Step-by-Step Protocol for Application of the nonideal c(s) model in SEDFIT

Generally, when using this model keep in mind that nonideality coefficients will be defined well only for experimental data sets that show significant nonideality. Typically these are data at high concentration with boundary sharpening. Such data cannot be fit well with standard c(s), resulting in large systematic errors in the boundary shape and in unrealistically high best-fit frictional ratio parameter. Application of the nonideal $c_{NI}(s_0)$ will typically provide well-defined k_S -values but not so well-defined k_D-values. For details see *"Measuring macromolecular size distributions and interactions at high concentrations by sedimentation velocity"*, 2018, Nature Communications 9 (4415)
https://www.nature.com/articles/s41467-018-06902-x

1. Open the SEDFIT v 16.1C and select the "Load files" function from data menu and load the desired files similar to standard c(s) analysis.
2. Do a standard c(s) analysis to get estimates for TI and RI noise.
3. Subtract TI and RI noise: now fringes are proportional to concentration.
4. Go to "Model" in menu section and switch to "nonideal c(s) model" (can be found in the subset of "nonideal sedimentation").
5. Fix the frictional ratio to the value obtained under very dilute condition.
6. Enter s_{min} >0 (s<=0 models are not implemented yet) check the baseline, RI, TI noise and meniscus for refinement.
7. In the field "signal/[mg/ml]": enter 3.3 when using 12 mm pathlength centerpiece and 0.825 when using 3 mm pathlength centerpiece, respectively.
8. In the field "log[ks/(mg/ml)]" and "log[kD/(mg/ml)]": enter -2 (this corresponds to a value of 0.01 ml/mg) for log[ks/(mg/ml)] and -3 for log[kD/(mg/ml)].
9. In the section "nonideal sedimentation based on", switch on the radio button "data mode".
10. First, run the model with fixed "log[kD/(mg/ml)]" and floating "log[ks/(mg/ml)]" then fit the model.
11. Do another fit to float both "log[kD/(mg/ml)]" and "log[ks/(mg/ml)]" for refinement.
12. In the section "nonideal sedimentation based on", switch on the radio button "best simulation mode".
13. Run and fit the model.
14. Do not stop fit too early at any step, wait until fit fully converged and repeat the fit with alternating optimization methods (Simplex and Marquardt-Levenberg).