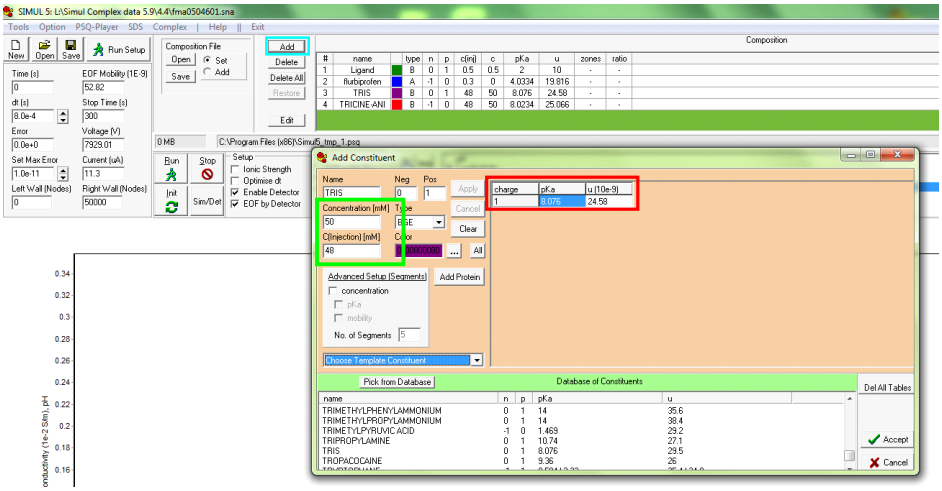
Simul 5 Complex ï Instructions

Complexation mode of the Simul 5 enables to simulate electrophoretical separations in systems with complexation agents (further ligands), e.g., enantioseparation in electrophoresis. The following text describes the setup for simulations using the Complexation mode of Simul 5. The general setup is the same as in the previous version of the Simul 5, so the instructions concerning the general setup are mentioned just briefly here (for more details see the Simul 5 manual). Thus, the following text is focused mainly on the detailed setting of the Complexation mode parameters.

1. Constituents:
Constituents table can be opened by clicking on the button "Add" in the Main window.
Necessary input data:

- Concentrations of all compounds in the BGE ("Concentration [mM]") and concentrations of all compounds in the sample zone ("C(Injection) [mM]")
- pKa values ("pK_a") of all constituents
- Limit mobilities ("u [$10^{ï 9}$ $m^2V^{ï 1}s^{ï 1}$]") of all constituents



Simul 5 offers the database of constituents, based on the Hirokawa's tables, which contains the data of many ions (pKa values and limit mobilities). So, in many cases the compounds of interest can be easily selected from this database and only concentrations of constituents must be added.

The complexation mode requires that the ligand is entered as the first constituent in the constituents list. Only one ligand is allowed.

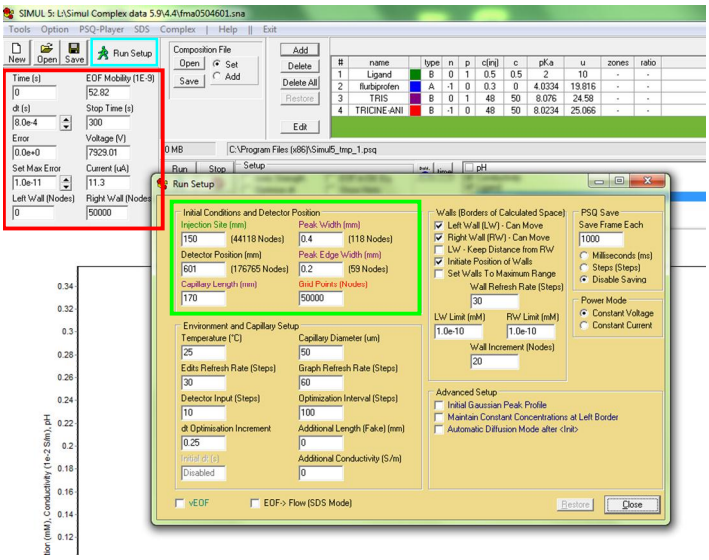
2. Run setup and system parameters:
The detailed setup of simulations is described in the Simul 5 manual.
Necessary input data:
a) Main Simul 5 window:

- EOF mobility
- Applied voltage

b) Run Setup window (click on the "Run Setup" button in the Main window):

- Capillary Parameters ("Injection Site (mm)", "Capillary Length (mm)", "Detector Position (mm)")

- Parameters of injection zone/peak ("Peak Width (mm)", "Peak Edge Width (mm)", "Grid Points (Nodes)).
- Other settings can also be specified in the Run Setup window (e.g. "Environment and Capillary Setup", "Wall (Border of Calculated Space)", "Advanced setup", "PSQ save", "Power mode"). For these advanced setup options see the Simul 5 Manual.

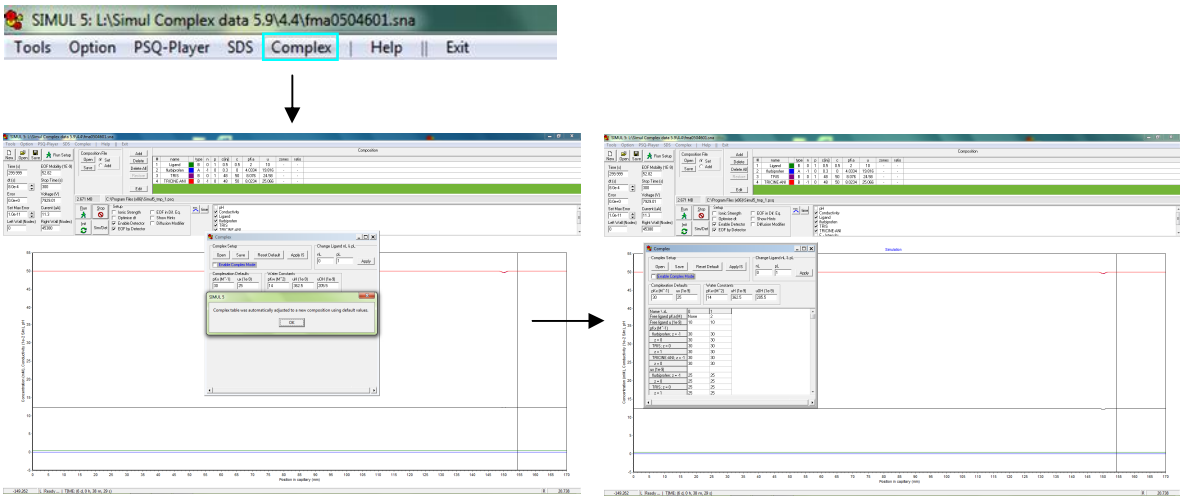


3. Complexation mode

Necessary input data:

- Complexation constants "pK_x [M^{-1}]"
- Mobilities of complexes "u_x [10^{-9} $m^2V^{-1}s^{-1}$]"
- Characteristics of the ligand:
  - pK_a values of all free ligand dissociation forms ("Free ligand pK_a")
  - Mobilities of all free ligand dissociation forms ("Free ligand u [10^{-9} $m^2V^{-1}s^{-1}$]")

By clicking on the "Complex" button in the Main Menu Bar the default table of complexation characteristics (further Complex table) is created and opened.

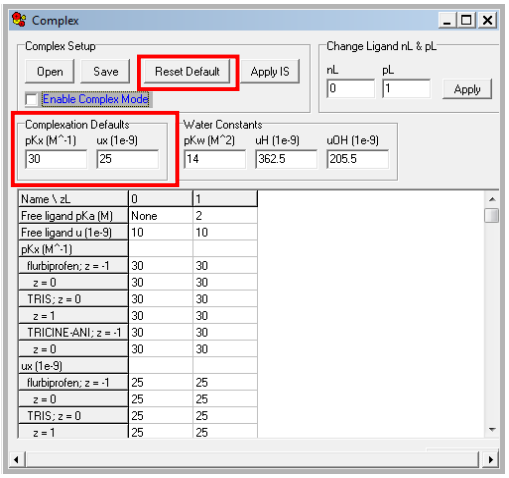


The Complex table requires to enter characteristics of the ligand (pK_a values and free ligand mobilities) and complexation parameters (complexation constants and actual mobilities of complexes) of the ALL constituents.

