Graphical Rules for Enzyme-Catalysed Rate Laws

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(Received 8 October 1979)

Two graphical rules for calculating the reaction rates of steady-state enzyme-catalysed systems are presented. According to Rule 1, the number of non-zero terms can be easily calculated, which is useful to check the results and avoid mistakes. Rule 2 provides a new graphical method, which will be much more effective than the existing methods in dealing with complicated problems. In addition, during the process of deriving Rule 1, the mathematical principles of the Wong–Hanes structural rule have been naturally obtained too.

In enzyme kinetics, one generally needs to derive the equation for the production rate of products. For example, for the steady-state enzyme-catalysed reaction system illustrated as the following:

the general interest is to calculate:

$$v = \frac{d[P]}{dt} = k_f [EP] - k_r [P][E] \quad (2)$$

For convenience, we use E_i ($i = 1, 2, \dots, n$) to express the various enzyme-containing species, and suppose that the rate constant for $E_i \rightarrow E_j$ be k_{ij} , and that for $E_j \rightarrow E_i$ be k_{ji} . Here k_{ij} stands for the first-order rate constant or the pseudo-first-order rate constant. For simplicity of mathematical formulation, we may specify $k_{ii} = 0$ according to the definition of k_{ij} . Without loss of generality, let $E_1 = E$, $E_n = EP$. Then, eqns. (1) and (2) will respectively become:

and:

$$v = \frac{dp}{dt} = k_{n1} e_n - k_{1n} e_1 \quad (4)$$

[As before (Chou *et al.*, 1979), the reactants are

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represented by capital letters and their concentrations by the corresponding italic lower-case ones.] It is extremely laborious and liable to errors for any but trivially simple reaction systems if one uses the general method to calculate v . Thus King & Altman (1956) first suggested applying a graphical method for the calculation. By their approach, the concentration e_m of enzyme-containing E_m in the reaction system can be expressed as:

$$e_m = \frac{N_m}{D} e_0 \quad (m = 1, 2, \dots, n) \quad (5)$$

where e_0 is the total enzyme concentration, and the algebraic expressions for N_m and D are respectively as follows (King & Altman, 1956; Cornish-Bowden, 1976; Chou *et al.*, 1979):

$$N_m = \begin{vmatrix} \sum_{i=1}^n k_{1i} & -k_{12} & \dots & \dots & -k_{1n} \\ -k_{21} & \sum_{i=1}^n k_{2i} & \dots & \dots & -k_{2n} \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ -k_{n1} & -k_{n2} & \dots & \dots & \sum_{i=1}^n k_{ni} \end{vmatrix} \quad \begin{matrix} \text{(the } m\text{th} \\ \text{column} \\ \text{is removed)} \end{matrix} \quad \begin{matrix} \text{(the } m\text{th} \\ \text{row is} \\ \text{removed)} \end{matrix} \quad (6)$$

$$D = \sum_{m=1}^n N_m \quad (7)$$

Instead of developing determinants, they presented a graphical method to calculate N_m so as to make the calculation of e_m much more intuitive and simple.

Later Wong & Hanes (1962) and Wong (1975) pointed out that, when e_1 and e_n are substituted into eqn. (4), in the numerator some terms appear to be reciprocally cancelled. So, Wong & Hanes (1962) and Wong (1975) suggested another graphical rule, namely the structural rule for rate laws, to calculate the reaction rate. With such a rule, one can immediately write out the non-zero terms in the numerator one by one without the necessity to make the reciprocal cancel calculation, which is prone to errors.

However, when the Wong-Hanes rule is applied to more complicated reaction systems, the number of the sub-graphs to be considered increases also, and some sub-graphs would be easily missed, so as to lead the loss of some corresponding terms. In such a case, can we devise a convenient method that may serve to calculate the number of sub-graphs required beforehand? Moreover, for more complicated reaction systems, even if the number is known, it is still laborious to find out all the sub-graphs. For these systems, is there any easier graphical method? In the present paper we discuss these problems. Below, we shall start from the general algebraic equations.

