SEDFIT MSTAR

Standard procedure

SEDFIT MSTAR includes the 'MSTAR' button on the menu bar
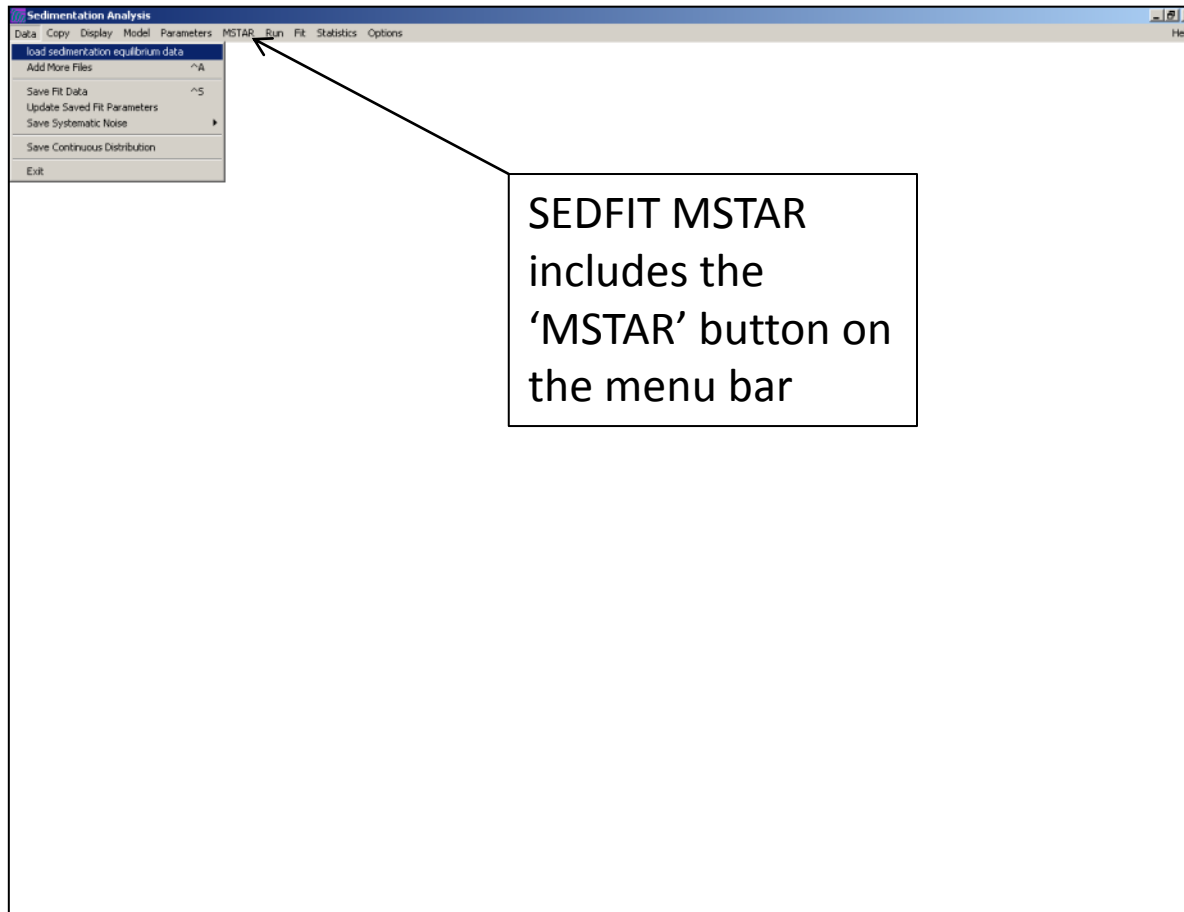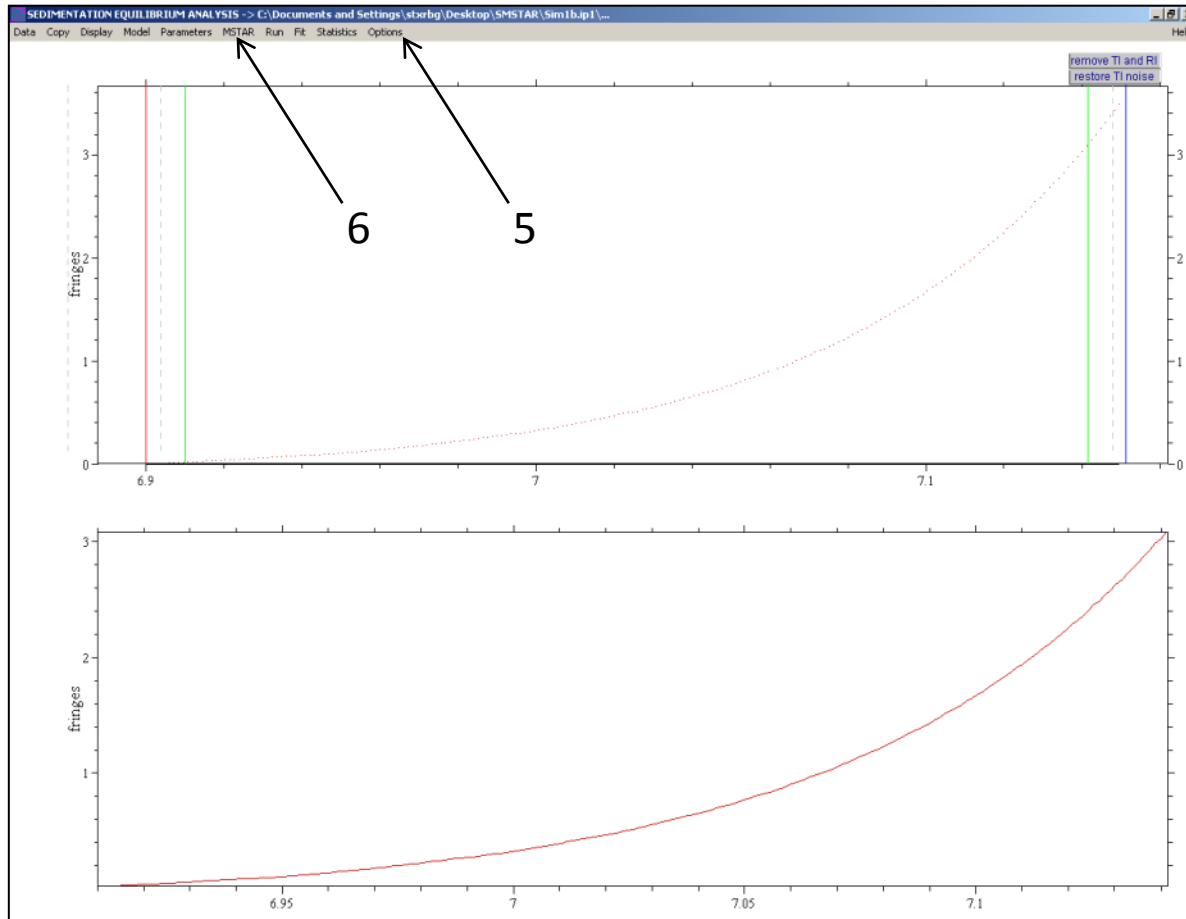
1. **Open SEDFIT MSTAR**
2. **Data → Load sedimentation equilibrium data**