Complexation data of ALL dissociation forms of ALL constituents with ALL dissociation forms of the ligand are required!

Note: If the complexation of a constituent with the ligand is regarded as negligible (e.g., valid for the buffer constituents), we recommend to use the following default values of the complexation parameters: pK_x [M^{-1}] = 30, u_x [10^{-9} $m^2V^{-1}s^{-1}$] = 25.

The newly created Complex table is filled in with the default values. These values can be changed in the section Complexation Defaults followed by clicking on the button Reset Default.



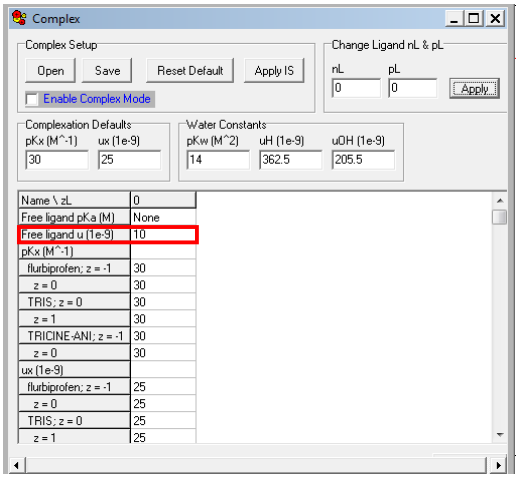
3.1. Characteristics of ligand

In the next step characteristics of the ligand must be specified. The pK_a values and limit mobilities are transferred from the constituent list but can also be modified in this section.

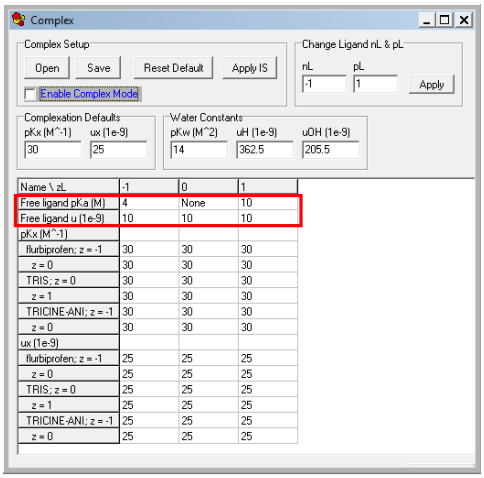
The values from the Complex table are used for the simulation!

The ligands are divided into three specific groups.

I. Neutral ligand:
Only the Free ligand mobility ("Free ligand u [10^{-9} $m^2V^{-1}s^{-1}$]") is required. This mobility is used for calculation of the diffusion constant of the complex.

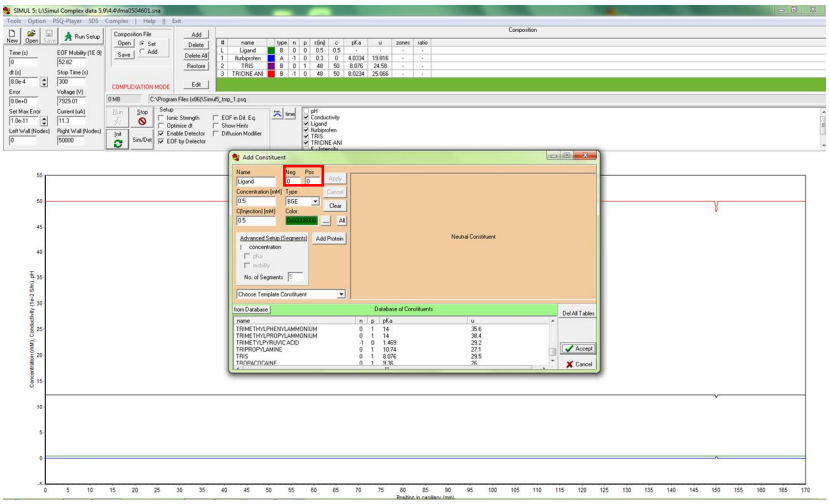


II. Weak electrolyte ligand:
The pKa values of charged forms of the ligand ("Free ligand pK_a") and the mobilities of ALL forms of free ligand even the neutral form as in the group I., ("Free ligand u [$10^{ï\,9}$ $m^2V^{ï\,1}s^{ï\,1}$]") are necessary .



