only for the purpose of demonstration. As a matter of fact the elements of the vector (A.9) can be obtained directly from the matrix (A.4) by multiplying each value Δc_{pqr} by q or r (or by $q(q-1)$ or $r(r-1)$), depending on which coefficient $a_{j'}$ is being calculated. This is a consequence of the application of eqn. (45):

$$\partial(\delta Q_n)/\partial(\Delta h_j) = -\sum_{i_j} i_j \, \Delta c_{pqr} \tag{A.1}$$

and

$$\partial(\delta Q_n)/\partial(\Delta h_{\gamma j}) = -\sum_{i_j} i_j(i_j - 1) \, \Delta c_{pqr} \tag{A.2}$$

In fact eqns. (A.1) and (A.2) give the coefficients of eqn. (A.8) and hence the elements of $\mathbf{a}_{n,j'}$ in eqns. (A.9) and (A.11).

It should be mentioned that eqn. (A.13) and its compact form (A.14) represent the set of normal equations for the determination of the parameters by a linear least-squares method.

Worked example

From the binary generating functions of Table 1 and from the multiplication rules of Table 3 the following matrix is obtained:

$\mathbf{L}_4$

q_4 \ r_5	0	1	2	3
0	1	$3k_5[\mathrm{H}]\exp(2b_5)$	$3(k_5[\mathrm{H}])^2\exp(6b_5)$	$(k_5[\mathrm{H}])^3$
1	$3k_4[\mathrm{A}]$	$3k_4[\mathrm{A}]2k_5[\mathrm{H}]\exp(2b_5)$	$3k_4[\mathrm{A}](k_5[\mathrm{H}])^2$	
2	$3(k_4[\mathrm{A}])^2\exp(2b_4)$	$3(k_4[\mathrm{A}])^2k_5[\mathrm{H}]\exp(2b_4)$		
3	$(k_4[\mathrm{A}])^3\exp(6b_4)$			

(A.3)

At the nth experimental point, the following concentration changes can be calculated from the mass balance equations:

Δc_{qr}

q_4 \ r_5	0	1	2	3
0	Δc_{00}	Δc_{01}	Δc_{02}	Δc_{03}
1	Δc_{10}	Δc_{11}	Δc_{12}	
2	Δc_{20}	Δc_{21}		
3	Δc_{30}			

(A.4)

From vectors h_j and $h_{\gamma j}$, the total heat matrix can be obtained:

$\mathbf{H}_\mathrm{T}$

q_4 \ r_5	0	1	2	3
0	0	Δh_5	$2\,\Delta h_5 + 2\,\Delta h_{\gamma 5}$	$3\,\Delta h_5 + 6\,\Delta h_{\gamma 5}$
1	Δh_4	$\Delta h_4\,\Delta h_5$	$\Delta h_4\,2\,\Delta h_5 + 2\,\Delta h_{\gamma 5}$	
2	$2\,\Delta h_4 + 2\,\Delta h_{\gamma 4}$	$2\,\Delta h_4\,\Delta h_5 + 2\,\Delta h_{\gamma 4}$		
3	$3\,\Delta h_4 + 6\,\Delta h_{\gamma 4}$			

(A.5)

Then by matrix multiplication the enthalpy matrix $\delta\mathbf{H}_n$ can be obtained:

$$\delta\mathbf{H}_n = \Delta\mathbf{c}^\mathrm{T}\mathbf{H}_\mathrm{T}$$

q_4 \ r_5	0	1	2	3
0	0	$\Delta h_5\,\Delta c_{01}$	$(2\,\Delta h_5 + 2\,\Delta h_{\gamma 5})\,\Delta c_{02}$	$(3\,\Delta h_5 + 6\,\Delta h_{\gamma 5})\,\Delta c_{01}$
1	$\Delta h_4\,\Delta c_{10}$	$(\Delta h_4 + \Delta h_5)\,\Delta c_{11}$	$(\Delta h_4\,2\,\Delta h_5 + 2\,\Delta h_{\gamma 5})\,\Delta c_{12}$	
2	$(2\,\Delta h_4 + 2\,\Delta h_{\gamma 4})\,\Delta c_{20}$	$(2\,\Delta h_4 + \Delta h_5 + 2\,\Delta h_{\gamma 4})\,\Delta c_{21}$		
3	$(3\,\Delta h_4 + 6\,\Delta h_{\gamma 4})\,\Delta c_{30}$			

(A.6)