The data files from Schuck P, Gillis R, Besong D, Almutairi F, Adams G, Rowe A, Harding S (2014) *Analyst*, **139**, 79-92 are available for download. This tutorial will use Sim1b (ideal single solute) and NISS1e (very non-ideal single solute) from that paper

Sim1b:

3. **Move red (meniscus) and blue (base) lines to appropriate positions.**
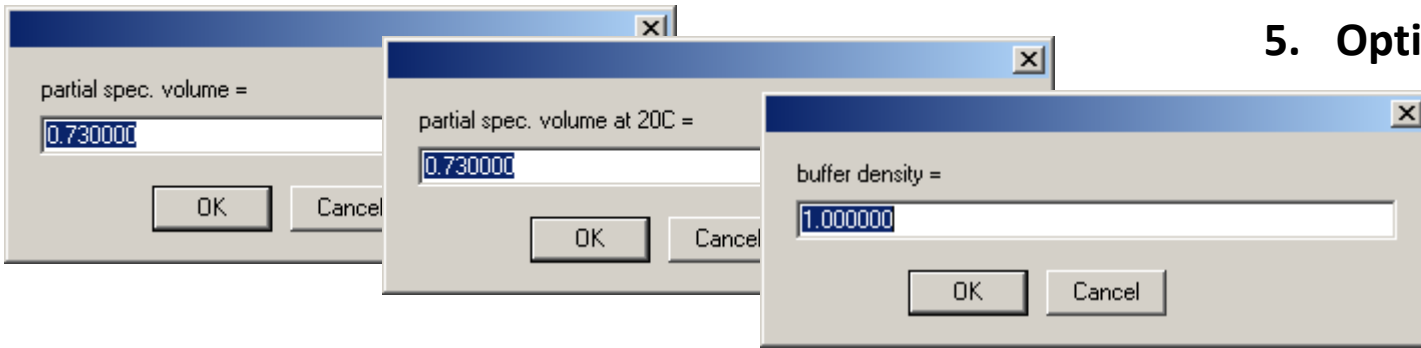
The dotted fitting limit lines do not perform any function in this version of SEDFIT so their position does not matter.

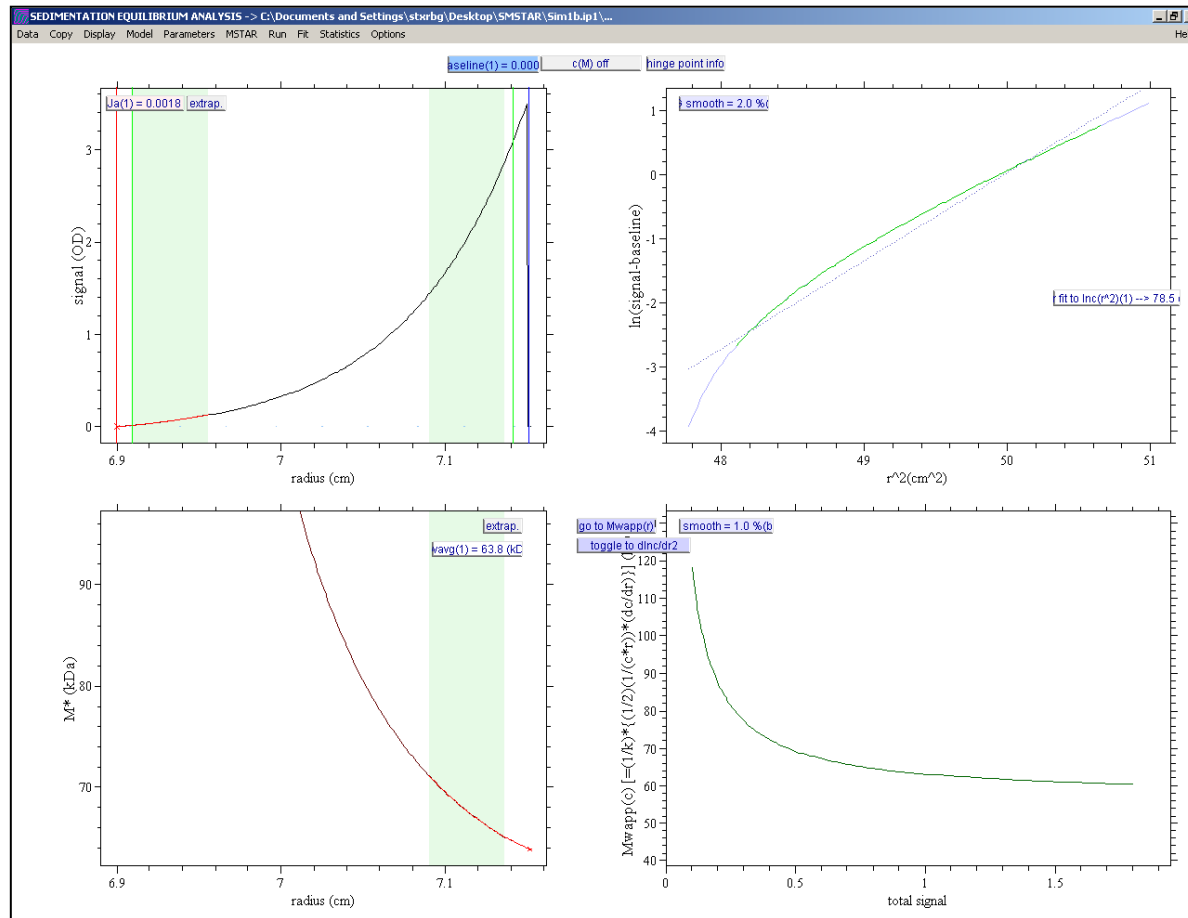4. **Move the green lines to the data in between meniscus and base.**

SEDFIT will not allow you to move them too close to the meniscus and base.

5. **Options → Set vbar, rho.**

6. **Click MSTAR button.**

partial spec. volume =
0.730000
OK   Cancel

partial spec. volume at 20C =
0.730000
OK   Cancel

buffer density =
1.000000
OK   Cancel

This is the "MSTAR" page.

Top left: signal vs. r, with green areas shaded for evaluating the missing signal data near the meniscus and extrapolation to base.

