

# Calculation of Equilibrium Compositions of Systems of Enzyme-Catalyzed Reactions

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When a system of enzyme-catalyzed reactions does not involve H<sub>2</sub>O as a reactant, the equilibrium composition at specified temperature, pH, and ionic strength can be calculated using the Mathematica programs *equalcc*, which uses the conservation matrix, or *equalcrx*, which uses the stoichiometric number matrix. When H<sub>2</sub>O is involved as a reactant, *equalcrx* must be used because H<sub>2</sub>O is not in the stoichiometric number matrix. It is shown here that the use of *equalcrx* is equivalent to using the further transformed Gibbs energy  $G''$  that eliminates oxygen from the conservation matrix. Calculations presented here show that the calculation of further transformed Gibbs energies of formation of reactants other than coenzymes can be avoided by using *equalcrx* to calculate equilibrium concentrations.

## Introduction

When a system of enzyme-catalyzed reactions does not involve H<sub>2</sub>O as a reactant, the equilibrium composition at specified temperature, pH, and ionic strength can be calculated using the Mathematica programs *equalcc*, which involves the conservation matrix, and *equalcrx*, which involves the stoichiometric number matrix.<sup>1–5</sup> These programs are given in Appendix. But when H<sub>2</sub>O is a reactant in even one reaction in the system, it is necessary to use *equalcrx* because the conservation matrix is not consistent with the stoichiometric number matrix. This problem arises because [H<sub>2</sub>O] is not included in the expression for the apparent equilibrium constant. This article is about systems of enzyme-catalyzed reactions that involve H<sub>2</sub>O and may involve coenzymes.

The program *equalcrx* does three things: (1) It applies the Mathematica<sup>6</sup> operation *NullSpace* to the stoichiometric number matrix that omits H<sub>2</sub>O to derive a basis for a consistent conservation matrix. (2) It uses the Mathematica operation *LinearSolve* to calculate  $-(1/RT)$  times the vector of standard transformed Gibbs energies of reactants other than H<sub>2</sub>O. (3) Then it calls on the program *equalcc* to calculate the equilibrium composition using an iterative method. This article shows that this is equivalent to using a Legendre transformation to define a further transformed Gibbs energy  $G''$  that provides the criterion for spontaneous change and equilibrium when the availability of oxygen atoms is independent of the extent of the reaction.<sup>3–5</sup> The Legendre transform that defines the further transformed Gibbs energy  $G''$  is

$$G'' = G' - n_c(O) \Delta_f G'^{\circ}(H_2O) \quad (1)$$

$G'$  is the transformed Gibbs energy of the system at specified pH,  $n_c(O)$  is the amount of the oxygen component in the system, and  $\Delta_f G'^{\circ}(H_2O)$  is the standard transformed Gibbs energy of H<sub>2</sub>O at the specified temperature, pH, and ionic strength.

Since the computer programs *equalcc* and *equalcrx* refer to Gibbs energies of formation, equilibrium constants, conservation matrices, and stoichiometric number matrices, it is important to understand that the Gibbs energies of formation can be standard Gibbs energies of formation  $\Delta_f G^{\circ}$  of species, standard transformed Gibbs energies of formation  $\Delta_f G'^{\circ}$  of reactants

(sums of species) at specified pH, or standard further transformed Gibbs energies of formation  $\Delta_f G''^{\circ}$  of reactants at specified pH without conservation of oxygen atoms and coenzymes. Each of these Gibbs energies of formation comes with corresponding equilibrium constants  $K$ ,  $K'$ , and  $K''$ , conservation matrices  $A$ ,  $A'$ , and  $A''$ , and stoichiometric number matrices  $\nu$ ,  $\nu'$ , and  $\nu''$ . All of these Gibbs energies have partial derivatives that are familiar in chemical thermodynamics, but transformed and further transformed Gibbs energies have additional partial derivatives because they have more independent variables.