From the matrix (A.6), the heat evolved at the nth experimental point is calculated as

$$\begin{aligned}-\delta Q_{n,\mathrm{obs}} = {} & \Delta h_4(1\,\Delta c_{10} + 2\,\Delta c_{20} + 3\,\Delta c_{30} + 1\,\Delta c_{11} + 2\,\Delta c_{12}) \\ & + \Delta h_{\gamma 4}(2\,\Delta c_{20} + 6\,\Delta c_{30} + 2\,\Delta c_{21}) \\ & + \Delta h_5(1\,\Delta c_{01} + 2\,\Delta c_{02} + 3\,\Delta c_{03} + 1\,\Delta c_{11} + 1\,\Delta c_{21} + 2\,\Delta c_{12}) \\ & + \Delta h_{\gamma 5}(2\,\Delta c_{02} + 6\,\Delta c_{03} + 2\,\Delta c_{12})\end{aligned} \tag{A.7}$$

and in more general form as

$$-\delta Q_{n,\mathrm{obs}} = \Delta h_4\Big(\sum q\ \Delta c_{qr}\Big) + \Delta h_5\Big(\sum r\ \Delta c_{qr}\Big) + \Delta h_{\gamma 4}\Big[\sum q(q-1)\ \Delta c_{qr}\Big] + \Delta h_{\gamma 5}\Big[\sum r(r-1)\ \Delta c_{qr}\Big] = \Delta h_4 a'_4 + \Delta h_5 a'_5 + \Delta h_{\gamma 4} b'_4 + \Delta h_{\gamma 5} b'_5 \tag{A.8}$$

where the summations are extended to all the elements of the matrix (A.6).

The coefficients of eqn. (A.8) can be represented in an array of index j' forming a row vector

$$\left[\mathbf{a}_{j'}\right]_n = \left[a'_4\ a'_5\ b'_4\ b'_5\right]_n = \left[a_{j'}\right]_n \tag{A.9}$$

whereas the specific heats of eqn. (A.8) are arranged in a column vector of index j'

$$\{\mathbf{x}_{j'}\} = \left[\Delta h_4\ \Delta h_5\ \Delta h_{\gamma 4}\ \Delta h_{\gamma 5}\right] \tag{A.10}$$

so that

$$\left[\mathbf{a}_{j'}\right]_n \{\mathbf{x}_{j'}\} = \delta Q_n \tag{A.11}$$

which is the generalized form of eqn. (A.8).

By pre-tensor multiplication of both members of eqn. (A.11) by $\{\mathbf{a}_{j'}\}_n = [\mathbf{a}_{j'}]_n^{\mathrm{T}}$, and by defining

$$\mathbf{A}_n = \{\mathbf{a}_{j'}\}_n \left[\mathbf{a}_{j'}\right]_n \tag{A.12}$$

the following equation is obtained:

$$\mathbf{A}_n \mathbf{x}_{j'} = \mathbf{a}_{j'n}\ \delta Q_{n,\mathrm{obs}} = \delta \mathbf{Q}_{n,a_{j'}} \tag{A.13}$$

For n experimental points, n matrices $\mathbf{A}_n$ and n vector $\mathbf{a}_{j'n}$ can be added element by element, thus obtaining

$$\begin{bmatrix} \sum_n a'_4 a'_4 & \sum_n a'_4 a'_5 & \sum_n a'_4 b'_4 & \sum_n a'_4 b'_5 \\ \sum_n a'_5 a'_4 & \sum_n a'_5 a'_5 & \sum_n a'_5 b'_4 & \sum_n a'_5 b'_5 \\ \sum_n b'_4 a'_4 & \sum_n b'_4 a'_5 & \sum_n b'_4 b'_4 & \sum_n b'_4 b'_5 \\ \sum_n b'_5 a'_4 & \sum_n b'_5 a'_5 & \sum_n b'_5 b'_4 & \sum_n b'_5 b'_5 \end{bmatrix} \begin{bmatrix} \Delta h_4 \\ \Delta h_5 \\ \Delta h_{\gamma 4} \\ \Delta h_{\gamma 5} \end{bmatrix} = \begin{bmatrix} \sum_n a'_4\ \delta Q_{n,\mathrm{obs}} \\ \sum_n a'_5\ \delta Q_{n,\mathrm{obs}} \\ \sum_n b'_4\ \delta Q_{n,\mathrm{obs}} \\ \sum_n b'_5\ \delta Q_{n,\mathrm{obs}} \end{bmatrix} \tag{A.14}$$

$$\mathbf{A}\ \mathbf{x}_{j'} = \Delta \mathbf{Q}_{\mathrm{obs},a_{j'}} \tag{A.15}$$

where the elements of $\Delta \mathbf{Q}_{\mathrm{obs},a_{j'}}$ are obtained from

$$\sum_n a_{j'n}\ \delta Q_{n,\mathrm{obs}} = \left[\Delta Q_{\mathrm{obs},a_{j'}}\right] \tag{A.16}$$

at constant j'.

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