Theory

For the reaction system expressed by scheme (3), according to the steady-state assumption we have:

$$-\frac{de_j}{dt} = e_j \sum_{i=1}^n k_{ji} - \sum_{i=1}^n k_{ij} e_i = 0 \quad (8)$$

($j = 1, 2, \dots, n$)

$$\sum_{j=1}^n e_j = e_0 \quad (9)$$

It is readily seen that the sum of n equations in eqn.

$$= (-1)^{2n+1} \frac{e_0}{D} \begin{vmatrix} \sum_{i=1}^n k_{1i} & -k_{21} & \dots & -k_{(n-1)1} & -k_{n1} \\ -k_{12} & \sum_{i=1}^n k_{2i} & \dots & -k_{(n-1)2} & -k_{n2} \\ \vdots & \vdots & & \vdots & \vdots \\ -k_{1(n-1)} & -k_{2(n-1)} & \dots & \sum_{i=1}^n k_{(n-1)i} & -k_{n(n-1)} \\ k_{1n} & 0 & \dots & 0 & -k_{n1} \end{vmatrix} = -\frac{e_0}{D} \begin{vmatrix} \sum_{i=1}^{n-1} k_{1i} & -k_{21} & \dots & -k_{(n-1)1} & 0 \\ -k_{12} & \sum_{i=1}^n k_{2i} & \dots & -k_{(n-1)2} & -k_{n2} \\ \vdots & \vdots & & \vdots & \vdots \\ -k_{1(n-1)} & -k_{2(n-1)} & \dots & \sum_{i=1}^n k_{(n-1)i} & -k_{n(n-1)} \\ k_{1n} & 0 & \dots & 0 & -k_{n1} \end{vmatrix} \quad (11)$$

(8) is always equal to zero, i.e. they are linearly dependent. Therefore we can replace any one (e.g. the n th) of them with eqn. (9) so as to find out e_j ($j = 1, 2, \dots, n$). Then, substitution of the results into eqn. (4) will yield v . However, we can also take v as an unknown, put eqns. (4), (8) and (9) together, and

directly set out $n+1$ simultaneous equations with $n+1$ unknowns, i.e.:

$$\left. \begin{aligned} e_j \sum_{i=1}^n k_{ji} - \sum_{i=1}^n k_{ij} e_i &= 0 \\ (j &= 1, 2, \dots, n-1) \\ \sum_{j=1}^n e_j &= e_0 \\ k_{1n} e_1 - k_{n1} e_n + v &= 0 \end{aligned} \right\} \quad (10)$$

Then, by Cramer's rule, we get:

$$v = \frac{\begin{vmatrix} \sum_{i=1}^n k_{1i} & -k_{21} & \dots & -k_{(n-1)1} & -k_{n1} & 0 \\ -k_{12} & \sum_{i=1}^n k_{2i} & \dots & -k_{(n-1)2} & -k_{n2} & 0 \\ \vdots & \vdots & & \vdots & \vdots & \vdots \\ -k_{1(n-1)} & -k_{2(n-1)} & \dots & \sum_{i=1}^n k_{(n-1)i} & -k_{n(n-1)} & 0 \\ 1 & 1 & \dots & 1 & 1 & e_0 \\ k_{1n} & 0 & \dots & 0 & -k_{n1} & 0 \end{vmatrix}}{\begin{vmatrix} \sum_{i=1}^n k_{1i} & -k_{21} & \dots & -k_{(n-1)1} & -k_{n1} & 0 \\ -k_{12} & \sum_{i=1}^n k_{2i} & \dots & -k_{(n-1)2} & -k_{n2} & 0 \\ \vdots & \vdots & & \vdots & \vdots & \vdots \\ -k_{1(n-1)} & -k_{2(n-1)} & \dots & \sum_{i=1}^n k_{(n-1)i} & -k_{n(n-1)} & 0 \\ 1 & 1 & \dots & 1 & 1 & 0 \\ k_{1n} & 0 & \dots & 0 & -k_{n1} & 1 \end{vmatrix}}$$

where D is given by eqn. (7) (Chou *et al.*, 1979). By means of the properties of the determinant, eqn. (11) may be further condensed to:

$$v = \frac{e_0}{D} \{k_{n1} N_n^{\overline{1n}} - k_{1n} N_1^{\overline{n1}}\} \quad (n \geq 3) \quad (12)$$

where:

$$N_1^{-1} = \begin{vmatrix} \sum_{i=1}^n k_{2i} & \cdots & -k_{2(n-1)} & -k_{2n} \\ \vdots & & \vdots & \vdots \\ -k_{(n-1)2} & \cdots & \sum_{i=1}^n -k_{(n-1)i} & -k_{(n-1)n} \\ -k_{n2} & \cdots & -k_{n(n-1)} & \sum_{i=2}^n k_{ni} \end{vmatrix}$$

$$N_n^{-1} = \begin{vmatrix} \sum_{i=1}^{n-1} k_{1i} & -k_{12} & \cdots & -k_{1(n-1)} \\ -k_{21} & \sum_{i=1}^n k_{2i} & \cdots & -k_{2(n-1)} \\ \vdots & \vdots & & \vdots \\ -k_{(n-1)1} & -k_{(n-1)2} & \cdots & \sum_{i=1}^n k_{(n-1)i} \end{vmatrix} \quad (13)$$