## Methods

**1. Calculations of Equilibrium Concentrations for a Reaction System Involving H<sub>2</sub>O Using *Equalcrx*.** As a first example, consider the calculation of the equilibrium composition when the following two enzyme-catalyzed reactions occur at 298.15 K, pH 7, and 0.25 M ionic strength:



Since these reactions involve H<sub>2</sub>O, the program *equalcc* cannot be used, but *equalcrx* can be used. This program requires the transposed stoichiometric number matrix (*nt*), a list of  $\ln K'$  values (*lnk*), and the initial composition vector (*no*).

The forms of reactions 2 and 3 that omit H<sub>2</sub>O and correspond with the expressions for the two apparent equilibrium constants are  $\text{ATP} = \text{ADP} + \text{P}_i$  and  $\text{glucose 6-phosphate} = \text{glucose} + \text{P}_i$ . The stoichiometric number matrix *nu3* for these reactions is shown in Table 1.

The input for *equalcrx* is the transposed stoichiometric number matrix, represented here by *nt3*. Given the standard Gibbs energies of formation of the species of the five reactants and H<sub>2</sub>O, the two apparent equilibrium constants can be calculated using *calckprime*.<sup>7</sup> The apparent equilibrium constants  $K'$  for reactions 2 and 3 at 298.15 K, pH 7, and 0.25 M ionic strength calculated using *data8*<sup>8</sup> in *BasicBiochemData3* are  $2.06 \times 10^6$  and 108, and so the input *lnk* for *equalcrx* is *lnkr3* = {14.53, 4.69}. Although H<sub>2</sub>O is not in the stoichiometric number

**TABLE 1: Stoichiometric Number Matrix nu3 for Reactions 2 and 3**

reactant	eq 2	eq 3	reactant	eq 2	eq 3
ATP	-1	0	P <sub>i</sub>	1	1
ADP	1	0	glucose 6-phosphate	0	-1
glucose	0	1			

matrix,  $\Delta_f G^\circ(\text{H}_2\text{O})$  is involved in the calculation of the apparent equilibrium constants  $K'$ .

In this calculation of the equilibrium composition, it is assumed that the five reactants are initially all at 1 mM so that the initial composition vector is  $\text{no3} = \{0.001, 0.001, 0.001, 0.001, 0.001\}$ . The equilibrium composition for the two-reaction system obtained by typing `equalcrx[nt3,lnkr3,no3]` into Mathematica is  $\{2.92 \times 10^{-12}, 0.002, 0.002, 0.003, 5.54 \times 10^{-8}\}$ , where the reactants are in the same order as in Table 1. These calculated equilibrium concentrations can be tested by using them to calculate the two apparent equilibrium constants. The vector of apparent equilibrium constants  $K'$  can be calculated using the vector equation

$$K' = \exp[\text{ln}c \cdot \text{nu}'] \quad (4)$$

where **ln**c is the vector of natural logarithms of equilibrium concentrations, **nu'** is the stoichiometric number matrix for reactions 2 and 3 when water is omitted, and the dot indicates the dot product. The two apparent equilibrium constants calculated from the equilibrium composition using eq 4 agree with the input data, and so this validates the calculated equilibrium composition.

**2. Since Equalcrx Uses Equalcc, It Is of Interest To See How the Input for Equalcc Is Calculated.** The first line of `equalcrx` calculates  $-(1/RT)$  times the vector of standard transformed Gibbs energies of formation (that is, **ln**k in `equalcc`) by setting up linear equations relating  $-(1/RT)\Delta_f G^\circ(i)$  for reactants to  $\ln K'$  for reactions. This is an underdetermined set of linear equations (two linear equations and five unknowns), but the two linear equations have solutions, and `LinearSolve` finds one.

`LinearSolve[Transpose[nt3],lnkr3]` yields the following solution that is referred to as **lnkLS**:

$$\text{lnkLS} = \{-4.87, 4.87, -0.600, 4.81, 0.0600\} \quad (5)$$

where the reactants are in the same order as in Table 1. These values can be checked by using them to calculate the vector **ln**k using the following dot product:

$$\text{lnkLS} \cdot \text{nu3} = \{14.53, 4.69\} \quad (6)$$

These values are as expected for  $\ln K_2'$  and  $\ln K_3'$ , as mentioned in the preceding section. The values given in eq 5 will be interpreted in the next section.