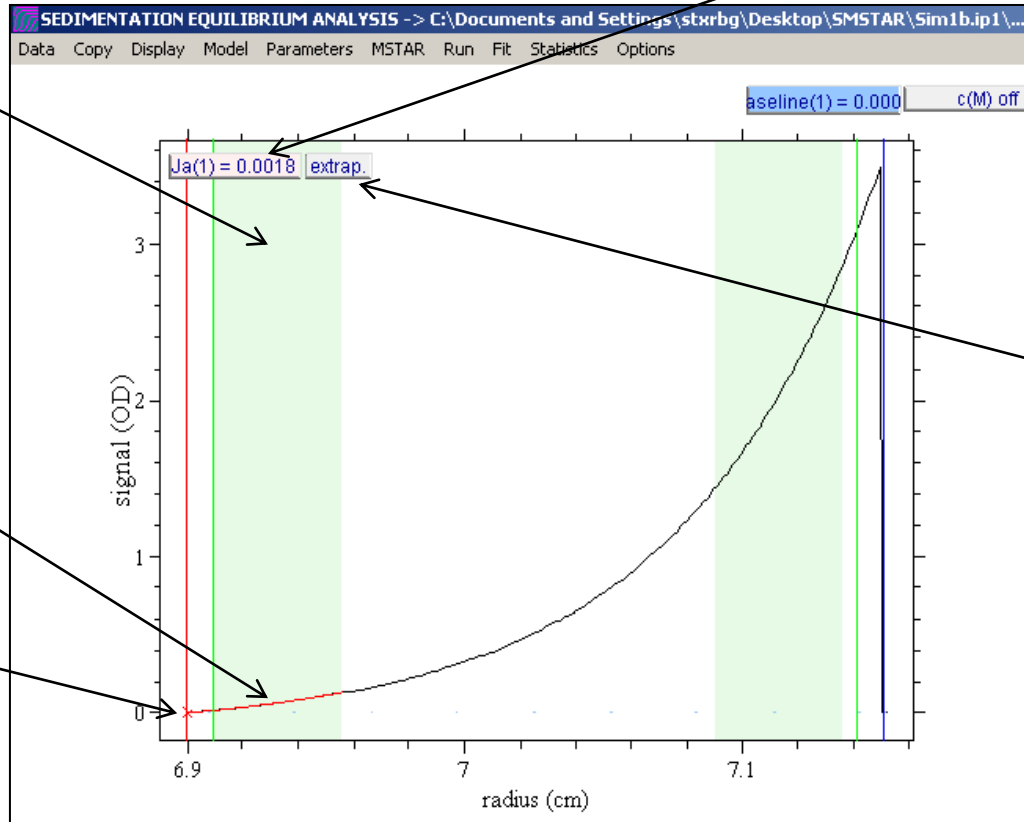Top right: ln(c) vs. r^2, where c is the signal – baseline (baseline currently set at 0)

Bottom right: $dln(c)/dr^2$ vs. signal.

Bottom left: M* vs. r plot, with green area for extrapolation to base.

NB at this stage, the data has not been baseline corrected (including evaluation of the meniscus concentration "Ja"). Before we do anything else, we now explain each of these 4 plots:

The black plot shows the raw data. Limits can be changed in this view like in the normal data plot.

You can manually re-enter the meniscus concentration and override the value from extrapolation.
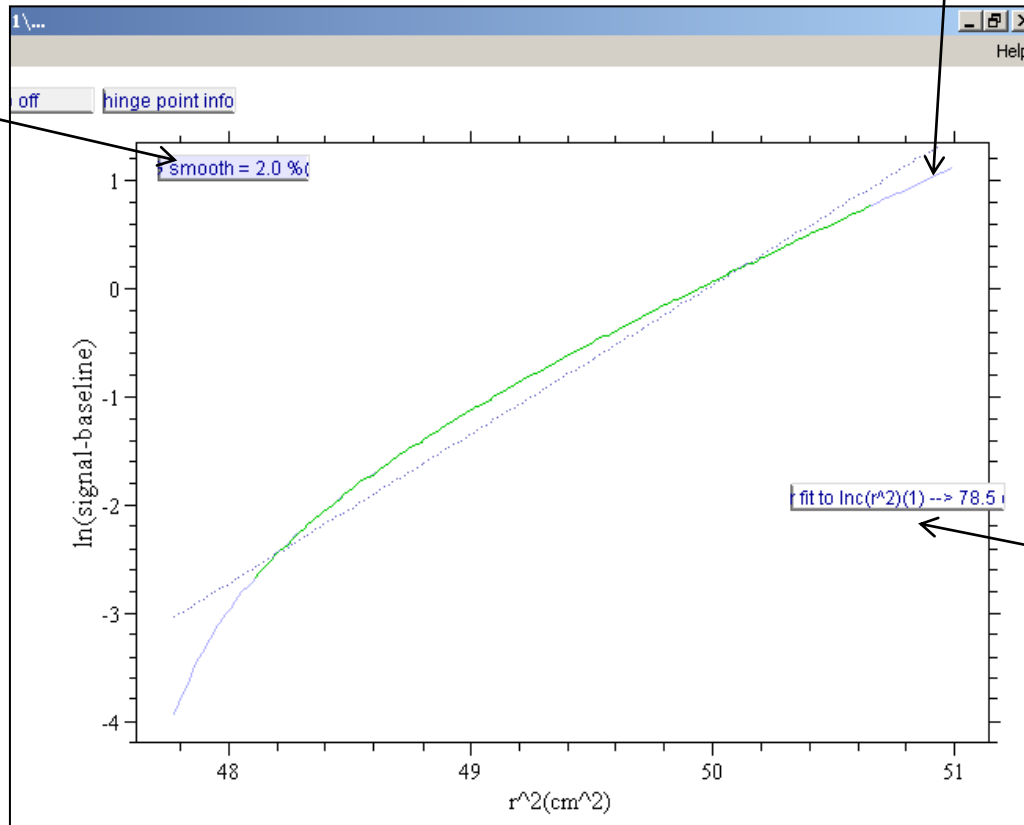
This green region is the range used to extrapolate the signal to the meniscus. This extrapolation is shown as the red curve segment, with the <u>red cross</u> at the extrapolated value.

Hit the "extrap." button to undo the manual override. SEDFIT will ask you to select the polynomial order of extrapolation and allow you to change the size of the region.

SEDIMENTATION EQUILIBRIUM ANALYSIS -> C:\Documents and Settings\stxrbg\Desktop\SMSTAR\Sim1b.ip1\...

Data Copy Display Model Parameters MSTAR Run Fit Statistics Options

aseline(1) = 0.000   c(M) off

Ja(1) = 0.0018 extrap.

signal (OD)

radius (cm)

