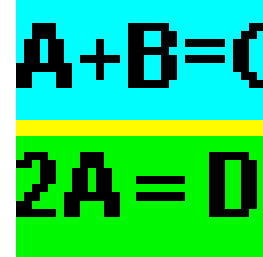
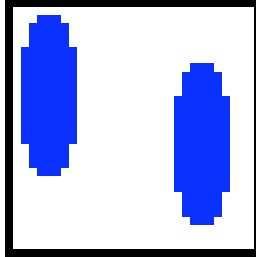


Chemical reactions in **SedAnal** and **Model editor**



- **SedAnal** is designed to handle arbitrary chemical reaction schemes in conjunction with sedimentation analysis.
- User specifies the reaction scheme using **Model editor**, which maintains a file `ModelInfo.txt`.
- A reaction scheme includes the number of species, number of reactions, and their stoichiometry.
- **Model editor** also specifies relations among species and reaction parameters.

- All kinetics in **SedAnal** are computed using the law of mass action; i.e., reaction rate is proportional to product of concentrations.

Example: $2A = A_2$

$$\text{rate} = dA_2/dt = \text{forward rate} - \text{reverse rate} = k_f(A)^2 - k_r(A_2) \quad K = k_f / k_r$$

Either the kinetic constants k_f and k_r or the equilibrium constant K are specified by the user on the control screen.

- **SedAnal** assumes the reaction rates are determined by the stoichiometry; this is unlikely to be true unless the reaction scheme expresses the mechanism, because the mechanism is a series of elementary steps that correspond to the physical interactions of molecules in solution.

Example: $3A = A_3$

or

$2A = A_2$

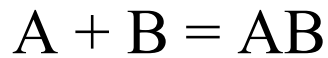
$A + A_2 = A_3$

$$\text{rate} = dA_3/dt = k_f(A)^3 - k_r(A_3)$$

$$\text{rate} = dA_3/dt = k_f(A)(A_2) - k_r(A_3)$$

Example: 6 species, 4 reactions

4 Number of reactions 6 Number of species

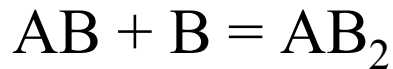


Species: A, B, AB, A₂B, AB₂, A₃

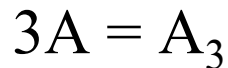


Some of the constraints:

$$M_{AB} = M_A + M_B$$



$$M_{AB_2} = M_{AB} + M_B$$



$$M_{A_2B} = M_{AB} + M_A$$

$$M_{A_3} = 3M_A$$

Species 1	Species 2	Species 3	Species 4	Species 5	Species 6	Species 7	
A	B	AB	A2B	AB2	A3		
-1	-1	1	0	0	0	0	A + B = AB
-1	0	-1	1	0	0	0	A + AB = A2B
0	-1	-1	0	1	0	0	B + AB = AB2
-3	0	0	0	0	1	0	3A = A3

Stoichiometry
in Model editor

Species 1	Species 2	Species 3	Species 4	Species 5	Species 6
A2B					
Molecular mass (g/mole) <input type="checkbox"/>					
Calculate as					
Default is fit					
Sum <input checked="" type="checkbox"/> MW-wtd avg <input type="checkbox"/> Copy <input type="checkbox"/>					

Constraint on molar mass in Model editor

Sum means
$$p_j = (1/v_j) \sum_{v_i \cdot v_j < 0} v_i p_i$$

MW-wtd avg means
$$p_j = \sum_{v_i \cdot v_j < 0} M_i p_i / \sum_{v_i \cdot v_j < 0} M_i$$

Kinetics and equilibria in SedAnal

Kinds of concentrations

- **Loading concentration:** Nominal concentrations based on how much material was added to the cell.
- **Initial concentration:** The concentrations after all species have reached equilibrium before the start of the run.
- **Instantaneous concentration:** Species concentrations during a run, $c_i(r, t)$, dependent on both position and time. This is what is observed during a velocity run.
- **Equilibrium concentration:** Species concentrations after sedimentation, diffusion and chemical kinetics have stopped changing, $c_i(r)$, dependent on position only. This is the only data taken for an equilibrium run; for a sedimentation velocity run, it will be data taken after the time required to reach complete equilibrium.