The second line of `equalcrx` uses the Mathematica operation `NullSpace` to calculate a basis for the conservation matrix **A''** from the stoichiometric number matrix **v''** in Table 1. The symbol **A''** is used for this conservation matrix since  $\text{H}_2\text{O}$  is not included in the stoichiometric number matrix or the conservation matrix. Since `NullSpace` yields a basis for the conservation matrix, it is row reduced to facilitate comparison with other conservation matrices. This row reduced conservation matrix is given in Table 2.

The last two columns in Table 2 indicate the following two reactions:  $\text{P}_i + \text{ADP} = \text{ATP}$  and  $\text{glucose 6-phosphate} + \text{ADP} = \text{ATP} + \text{glucose}$ . These are not the same as eqs 2 and 3, but they can represent the system of two reactions. In calculating

**TABLE 2: Row Reduced Conservation Matrix A'' Calculated from the Stoichiometric Number Matrix in Table 1**

reactant	ATP	ADP	glucose	P <sub>i</sub>	glucose 6-phosphate
ATP	1	0	0	1	1
ADP	0	1	0	-1	-1
glucose	0	0	1	0	1

equilibrium compositions in multi-reaction systems, it is important to remember that when a chemical reaction system is at equilibrium, each of the independent reactions used in the equilibrium calculation is at equilibrium, but so are the infinite number of reactions that can be obtained by adding and subtracting the independent reactions or by multiplying reactions by integers and adding and subtracting them. This statement also applies to enzyme-catalyzed reactions even though there may not be known enzymes for these reactions. This means that when some reactions in the system have to occur two, three, and so forth times when other reactions occur only once, these reactions do not have to be multiplied by 2, 3, and so forth in the stoichiometric number matrix, as is necessary in calculating the net reaction. It is not possible to obtain a correct conservation matrix by counting atoms of C, O, and P because oxygen atoms are not conserved in the reaction system, as discussed in the next section. The components in the conservation matrix in Table 2 are ATP, ADP, and glucose. When a biochemical reaction system is considered from the point of view of  $G''$ , the number of components  $C''$ , number of independent reactions  $R''$ , and number of reactants  $N''$  are related by  $N'' = C'' + R''$ .

**3. Calculation of the Equilibrium Composition for the System of Two Reactions Using Equalcc.** There is a way to use `equalcc` to calculate the equilibrium composition for the reaction system consisting of reactions 2 and 3 without using `equalcrx`. That is to use a Legendre transform to define a further transformed Gibbs energy  $G''$  that eliminates the conservation equation for oxygen atoms. This is like the use of the Legendre transform defining  $G'$  to eliminate the conservation equation for hydrogen atoms. Actually, oxygen atoms are conserved, but the supply of oxygen atoms in the solvent is many orders of magnitude larger than the requirements for the enzyme-catalyzed reactions so that  $[\text{H}_2\text{O}]$  is omitted in the expressions for the apparent equilibrium constants. The fact that  $[\text{H}_2\text{O}]$  is omitted means that there is no conservation equation for oxygen atoms. The further transformed Gibbs energy  $G''$  that provides the criterion for spontaneous change and equilibrium when the availability of oxygen atoms is independent of the extent of reaction is defined by the Legendre transform<sup>3</sup> in eq 1. This leads to the following expression for the standard further transformed Gibbs energy of reactant  $i$ :

$$\Delta_f G''(i) = \Delta_f G^\circ(i) - N_{\text{O}}(i) \Delta_f G^\circ(\text{H}_2\text{O}) \quad (7)$$

$N_{\text{O}}(i)$  is the number of oxygen atoms in reactant  $i$ . Note that  $\Delta_f G''(\text{H}_2\text{O}) = 0$ , and so  $\text{H}_2\text{O}$  does not count as a reactant.

The standard further transformed Gibbs energies of formation  $\Delta_f G''(i)$  of the five reactants at 298.15 K and 0.25 M ionic strength are calculated using eq 7. The calculations, here, are carried out using  $\text{kJ mol}^{-1}$ . These calculations are summarized in Table 3.