6.9   7   7.1

The green plot is the smoothed raw data. Smoothing can be modified with this button.
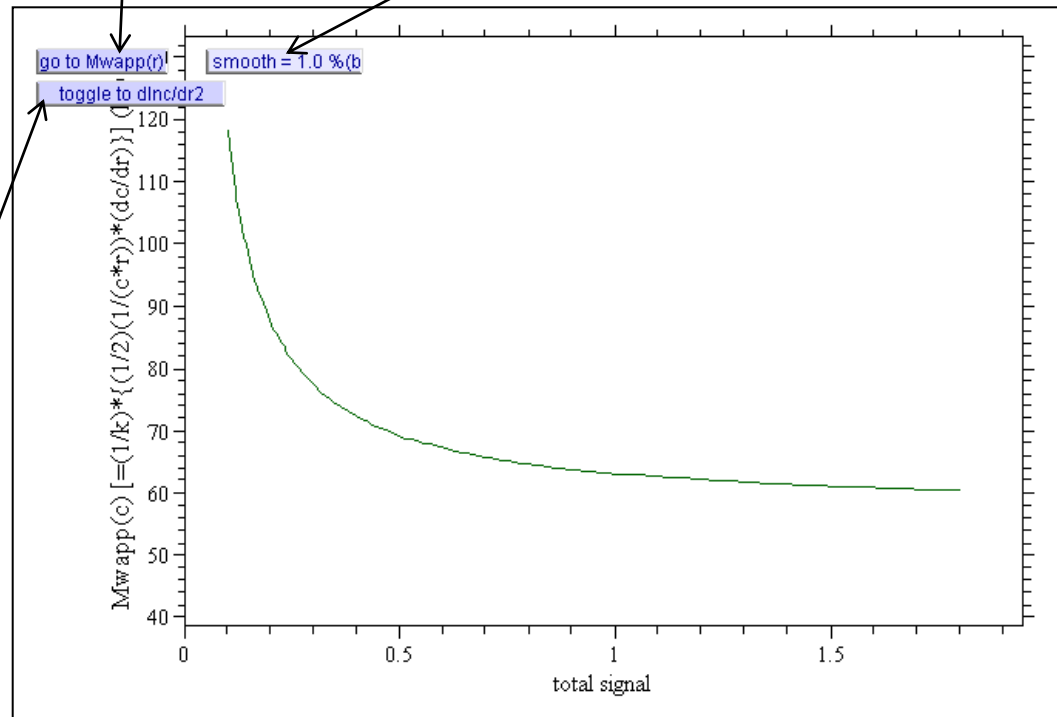
The light blue plot is the transformed raw data.

The dotted line is the linear regression, from which a 'ballpark' weight average molar mass is calculated. Unless the fit perfectly superimposes the data, this number will not be representative.

1\...

Help

off   hinge point info

smooth = 2.0 %

fit to lnc(r^2)(1) --> 78.5

ln(signal-baseline)

1

0

-1

-2

-3

-4

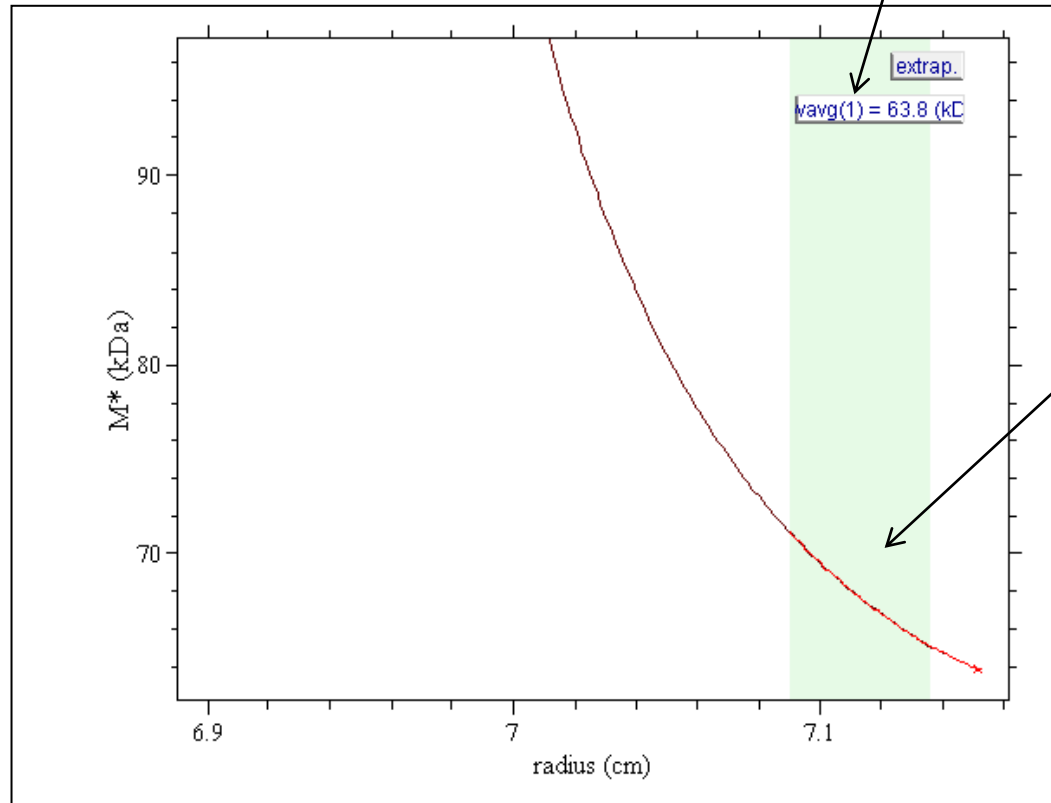48   49   50   51

r^2(cm^2)

The graph can also be plotted against radius by toggling this button.

Further smoothing can be changed with this button.

This data is the derivative of the smoothed ln(c) vs. r^2 plot. Clicking this button will change the derivation method.