Example

Iter	(A), mol/L	(B), mol/L	(AB), mol/L	(A2B), mol/L	(AB2), mol/L	(A3), mol/L
1	1.00000000E-05	3.00000000E-05	3.00000000E-05	3.00000000E-05	9.00000000E-05	1.00000000E-05
7...	1.56844122E-06	1.73760799E-05	2.72533599E-06	4.27452931E-07	4.73556559E-06	3.85837773E-08

The first line above shows *loading* concentrations (components only). The last (highlighted) line shows the *initial* concentrations (all species), the equilibrium before the start of the run.

Equilibrium constants from control screen		Keq
A + B = AB		100000
A + AB = A2B		100000
B + AB = AB2		100000
3A = A3		1e10

calculated K1	calculated K2	calculated K3	calculated K4
1.00000000E+05	1.00000000E+05	1.00000000E+05	1.00000000E+10
1.00000000E+05	1.00000000E+05	1.00000000E+05	1.00000000E+10

These calculations were done by SedAnal's equilibrium calculator. As a check, the equilibrium constants computed from the equilibrium concentrations are shown.

Components in SedAnal

SedAnal assumes you load the cell with only some of the species, the *components*.

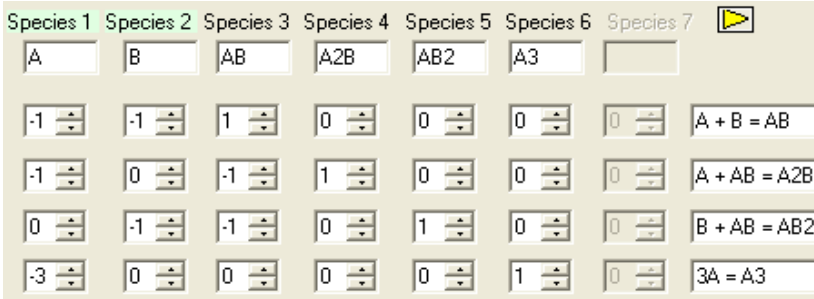
In thermodynamics, a component is an independent species. The composition of any system at equilibrium can be completely specified by the concentrations of its components.

The number of components $n_c = n_s - n_r$ in a single-phase system (n_s = number of species; n_r = number of independent chemical reactions). At constant T and p , n_c is the number of degrees of freedom of the system.

Any choice of equilibrium concentrations can be attained by starting with component species only (loading concentrations of non-components zero).

In **SedAnal**, components are chosen by the program. There is often more than one way to choose the component species; all are equivalent mathematically, but some are more intuitive. **SedAnal** tries to find the choice that makes most sense to humans.

The green tint indicates Model editor has chosen A and B as the components (independent species)



Species 1	Species 2	Species 3	Species 4	Species 5	Species 6	Species 7	
A	B	AB	A2B	AB2	A3		
-1	-1	1	0	0	0	0	A + B = AB
-1	0	-1	1	0	0	0	A + AB = A2B
0	-1	-1	0	1	0	0	B + AB = AB2
-3	0	0	0	0	1	0	3A = A3

Here, any pair of species except A and A₃ could have been chosen as the component species.

More on components

The simplest systems have a single component:

1. A (a single species)
2. $A = A_2$ (monomer-dimer)
3. $A = A_3 = A_6$ (monomer-trimer-hexamer)
4. $A + A = A_2$ $A + A_2 = A_3$ $A + A_3 = A_4$ $A + A_4 = A_5$... (indefinite self-association)

We can write as many *conservation-of-mass relations* as there are components.

Examples of conservation relations in homogeneous solution, for the examples above:

1. $(A) = A_0$ () indicate concentrations in mol/L
2. $(A) + 2(A_2) = A_0$ A_0 is the loading concentration of A, etc
3. $(A) + 3(A_3) + 6(A_6) = A_0$
4. $(A) + 2(A_2) + 3(A_3) + 4(A_4) + 5(A_5) + \dots = A_0$

Model editor chooses the components and stores them in the file `ModelInfo.txt`. **SedAnal** uses this choice of components when it asks you for loading concentrations on the control screen.