Comparing eqn. (6) with eqn. (13), we consequently obtain the following relations:

$$N_1^{-1} = N_1|_{k_{n1}=0}, N_n^{-1} = N_n|_{k_{1n}=0} \quad (14)$$

In other words, N_1^{-1} and N_n^{-1} are respectively the special cases of N_1 and N_n , and hence can be calculated directly by the existing graphical methods of calculating N_m (e.g. King & Altman, 1956; Chou *et al.*, 1979). But, in calculation, we ought to first eliminate the arc from E_n to E_1 and that from E_1 to E_n in the graph expressing the enzyme-catalysed reaction system. Note that, for the mechanisms with branched pathways, instead of eqn. (4) we generally have:

$$v = \frac{dp}{dt} = \sum_{\alpha \neq \beta} (k_{\beta\alpha} e_\beta - k_{\alpha\beta} e_\alpha) \quad (4')$$

Here, what indexes should be taken for α and β will depend on the concrete mechanism. Through nearly the same but a little more complicated derivation, we can obtain:

$$v = \frac{e_0}{D} \sum_{\alpha \neq \beta} \{k_{\beta\alpha} N_{\beta}^{\alpha\beta} - k_{\alpha\beta} N_{\alpha}^{\beta\alpha}\} \quad (12')$$

where:

$$N_{\alpha}^{\beta\alpha} = N_{\alpha}|_{k_{\beta\alpha}=0}, N_{\beta}^{\alpha\beta} = N_{\beta}|_{k_{\alpha\beta}=0} \quad (14')$$

Therefore we immediately obtain a general rule for calculating $N_{\alpha}^{\beta\alpha}$.

Rule 1

(1) Draw a directed graph $\mathcal{D}$ expressing the enzyme-catalysed reaction system. This graph consists of points and arcs. Each point denotes an enzyme species and each arc reflects the relationship between two enzyme species. If there is a direct chemical conversion between some two enzyme species, then there will be an arc to link the two corresponding points. The arc bears an arrow to indicate the conversion direction and is weighted by a rate constant to show the conversion rate.

(2) Eliminate the arc from E_β to E_α in $\mathcal{D}$, and the graph thus obtained is denoted by $\mathcal{D}^{\beta\alpha}$, and we then have:

$$N_{\alpha}^{\beta\alpha} = \sum_i f(T_i^{\alpha}) \quad (15)$$

Here T_i^{α} is the i th of those sub-graphs in $\mathcal{D}^{\beta\alpha}$ each of which consists of $n-1$ arcs, but contains no

cycle, no two or more than two arcs coming out of one point, and also no arc coming out from E_α . And $f(T_i^{\alpha})$ denotes the product of the weights of all arcs in T_i^{α} .

(3) In order to facilitate checking and avoid missing any sub-graph, the number, $P_{\alpha}^{\beta\alpha}$, of sub-graphs involved in calculating $N_{\alpha}^{\beta\alpha}$ can also be obtained beforehand by the following method. In accordance with the graph $\mathcal{D}^{\beta\alpha}$, construct a matrix $A(\mathcal{D}^{\beta\alpha}) = [a_{ij}]$, where:

$$a_{ij} = \begin{cases} 1, & \text{if there is a connecting line from } E_i \text{ to } E_j \\ 0, & \text{if there is no connecting line from } E_i \text{ to } E_j \end{cases} \quad (16)$$

And make the matrix $B(\mathcal{D}^{\beta\alpha}) = [b_{ij}]$, where:

$$b_{ij} = \begin{cases} \sum_{t=1}^n a_{it} & \text{if } i=j \\ -a_{ij} & \text{if } i \neq j \end{cases} \quad (17)$$

Then the desired number of the sub-graphs:

$$P_{\alpha}^{\beta\alpha} = \det[B(\mathcal{D}^{\beta\alpha})]_{\bar{\alpha}, \bar{\alpha}} \quad (18)$$

where $[B(\mathcal{D}^{\beta\alpha})]_{\bar{\alpha}, \bar{\alpha}}$ denotes the sub-matrix obtained by removing the $\bar{\alpha}$ th row and $\bar{\alpha}$ th column, and the symbol $\det$ denotes taking the determinant value of the matrix following it.