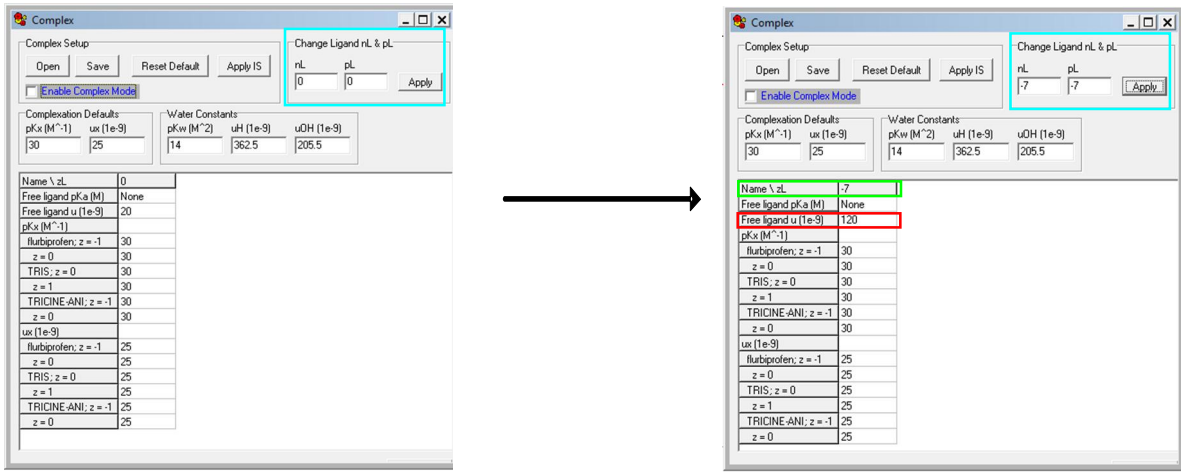
III. Fully charged ligand:
The case of a fully charged ligand (e.g. sulfated cyclodextrin with the charge number ï 7).

(i) First, the ligand is set as a neutral compound in the Constituent table.



(ii) The charge numbers are modified in the Complex table. In the section Change Ligand nL & pL change the maximum positive and negative charge numbers. For example by setting both values ï 7 followed by click on "Apply" button, the charge number of ligand is changed and the ligand is regarded as fully charged strong electrolyte with charge number ï 7.

(iii) The mobility of the free ligand ("Free ligand u [$10^{ï\,9}$ $m^2V^{ï\,1}s^{ï\,1}$]") is entered.



3.2 Complexation parameters

In the next step the complexation parameters of ALL dissociation forms of ALL constituents have to be specified.

We recommend to use the default values for constituents with negligible complexation with the ligand.

Example

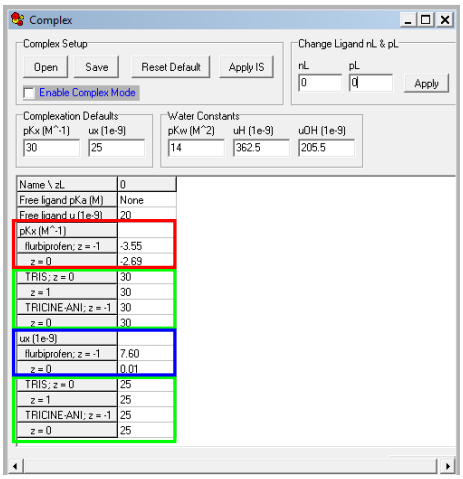
Ligand: neutral cyclodextrin (6-O-α-maltosyl-ß-cyclodextrin hydrate)
Analyte: Flurbiprofen
Buffer: TRIS and Tricine

Only complexation of flurbiprofen with the ligand is considered.