Model editor screen showing components chosen as **A** and **B**

Species 1	Species 2	Species 3	Species 4	Species 5	Species 6	Species 7	
A	B	AB	A2B	AB2	A3		
-1	-1	1	0	0	0	0	A + B = AB
-1	0	-1	1	0	0	0	A + AB = A2B
0	-1	-1	0	1	0	0	B + AB = AB2
-3	0	0	0	0	1	0	3A = A3

```

"A+B=AB=A2B;AB2 A3"           // name of the model
2008-5-22 15:35:52           // date/time last stored
 4 6 4 3 2                   // reactions, species, params/species (vel), params/species (eq), components
 1 2                           // species numbers of components
...
// Equilibrium information
Cons 1    0 1/2 1/2 1/2    1    0    [ i e, (B) + (AB) + (A2B) + 2(AB2) = constant ]
Cons 2   1/3 -1/6 1/6 1/2    0    1    [ i e, 2(A) - (B) + (AB) + 3(A2B) + 6(A3) = constant ]
  
```

Component information stored in the file `ModelInfo.txt` by **Model editor**

Loading concentration input on the **SedAnal** control screen

The image shows several screenshots of the SedAnal control screen. The top-left screenshot shows a table for molecular mass (g/mole) for species A (1e4) and B (2e4). The middle-left screenshot shows input fields for 'Loading conc of A, mol/L' (1e-5) and 'Loading conc Mratio B/A' (3). A tooltip below it shows the conversion: '3e-5 mol/L = 0.6 g/L 85.7143% by weight'. The top-right and bottom-right screenshots show input fields for 'Loading conc of A, g/L' (0.1) and 'Loading conc of B, mol/L' (0.0003) or 'g/L' (6). The middle-right screenshot shows 'Loading conc of A, g/L' (0.1) and 'Loading conc Wratio B/A' (6). Colored squares (yellow, red, blue, green) are present next to the input fields to indicate unit selection.

By clicking the colored squares, the input units can be changed. These are all equivalent. "Wratio" is the ratio of weight concentrations.

Original input: (A) in units of mol/L, and (B) given as the ratio of molar concentrations. The tooltip shows the conversions to other units.

Kinetics

- **SedAnal** can deal with either rapid equilibria or slow reactions. “Rapid” and “slow” are with respect to the sedimentation time.
- **SedAnal** always assumes a sedimentation velocity experiment starts at chemical equilibrium.
- **SedAnal** allows input of either K_{eq} or any pair from (K_{eq}, k_f, k_r) .
 - If K_{eq} only, a user-specified value for k_r is assumed (default=0.01; settable in Preferences).
 - If K_{eq} and one rate constant, the other is calculated using $K_{eq} = k_f / k_r$.
- Analytic solutions are available for some reaction schemes; others require numerical methods. User can tell **SedAnal** which method to use (useful for some difficult cases).
- Tools built into **SedAnal** for understanding the kinetics and equilibria for specific experiments include

- Equilibrium calculator (main menu)