In `equalcc` the last row in Table 3 is referred to as **ln**k and is referred to here as **lnkfrans**. These many digits are required to preserve equilibrium constants determined to 1% accuracy. It is important to remember that the thermodynamic information about reactions in this type of table is in the differences between values, not in the absolute values.

**TABLE 3: Thermodynamic Properties of Reactants at 298.15 K, pH 7, and 0.25 M Ionic Strength<sup>a</sup>**

	ATP	ADP	glucose	P <sub>i</sub>	glucose 6-phosphate
$\Delta_f G'^{\circ}(i)$	-2292.50	-1424.70	-426.71	-1059.49	-1318.92
$\Delta_f G''^{\circ}(i)$	-268.92	131.90	507.25	-436.85	82.02
$-(1/RT)\Delta_f G''^{\circ}(i)$	108.49	-53.21	-204.62	176.22	-33.09

<sup>a</sup> The units in the first two rows are kJ mol<sup>-1</sup>. The property in the third row is dimensionless.  $\Delta_f G''^{\circ}(\text{H}_2\text{O})$  at 298.15 K, pH 7, and 0.25 M ionic strength is 155.66 kJ mol<sup>-1</sup>.

The dot product of the vector of values of  $\Delta_f G''^{\circ}(i)$  in Table 3 with the stoichiometric number matrix nu3 yields the  $\Delta_f G''^{\circ}$  values for the two enzyme-catalyzed reactions:

$$\text{df}G'' \cdot \text{nu3} = \{-36.03, -11.62\} \quad (8)$$

These are the values that are expected because  $K'' = K'$  when the Legendre transform in eq 1 is used.

The first input for equalccc is the conservation matrix **as**. There is a question here because oxygen atoms are not conserved, and so there is not an oxygen row in the conservation matrix. But the conservation matrix must be  $3 \times 5$  rather than  $2 \times 5$ . The conservation matrix can be calculated from the stoichiometric number matrix nu3 given in Table 1. This table shows that the three components can be taken to be ATP, ADP, and glucose. Since

$$(\nu'')^T (\mathbf{A}'')^T = 0 \quad (9)$$

the conservation matrix  $\mathbf{A}''$  calculated by applying NullSpace to Transpose[nu3] can be used for **as**, or its row reduced form in Table 2 can be used. When  $G''$  is used, the equilibrium concentrations are calculated using

$$\text{equalccc}[\text{NullSpace}[\text{Transpose}[\text{nu3}], \text{lnkfrans}, \text{no3}] \quad (10)$$

This yields  $\{2.92 \times 10^{-12}, 0.002, 0.002, 0.003, 5.53 \times 10^{-8}\}$ . This is the same equilibrium composition that was calculated in section 1 using equalcrx.

It is interesting to compare the two vectors lnkLS (eq 5) and lnkfrans (last row in Table 3) which might be expected to be the same. These vectors are  $\text{lnkLS} = \{-4.87, 4.87, -0.0600, 4.81, 0.0600\}$  and  $\text{lnkfrans} = \{108.48, -53.21, -204.62, 176.22, -33.09\}$ . These vectors are equivalent in the sense that when they are multiplied by the stoichiometric number matrix nu3, they both yield the same  $\text{lnkr} = -(1/RT) \ln\{2.06 \times 10^6, 108.\}$ . This means that when H<sub>2</sub>O is involved in a system of reactions, the use of equalccc with further transformed Gibbs energies of formation is equivalent to using equalcrx with apparent equilibrium constants. For larger systems of reactions, it is much easier to use equalcrx, and so this approach is used for glycolysis in the next section.

**4. Calculation of Equilibrium Concentrations for Glycolysis at Steady-State Concentrations of Coenzymes Using Equalcrx.** A more global view of the thermodynamics of systems of enzyme-catalyzed reactions can be obtained by use of a Legendre transform to define a further transformed Gibbs energy  $G''$  at specified concentrations of coenzymes.<sup>9–11</sup> This is of interest because coenzymes are involved in many reactions and tend to be in steady states. The further transformed Gibbs energy  $G''$  at specified concentrations of coenzymes that provides the criterion for spontaneous change and equilibrium is defined by