go to Mwapp(r)   smooth = 1.0 %(b

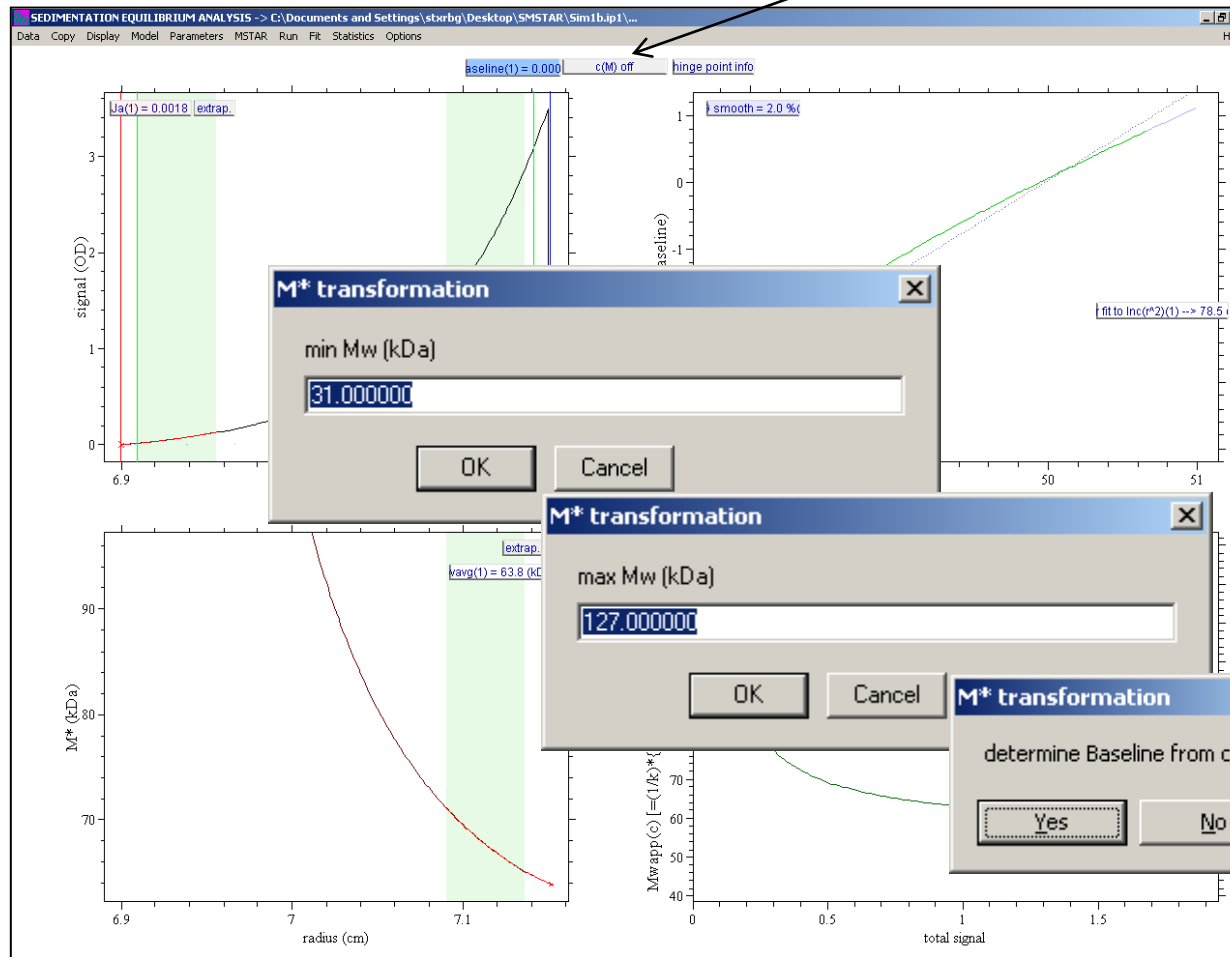toggle to dlnc/dr2

The extrapolated value is presented here.

The nature of the M* function means the plot is extrapolated to the base of the cell, accounting for all data preceding. Although real data will appear noisy near the meniscus, this will not matter too much.

extrap.

vavg(1) = 63.8 (kD

The extrapolation is based on the green area. The area can be changed by clicking the "extrap." button, selecting the polynomial order of extrapolation and changing the region on the c vs. r plot (analogous to the region on the M* plot).

7. Hit the c(M) button so it says "on":

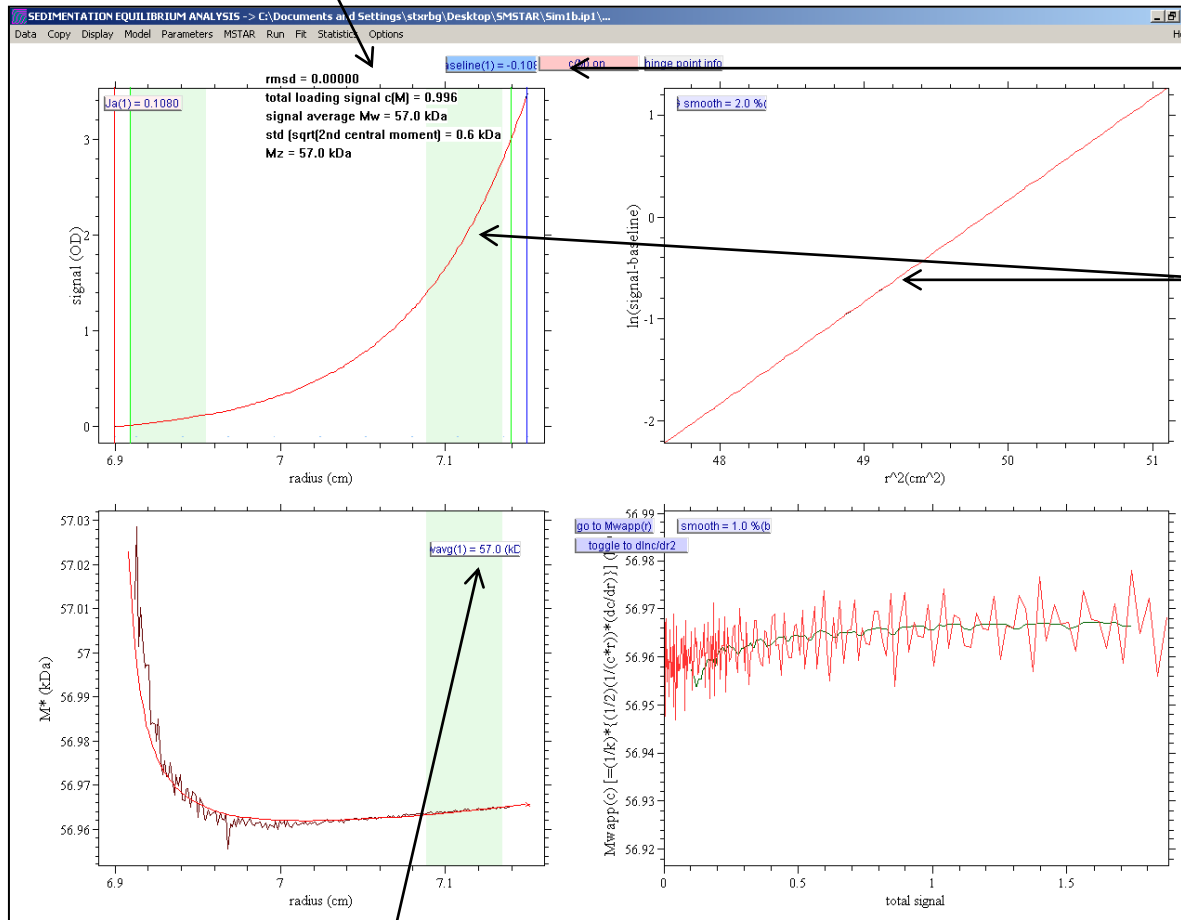**8. Set the molecular weight fitting limits.** The default is half (lower limit) and double (upper limit) the current estimate from the M* plot. Do not give too wide a range.

9. You will be asked to determine the baseline. Except in exceptional circumstances, you will want to **click Yes**.

This is a least-squares fit to the raw data performed with a continuous molar mass distribution c(M), according to:

$$a(r) \cong b + \int_{Mw_{min}}^{Mw_{max}} c_0(r,m) e^{\frac{M(1-\bar{v}\rho)}{2RT}(r^2-r_0^2)} dM$$

Refer to Schuck et al. 2014 for more details.