(4) If $\mathcal{D}$ is a symmetric directed graph (Chou *et al.*, 1979), i.e. in $\mathcal{D}$ whenever there is an arc $\widehat{E_i E_j}$, there must be an arc $\widehat{E_j E_i}$, then we must have $P_{\alpha}^{\beta\alpha} = P_{\beta}^{\alpha\beta}$.

Fig. 1. Directed graph $\mathcal{D}$ expressing the enzyme-catalysed reaction system in Example 1

Example 1. Consider the enzyme-catalysed reaction system illustrated in Fig. 1.

In accordance with step (2) of Rule 1, transform $\mathcal{D}$ of Fig. 1 into $\mathcal{D}^{\text{I}3}$ of Fig. 2. Then, from eqn. (15) we obtain:

$$N_3^{\text{I}3} = \begin{array}{c} \text{E}_1 \xrightarrow{k_{12}} \text{E}_2 \\ \text{E}_3 \xrightarrow{k_{23}} \text{E}_2 \end{array} = k_{12} k_{23}$$

Similarly:

$$N_1^{\text{I}1} = \begin{array}{c} \text{E}_1 \xleftarrow{k_{21}} \text{E}_2 \\ \text{E}_3 \xleftarrow{k_{32}} \text{E}_2 \end{array} = k_{32} k_{21}$$

Substituting the above results into eqn. (12), we get:

$$v = \frac{e_0}{D} (k_{31} k_{12} k_{23} - k_{13} k_{32} k_{21})$$

Check. According to Fig. 2, we obtain from eqns. (16) and (17):

$$A(\mathcal{D}^{\text{I}3}) = \begin{vmatrix} 0 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & 1 & 0 \end{vmatrix}, \quad B(\mathcal{D}^{\text{I}3}) = \begin{vmatrix} 1 & -1 & 0 \\ -1 & 2 & 1 \\ -1 & -1 & 2 \end{vmatrix}$$

Then from eqn. (18) it follows that:

$$P_3^{\text{I}3} = \begin{vmatrix} 1 & -1 \\ -1 & 2 \end{vmatrix} = 1$$

Also, since $\mathcal{D}$ of Fig. 1 is a symmetric directed graph, we must have $P_3^{\text{I}3} = P_3^{\text{I}3} = 1$. This shows that none of sub-graphs was lost in the above calculation.

From the above example we learn that the first part of Rule 1 is actually the Wong-Hanes rule (Wong & Hanes, 1962; Wong, 1975), whereas the second part of Rule 1 furnishes a check method for

Fig. 2. Transformed graph $\mathcal{D}^{\text{I}3}$ obtained from Fig. 1 in accordance with Rule 1

the calculated results, which is quite useful in dealing with complicated reaction systems. Moreover, the derivation of Rule 1 at the same time has also given the mathematical proof of the Wong-Hanes rule, and this has not yet been given elsewhere in the literature, so far as we are aware.

Example 2. Consider an enzyme-catalysed reaction system as shown in Fig. 3 (Cornish-Bowden, 1976).

In order to calculate $N_6^{\text{I}6}$, we first transform $\mathcal{D}$ of Fig. 3 into $\mathcal{D}^{\text{I}6}$ of Fig. 4 according to Rule 1. Then,

Fig. 3. Directed graph $\mathcal{D}$ expressing the enzyme-catalysed reaction system in Examples 2 and 3

Fig. 4. Transformed graph $\mathcal{D}^{\text{I}6}$ obtained from Fig. 3 in accordance with Rule 1

from eqns. (16) and (17) we have:

$$A(\mathcal{D}^{16}) = \begin{bmatrix} 0 & 1 & 1 & 0 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 \\ 0 & 1 & 1 & 0 & 1 & 1 \\ 1 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 & 0 & 0 \end{bmatrix}, \quad B(\mathcal{D}^{16}) = \begin{bmatrix} 3 & -1 & -1 & 0 & -1 & 0 \\ -1 & 2 & 0 & -1 & 0 & 0 \\ -1 & 0 & 2 & -1 & 0 & 0 \\ 0 & -1 & -1 & 4 & -1 & -1 \\ -1 & 0 & 0 & -1 & 2 & 0 \\ -1 & 0 & 0 & -1 & 0 & 2 \end{bmatrix}$$