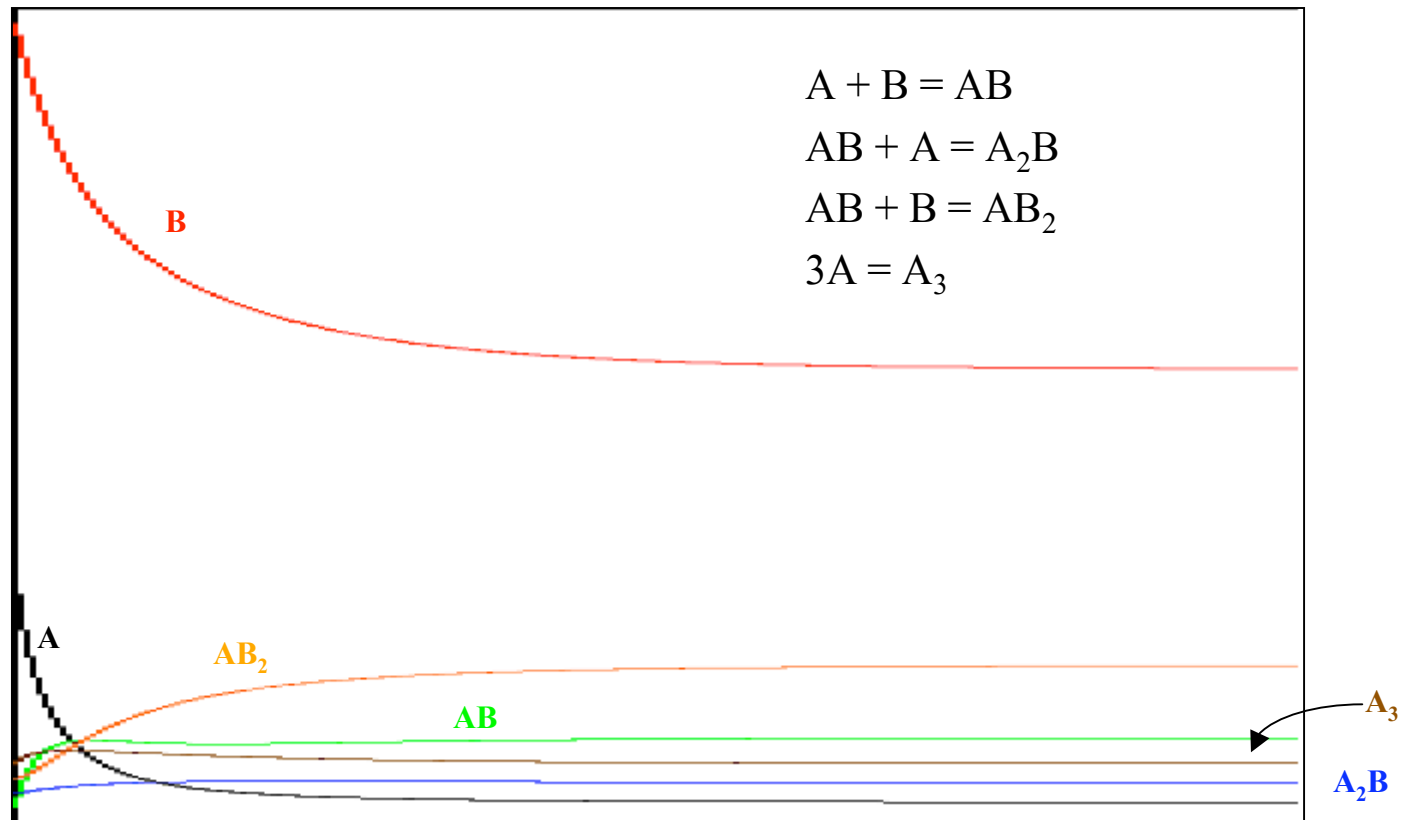
- Kinetic calculator (main menu)



- Concentration profiles (control screen/Outputs/conc(r))
- Signal profiles (control screen/Outputs/Minima)

- Allow enough time for solution to equilibrate after loading the cells.

If the reverse rate constants were 0.001 instead of 0.01, and the equilibrium constants the same, time-to-equilibrium would be about 50,000 sec instead of 5000 sec.



Output from Kinetic calculator for initial ~ 500 sec, for $A_0=10^{-5}$, $B_0=3 \cdot 10^{-5}$ mol/L; $K_1=K_2=K_3=10^5$ L/mol, $K_4=10^{10}$ (L/mol)², $k_r=0.01$. Equilibrium to 1 part in 10^6 is achieved in about 5000 sec for these values.

- Check expected results by simulation.

When you are designing an experiment, you can estimate all the parameters, and see what the results of the experiment should look like. From these simulation results, you may decide to use different concentrations, wavelengths, loading concentrations, etc.