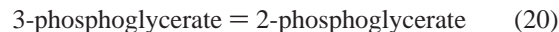
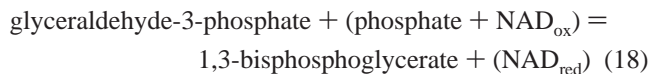
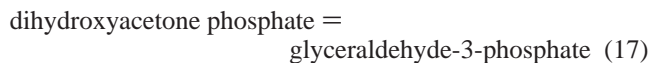
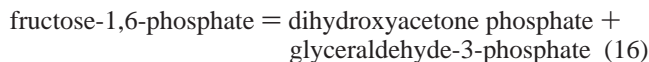
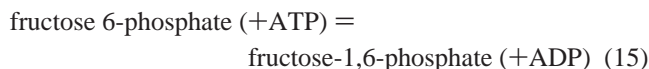
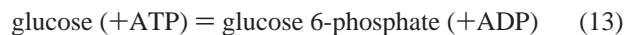
$$G'' = G' - \sum n_c(\text{coenz}) \mu'(\text{coenz}) \quad (11)$$

where  $n_c(\text{coenz})$  is the amount of a component for a coenzyme and  $\mu'(\text{coenz})$  is the transformed chemical potential of the coenzyme at its specified concentration and a specified pH. This leads to the following equation for the standard further transformed Gibbs energies of formation of reactants other than coenzymes:

$$\Delta_f G''^{\circ} = \Delta_f G'^{\circ} - \sum N_{\text{coenz}} (\Delta_f G'^{\circ}(\text{coenz}) + RT \ln[\text{coenz}]) \quad (12)$$

where  $N_{\text{coenz}}$  is the number of coenzyme components in a reactant. Row reduction of the conservation matrix for a system of enzyme-catalyzed reactions at specified pH provides the means for obtaining  $N_{\text{coenz}}$  for each coenzyme in each reactant. The use of eq 12 has made it possible<sup>10</sup> to use equalccc to calculate the equilibrium composition for the 10 reactions of glycolysis at specified concentrations of ATP, ADP, P<sub>i</sub>, NAD<sub>ox</sub>, and NAD<sub>red</sub>. An essential step in making this calculation is the identification of  $N_{\text{ATP}}(i)$ ,  $N_{\text{ADP}}(i)$ ,  $N_{\text{P}_i}(i)$ ,  $N_{\text{NAD}_{\text{ox}}}(i)$ , and  $N_{\text{NAD}_{\text{red}}}(i)$  for each of the 11 reactants in glycolysis.

The first part of this article has shown that the equilibrium composition can be calculated for a system of enzyme-catalyzed reactions more easily by using the apparent equilibrium constants of the reactions in a system of reactions. For glycolysis at specified concentrations of coenzymes, the enzyme-catalyzed reactions are represented by



In these reactions, the reactants ATP, ADP, P<sub>i</sub>, NAD<sub>ox</sub>, and NAD<sub>red</sub> have been put in parentheses because their steady-state concentrations are going to be specified. H<sub>2</sub>O has also been put in parentheses because its concentration is not given in expressions for apparent equilibrium constants. The net reaction (glucose = two pyruvate) is obtained by adding these 10 reactions with the last 5 reactions multiplied by 2. The stoichiometric matrix without the coenzymes and H<sub>2</sub>O is named nu6. This leads to the stoichiometric number matrix nu6 in Table 4. The transformed stoichiometric number matrix is named tnu6.

The apparent equilibrium constants for reactions 13–22 are calculated in a two-step process: (1)  $K'$  is calculated using<sup>8</sup> BasicBiochemData3. The apparent equilibrium constants for these 10 reactions at 298.15 K, pH 5, 6, 7, 8, and 9, and 0.25