The c(M) output gives rmsd, estimated loading concentration (in signal units), weight- and z- average molar mass and standard deviation.
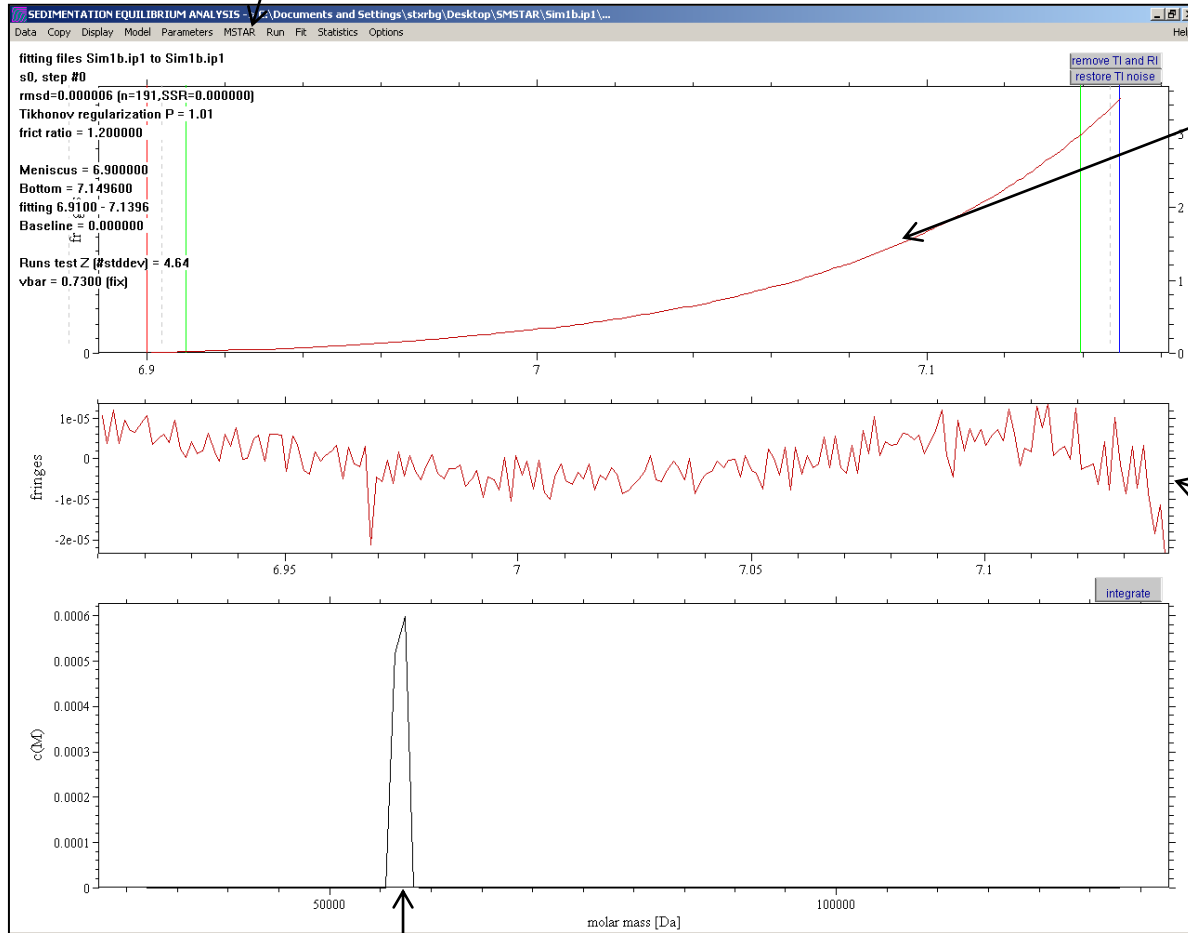

The baseline and Ja values will be estimated from the c(M) fit.

The red fit-line should superimpose onto the black raw data for both the signal vs. r and ln(signal) vs. r^2 plots.

An estimate for the M* extrapolation based on c(M) will be presented here.

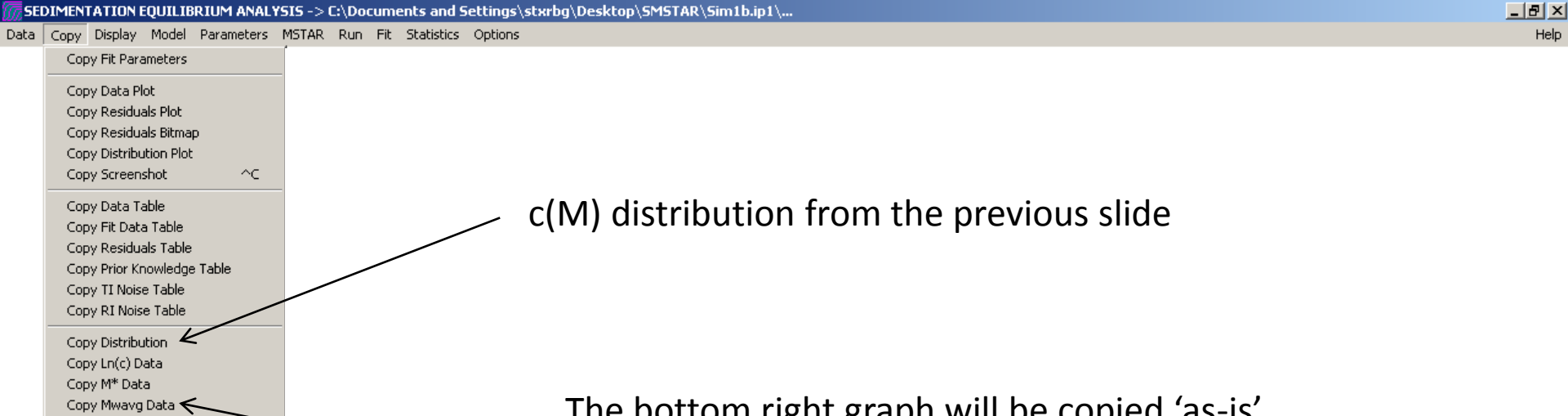**10. Finally press "Esc" on the keyboard to view the c(M) distribution**

11. When finished, you can go back to the MSTAR screen.

SEDIMENTATION EQUILIBRIUM ANALYSIS – C:\Documents and Settings\stxrbg\Desktop\SMSTAR\Sim1b.ip1\...

Data   Copy   Display   Model   Parameters   MSTAR   Run   Fit   Statistics   Options   Help

fitting files Sim1b.ip1 to Sim1b.ip1
s0, step #0
rmsd=0.000006 [n=191,SSR=0.000000]
Tikhonov regularization P = 1.01
frict ratio = 1.200000

Meniscus = 6.900000
Bottom = 7.149600
fitting 6.9100 - 7.1396
Baseline = 0.000000

Runs test Z (#stddev) = 4.64
vbar = 0.7300 (fix)

remove TI and RI
restore TI noise

integrate

molar mass [Da]

The red fit line should superimpose the black raw data as much as possible on the c vs. r.

As a general rule, the residuals should not show any patterns. However, in this case the residuals do not go above $\pm 10^{-5}$ fringes. A good fit is $\pm 5 \times 10^{-3}$ signal for Rayleigh interference.