Tris and tricine as buffer constituents do not complex with the ligand. Thus, the default values of complexation parameters are used in the simulations.

The analyte (flurbiprofen) occurs in the system in two dissociation forms, with charge numbers z = 0, z = ï 1. Thus, the complexation constants ("pK_x [$M^{ï\,1}$]") and the mobilities of the complexes ("u_x [$10^{ï\,9}$ $m^2V^{ï\,1}s^{ï\,1}$]") have to be specified for the both forms.

Note: If the analyte is present only in one dissociation state at the actual pH, the complexation constant for the other forms (not present in the separation system) do not influence the results of simulation. Thus, only a rough estimate of the complexation parameters for the NOT present form is sufficient.

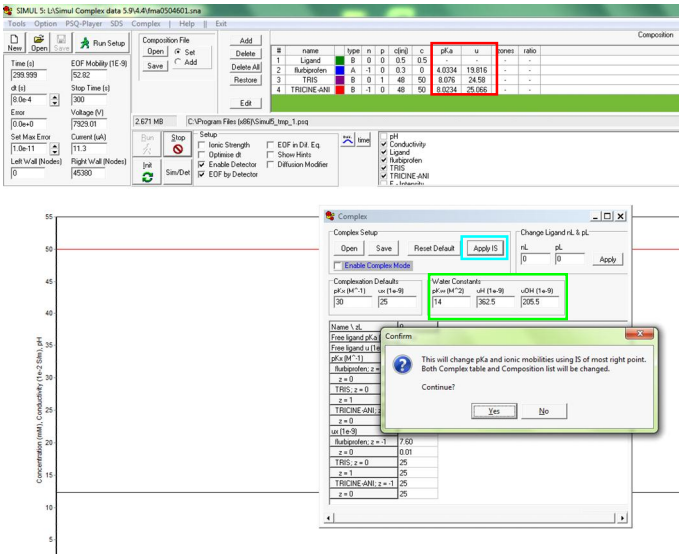


3.3. Ionic strength

The current version of the Simul 5 Complex does not have the possibility to apply the ionic strength correction during simulation. However, the input data can be corrected for the initial ionic strength.

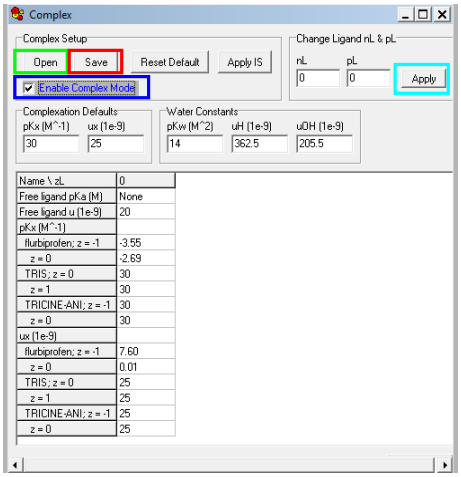
By clicking the button "Apply IS" the pKa values and limit mobilities in the Constituent table as well as the Water constants (pK_w, u_H and u_{OH}) shown in the Complex table are corrected for the actual ionic strength.

Note: It is expected that the complexation parameters and characteristics of the ligand are determined at the given ionic strength, or already corrected on it. So, these parameters are not corrected for the actual ionic strength by this procedure.



3.4 Complexation mode - general features

The Complex table can be saved as the TXT file by clicking on the "Save" button. The previously saved tables can be opened by clicking on the "Open" button.
All changes in the Complex table are registered by the program after clicking on the "Apply" button in the Change Ligans nL & pL section.
The Complex mode is activated by ticking the box Enable Complex Mode.



After the window of the Complex table is closed, initialization is necessary by clicking on the button "Init" in the Main window. If the initialization proceeds correctly, the Red label COMPLEXATION MODE appears in the main window and the ligand is denoted by ñLò.

By saving the whole simulation in the main window the complexation parameters are saved as well and will be opened together with the simulation file.