**TABLE 4: Stoichiometric Number Matrix (nu6) for Glycolysis at Specified Concentrations of Coenzymes**

	13	14	15	16	17	18	19	20	21	22
glucose	-1	0	0	0	0	0	0	0	0	0
glucose 6-phosphate	1	-1	0	0	0	0	0	0	0	0
fructose 6-phosphate	0	1	-1	0	0	0	0	0	0	0
fructose-1,6-phosphate	0	0	1	-1	0	0	0	0	0	0
dihydroxyacetone phosphate	0	0	0	1	-1	0	0	0	0	0
glyceraldehyde-3-phosphate	0	0	0	1	1	-1	0	0	0	0
1,3-bisphosphoglycerate	0	0	0	0	0	1	-1	0	0	0
3-phosphoglycerate	0	0	0	0	0	0	0	1	-1	0
2-phosphoglycerate	0	0	0	0	0	0	0	1	-1	0
phosphoenolpyruvate	0	0	0	0	0	0	0	0	1	-1
pyruvate	0	0	0	0	0	0	0	0	0	1

**TABLE 5: Apparent Equilibrium Constants  $K''$  and Their Natural Logarithms at Specified Concentrations of Coenzymes**

reaction	[NAD <sub>red</sub> ] = 10 <sup>-6</sup> M $K''$	[NAD <sub>red</sub> ] = 10 <sup>-6</sup> M ln $K''$	[NAD <sub>red</sub> ] = 10 <sup>-3</sup> M $K''$	[NAD <sub>red</sub> ] = 10 <sup>-3</sup> M ln $K''$
13	1.90 × 10 <sup>5</sup>	9.85	1.90 × 10 <sup>5</sup>	9.85
14	2.76 × 10 <sup>-1</sup>	-1.29	2.76 × 10 <sup>-1</sup>	-1.29
15	1.18 × 10 <sup>4</sup>	9.38	1.18 × 10 <sup>4</sup>	9.38
16	9.23 × 10 <sup>-5</sup>	-9.29	9.23 × 10 <sup>-5</sup>	-9.29
17	4.55 × 10 <sup>-2</sup>	-3.09	4.55 × 10 <sup>-2</sup>	-3.09
18	6.12 × 10 <sup>-1</sup>	-0.491	6.12 × 10 <sup>-1</sup>	-0.491
19	2.76 × 10	3.32	2.76 × 10	3.32
20	9.11 × 10 <sup>-2</sup>	-2.40	9.11 × 10 <sup>-2</sup>	-2.40
21	4.27	1.45	4.27	1.45
22	1.13 × 10 <sup>5</sup>	11.66	1.13 × 10 <sup>5</sup>	11.66

M ionic strength are available in Thermodynamics of Biochemical Reactions.<sup>4</sup> (2) Specified concentrations of coenzymes are substituted in the expressions for  $K'$  to obtain values of  $K''$ .

The apparent equilibrium constants  $K'$  for the 10 reactions of glycolysis at 298.15 K, pH 7, and 0.25 M ionic strength in Biochemical Thermodynamics<sup>4</sup> are adjusted by substituting the specified [ATP] = [ADP] = [phosphate] = 10<sup>-3</sup> M, [NAD<sub>ox</sub>] = 10<sup>-3</sup> M, and [NAD<sub>red</sub>] = 10<sup>-6</sup> M to obtain the corresponding  $K''$  for the first calculation of the equilibrium composition. This is illustrated for reaction 13 by  $K' = ([\text{glucose 6-phosphate}]/10^{-3})/([\text{glucose}]/10^{-3})$  so that  $K'' = [\text{glucose 6-phosphate}]/[\text{glucose}]$ . The vector of ln  $K''$  values for the first calculation is referred to as lnk6. In a second calculation of the equilibrium concentrations, [NAD<sub>red</sub>] is taken to be 10<sup>-3</sup> M. The vector of ln  $K''$  values for the second calculation is referred to as lnk7. The values of these apparent equilibrium constants and their natural logarithms are given in Table 5.

In the calculation of the equilibrium concentrations, it is assumed that only glucose is initially present at 0.01 M. The equilibrium concentrations calculated using equalcrx[tnu6, lnk6, no6] and equalcrx[tnu6, lnk7, no6] are given in Table 6.

Notice that raising [NAD<sub>red</sub>] raises all of the equilibrium concentrations up to GAP. These equilibrium concentrations have been verified by using eq 4.