The c(M) distribution allows you to assess the polydispersity of the system. This is of a much lower resolution than with sedimentation velocity c(s) distributions & components may not always resolve.   Non-ideal or noisy data may also not show this distribution very well, but you should still get a good baseline and Ja estimate
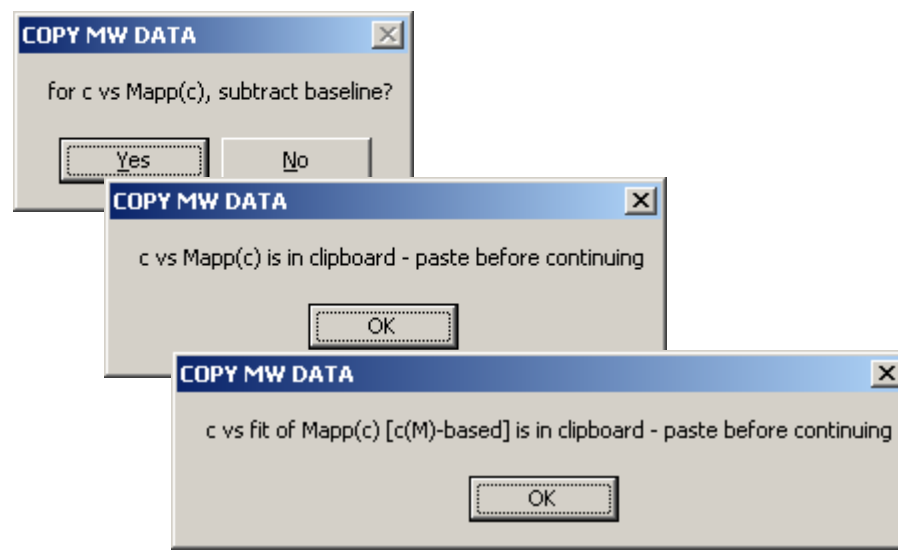
Data   Copy   Display   Model   Parameters   MSTAR   Run   Fit   Statistics   Options   Help

Copy Fit Parameters

Copy Data Plot
Copy Residuals Plot
Copy Residuals Bitmap
Copy Distribution Plot
Copy Screenshot   ^C

Copy Data Table
Copy Fit Data Table
Copy Residuals Table
Copy Prior Knowledge Table
Copy TI Noise Table
Copy RI Noise Table

Copy Distribution
Copy Ln(c) Data
Copy M* Data
Copy Mwavg Data

c(M) distribution from the previous slide

The bottom right graph will be copied 'as-is', so ensure that the x axis is toggled to either concentration signal or radius, and y axis toggled to preferred derivative method.

For concentration, you will be asked whether to subtract the baseline (Yes).

All graphs are exportable to the clipboard in ASCII format from the "Copy" menu. Just 'ctrl-v' in an appropriate spreadsheet/graphing software.
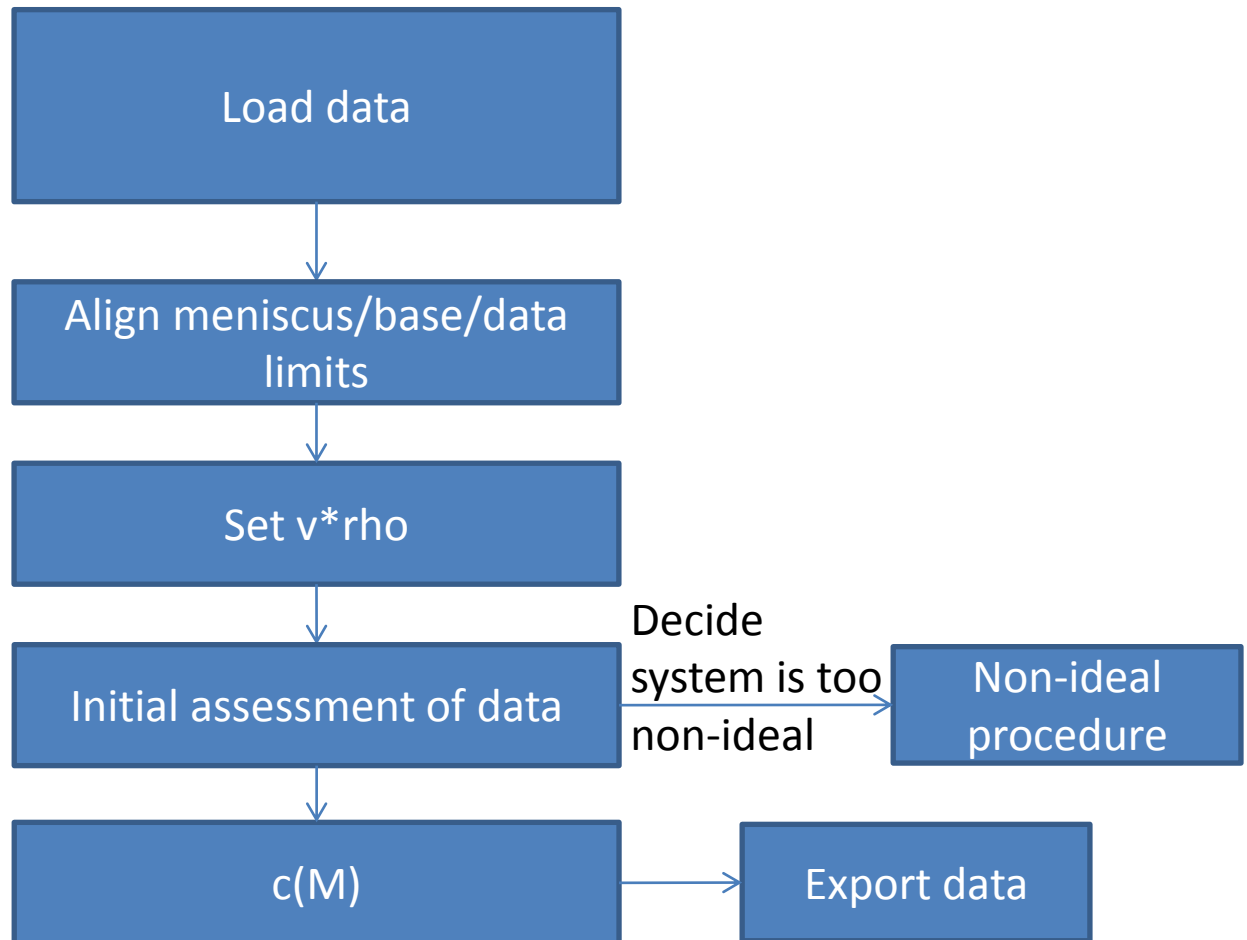
In cases where there is a fit, you will be asked to unload the clipboard of the 'raw' data first, then 'fit' data.

COPY MW DATA

for c vs Mapp(c), subtract baseline?