Then from eqn. (18) it follows that:

$$P_6^{16} = \begin{vmatrix} 3 & -1 & -1 & 0 & -1 \\ -1 & 2 & 0 & -1 & 0 \\ -1 & 0 & 2 & -1 & 0 \\ 0 & -1 & -1 & 4 & -1 \\ -1 & 0 & 0 & -1 & 2 \end{vmatrix} = 12$$

Moreover, since $\mathcal{D}$ in Fig. 3 is a symmetric directed graph, we have:

$$P_1^{61} = P_6^{16} = 12$$

which means that, if v is calculated in accordance with the Wong-Hanes rule, then 12 sub-graphs need to be counted in calculating N_6^{16} and N_1^{61} respectively (cf. eqn. 12). For such a reaction system, if calculated by the King-Altman method (cf. King & Altman, 1956; Cornish-Bowden, 1976), then 32 sub-graphs are required to be considered in calculating N_6 and N_1 respectively (Cornish-Bowden, 1976; Chou *et al.*, 1979). From this example we can see that it will save us from considering 40 sub-graphs that are due to be reciprocally cancelled. This is a great merit of the Wong-Hanes rule. However, even so, one still needs to discover 24 sub-graphs, which is by no means an easy job. And, for still more complicated reaction systems, the number of corresponding sub-graphs will be even more, increasing the size of the task and therefore the risk of errors in calculation. In such a case, we suggest the use of Rule 2 to make calculation.

Rule 2

(1) Draw a directed graph $\mathcal{D}$ expressing the enzyme-catalysed reaction system. Then eliminate the arc from E_β to E_α . The graph thus obtained is denoted by $\mathcal{D}^{\beta\alpha}$.

(2) Add a loop with weight $\sum_{i=1}^n k_{ii}$ to each point E_i ($i = 1, 2, \dots, n$) in $\mathcal{D}^{\beta\alpha}$, and alter the signs of the weights of all the arcs, i.e. change k_{ij} into $-k_{ij}$ ($i \neq j$).

(3) The new graph obtained by steps (1) and (2) is denoted by $\mathcal{D}^{\beta\alpha}$. Select any point E_q ($q \neq \alpha$) in $\mathcal{D}^{\beta\alpha}$ as a starting reference point, and we then have:

$$N_\alpha^{\beta\alpha} = (-1)^{n+1} \sum_i (-1)^{L_i^{\beta\alpha}} f(H_i^{\beta\alpha}) \quad (19)$$

Here $H_i^{\beta\alpha}$ is the i th of those sub-graphs in $\mathcal{D}^{\beta\alpha}$ each of which consists of one path from E_q to E_α , and all cycles that neither intersect with each other nor intersect with the path, and n is the number of the points in $\mathcal{D}^{\beta\alpha}$, $L_i^{\beta\alpha}$ the number of cycles in $H_i^{\beta\alpha}$, and $f(H_i^{\beta\alpha})$ denotes the product of the weights of all arcs in $H_i^{\beta\alpha}$.

(4) In order to facilitate checking, and avoid missing any sub-graph in the calculation, we can also calculate the number, $n_{q \rightarrow \alpha}^{\beta\alpha}$, of all $H_i^{\beta\alpha}$ in $\mathcal{D}^{\beta\alpha}$. The calculation method is as follows. Constructing the matrix $A(\mathcal{D}^{\beta\alpha})$ for graph $\mathcal{D}^{\beta\alpha}$ in accordance with eqn. (16), we then have:

$$n_{q \rightarrow \alpha}^{\beta\alpha} = \text{per } [A(\mathcal{D}^{\beta\alpha})]_{\alpha, q} \quad (20)$$

where $[A(\mathcal{D}^{\beta\alpha})]_{\alpha, q}$ denotes the sub-matrix obtained by removing the α th row and q th column, and per denotes taking such a value for a matrix that the result is a sum of the absolute values of all terms obtained by expanding the corresponding determinant. So it is very easy to carry out such an operation for a matrix defined by eqn. (16) where the elements are either 1 or 0.

Example 3. Now we apply Rule 2 to calculate v , the production rate of products, of the enzyme-catalysed reaction system illustrated in Fig. 3.