## Discussion

When even one reaction in a system of enzyme-catalyzed reactions involves H<sub>2</sub>O, equalcc cannot be used to calculate the equilibrium composition with the transformed Gibbs energy  $G'$  because the conservation matrix and stoichiometric number matrix are inconsistent. In section 1 (Methods), we show that equalcrx can be used, and section 2 (Methods) shows how this is accomplished. Section 3 (Methods) explains that if the further transformed Gibbs energy  $G''$  is used rather than the transformed Gibbs energy  $G'$ , then equalcc can be used to calculate the

**TABLE 6: Equilibrium Concentrations in Glycolysis at 298.15 K, pH 7, and Ionic Strength 0.25 M When Glucose Is Initially Present at 0.01 M**

reactant	equilibrium concn, M [NAD <sub>red</sub> ] = 10 <sup>-6</sup> M	equilibrium concn, M [NAD <sub>red</sub> ] = 10 <sup>-3</sup> M
glucose	2.79 × 10 <sup>-18</sup>	2.54 × 10 <sup>-12</sup>
glucose 6-phosphate	5.29 × 10 <sup>-14</sup>	4.82 × 10 <sup>-8</sup>
fructose 6-phosphate	1.46 × 10 <sup>-14</sup>	1.33 × 10 <sup>-8</sup>
fructose 1,6-bisphosphate	1.46 × 10 <sup>-14</sup>	1.57 × 10 <sup>-4</sup>
dihydroxyacetone phosphate	5.91 × 10 <sup>-7</sup>	5.65 × 10 <sup>-4</sup>
glyceraldehyde-3-phosphate	2.69 × 10 <sup>-8</sup>	2.57 × 10 <sup>-5</sup>
1,3-bisphosphoglycerate	1.65 × 10 <sup>-8</sup>	1.57 × 10 <sup>-8</sup>
3-phosphoglycerate	4.54 × 10 <sup>-7</sup>	4.34 × 10 <sup>-7</sup>
2-phosphoglycerate	4.14 × 10 <sup>-8</sup>	3.95 × 10 <sup>-8</sup>
phosphoenolpyruvate	1.77 × 10 <sup>-7</sup>	1.69 × 10 <sup>-7</sup>
pyruvate	2.00 × 10 <sup>-2</sup>	0.0191

equilibrium composition. The further transformed Gibbs energy  $G''$  that provides the criterion for spontaneous change and equilibrium at specified concentrations of coenzymes has been used earlier<sup>10</sup> to calculate equilibrium compositions for the 10 reactions of glycolysis using equalcc, but that involves some linear algebra in identifying the amounts of components (coenzymes) in the reactants. In section 4 (Methods), a new way to calculate equilibrium compositions of systems of enzyme-catalyzed reactions at specified concentrations of coenzymes is presented which avoids these complications with components. This method uses equalcrx. Once the basic equations are in the computer, it is easy to vary the pH, ionic strength, specified concentrations of coenzymes, and initial composition.

These calculations have been made for dilute aqueous solutions, but enzyme-catalyzed reactions can also occur in nonaqueous solvents. In this case, the concentration of H<sub>2</sub>O is not a constant, and the activity of H<sub>2</sub>O has to be treated differently.

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## Appendix

```

equalcc[as_,lnk_,no_] := Module[{l,x,b,ac,m,n,e,k},
(*as=conservation matrix
lnk=-(1/RT)(Gibbs energy of formation vector at T)
no=initial composition vector *)
(*Setup*)
{m,n}=Dimensions[as];
b=as.no;
ac=as;
(*Initialize*)
l=LinearSolve[ as.Transpose[as],-as.(lnk+Log[n]) ];
(*Solve*)
Do[e=b-ac.(x = E^(lnk+l.as));
If[(10^-10)>Max[Abs[e] ], Break[] ];
l=l+LinearSolve[ac.Transpose[as*Table[x,{m}]],e],{-
{k,100}];
If[k = 100,Return["Algorithm Failed"] ];
Return[x]
]
equalcrx[nt_,lnkr_,no_] := Module[{as,lnk},
(*nt=transposed stoichiometric number matrix
lnkr=ln of equilibrium constants of rxns (vector)
no=initial composition vector*)
(*Setup*)

```

```
lnk=LinearSolve[nt,lnkr];  
as=NullSpace[nt];  
equcalcc[as,lnk,no]
```

## References and Notes

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