In accordance with steps (1) and (2) of Rule 2, transform $\mathcal{D}$ of Fig. 3 into $\mathcal{D}_+^{16}$ of Fig. 5. Then we have:

$$A(\mathcal{D}_+^{16}) = \begin{bmatrix} 1 & 1 & 1 & 0 & 1 & 0 \\ 1 & 1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 0 & 1 & 1 & 0 \\ 1 & 0 & 0 & 1 & 0 & 1 \end{bmatrix}$$

Taking E_1 as the starting reference point, we obtain:

$$n_{1 \rightarrow 6}^{16} = \text{per } [A(\mathcal{D}_+^{16})]_{6, 1} = \text{per } \begin{bmatrix} 1 & 1 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 & 0 \\ 0 & 1 & 1 & 0 & 0 \\ 1 & 1 & 1 & 1 & 1 \\ 0 & 0 & 1 & 1 & 0 \end{bmatrix} = 3$$

Fig. 5. Transformed graph $\mathcal{D}_+^{T6}$ obtained from Fig. 3 in accordance with Rule 2

This indicates that only three sub-graphs are sufficient if we use Rule 2 for the calculation. Because the number of sub-graphs decreases remarkably, the following is readily obtained from eqn. (19).

$$N_1^{T6} = k_{64} k_{42} k_{21} (k_{31} + k_{34}) (k_{51} + k_{54}) \\ + k_{64} k_{43} k_{31} (k_{21} + k_{24}) (k_{51} + k_{54}) \\ + k_{64} k_{45} k_{51} (k_{21} + k_{24}) (k_{31} + k_{34})$$

$$N_6^{T6} = - \left\{ \begin{array}{c} \text{Sub-graph 1: } E_1 \rightarrow E_2 \xrightarrow{-k_{12}} E_3 \xrightarrow{-k_{24}} E_4 \xrightarrow{-k_{46}} E_6 \xrightarrow{-k_{64}} E_1, \text{ with } E_5 \text{ as a separate node with self-loop } k_{51} + k_{54}. \end{array} \right\} \\ - \left\{ \begin{array}{c} \text{Sub-graph 2: } E_1 \rightarrow E_3 \xrightarrow{-k_{13}} E_4 \xrightarrow{-k_{34}} E_6 \xrightarrow{-k_{64}} E_1, \text{ with } E_2 \text{ as a separate node with self-loop } k_{21} + k_{24}. \end{array} \right\} \\ - \left\{ \begin{array}{c} \text{Sub-graph 3: } E_1 \rightarrow E_5 \xrightarrow{-k_{15}} E_6 \xrightarrow{-k_{64}} E_4 \xrightarrow{-k_{46}} E_1, \text{ with } E_2 \text{ as a separate node with self-loop } k_{21} + k_{24} \text{ and } E_3 \text{ as a separate node with self-loop } k_{31} + k_{34}. \end{array} \right\} \\ = k_{12} k_{24} k_{46} (k_{31} + k_{34}) (k_{51} + k_{54}) \\ + k_{13} k_{34} k_{46} (k_{21} + k_{24}) (k_{51} + k_{54}) \\ + k_{15} k_{54} k_{46} (k_{21} + k_{24}) (k_{31} + k_{34})$$

The similar method yields $n_{6 \rightarrow 1}^{T6} = 3$, which means that, if one takes E_6 as the starting reference point, then again three sub-graphs are sufficient for calculation of N_1^{T6} . In fact, when familiar with such an operation, we do not need to depict the transformed graph $\mathcal{D}_+^{T6}$ as well as the decomposed sub-graphs therein one by one, but rather directly write down the desired results according to graph $\mathcal{D}$. For instance, for N_1^{T6} , when taking E_6 as the starting reference point, we immediately have:

Substituting the calculated results into eqn. (12), we obtain:

$$v = \frac{e_0}{D} \left\{ k_{61} N_6^{T6} - k_{16} N_1^{T6} \right\} \\ = \frac{e_0}{D} \{ (k_{31} + k_{34}) (k_{51} + k_{54}) \\ \times (k_{61} k_{12} k_{24} k_{46} - k_{16} k_{64} k_{42} k_{21}) + (k_{21} + k_{24}) \\ \times (k_{51} + k_{54}) (k_{61} k_{13} k_{34} k_{46} - k_{16} k_{64} k_{43} k_{31}) \\ + (k_{21} + k_{24}) (k_{31} + k_{34}) (k_{61} k_{15} k_{54} k_{46} - k_{16} k_{64} k_{45} k_{51}) \}$$

From this example we can see that for some complicated reaction systems, which could hardly be treated by the general methods, one can easily obtain

the desired results if Rule 2 is applied for the calculation.

The present work was supported by the Chemical Center of Lund. The authors are much indebted to Professor J. T.-F. Wong and Dr. R. E. Carter for helpful discussion.

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