    Yes   No

COPY MW DATA

c vs Mapp(c) is in clipboard - paste before continuing

            OK

COPY MW DATA

c vs fit of Mapp(c) [c(M)-based] is in clipboard - paste before continuing
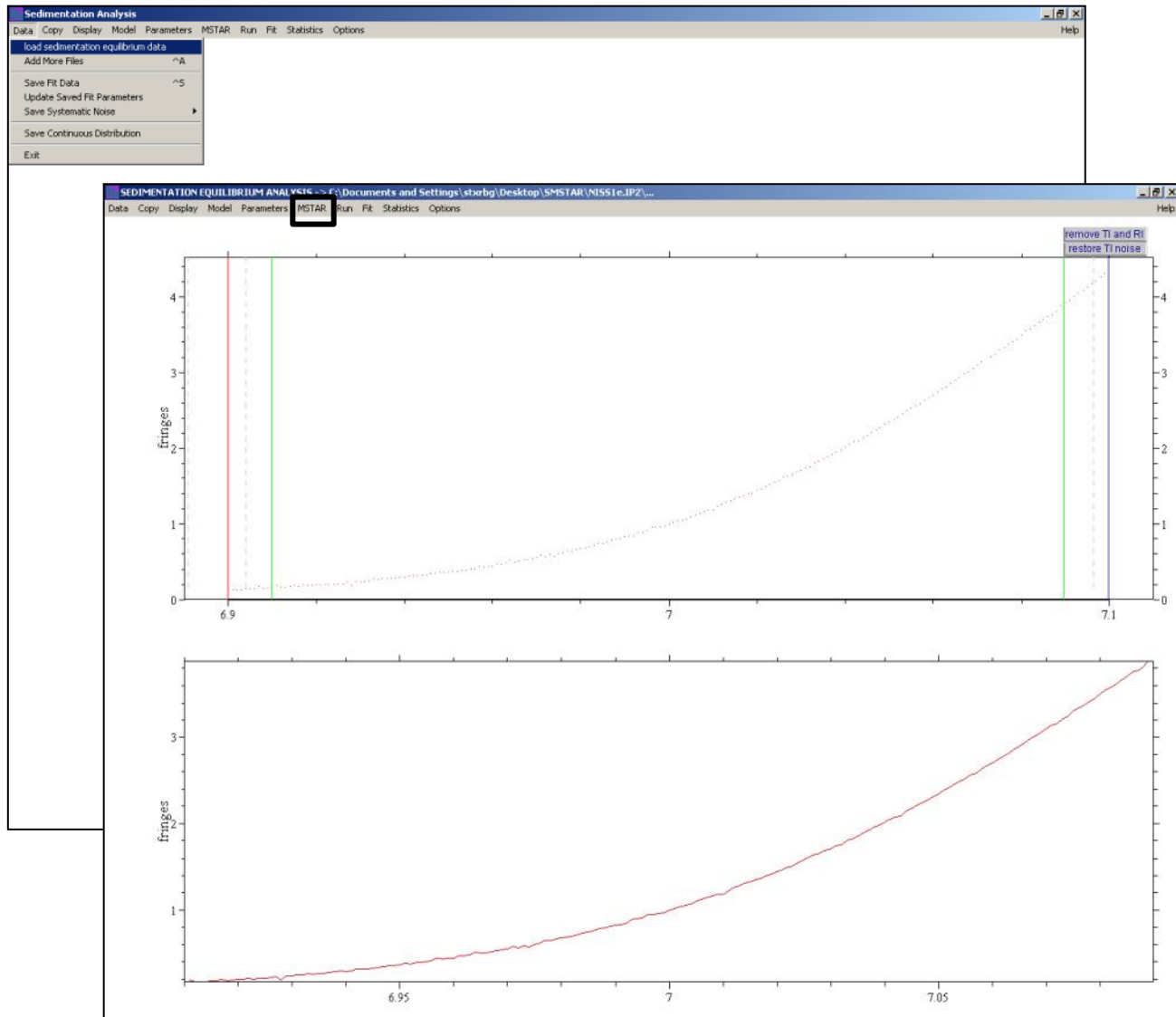
            OK

Other notes

- The procedure above was for interference optics. This method will also work for absorbance, but remember to set the green lines to be within the Lambert-Beer law (A<1.4).

- If your data goes below 0 signal units, the ln(signal) graph will give strange patterns. If it does not change after c(M), transpose your raw signal data +1, and check that the c(M) 'notices' by looking at the calculated baseline, which should also increase by ~1.

- There are various noise-reduction techniques (overspeeding/presedimenting initial scans/aged cells/higher wavelength etc.) It is suggested that your favoured technique is used before analysing your data with SEDFIT-MSTAR.

- For polydisperse systems keep the rotor speed as low as will allow to obtain a reduced molecular weight (k.M or σ, refer to Eq. 2 in paper) of 1.5-2.5.

- If your system has been identified as very non-ideal, please follow the procedure outlined below.
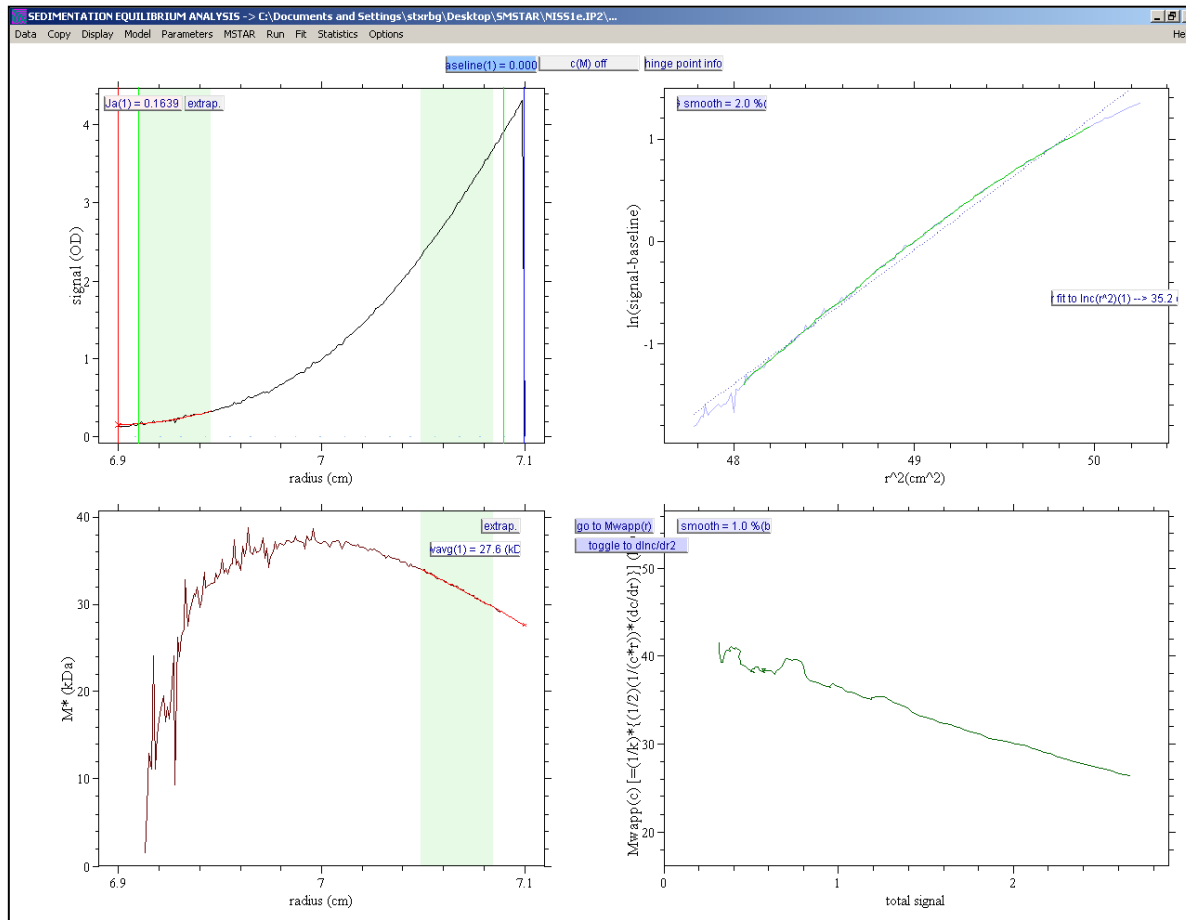
Summary

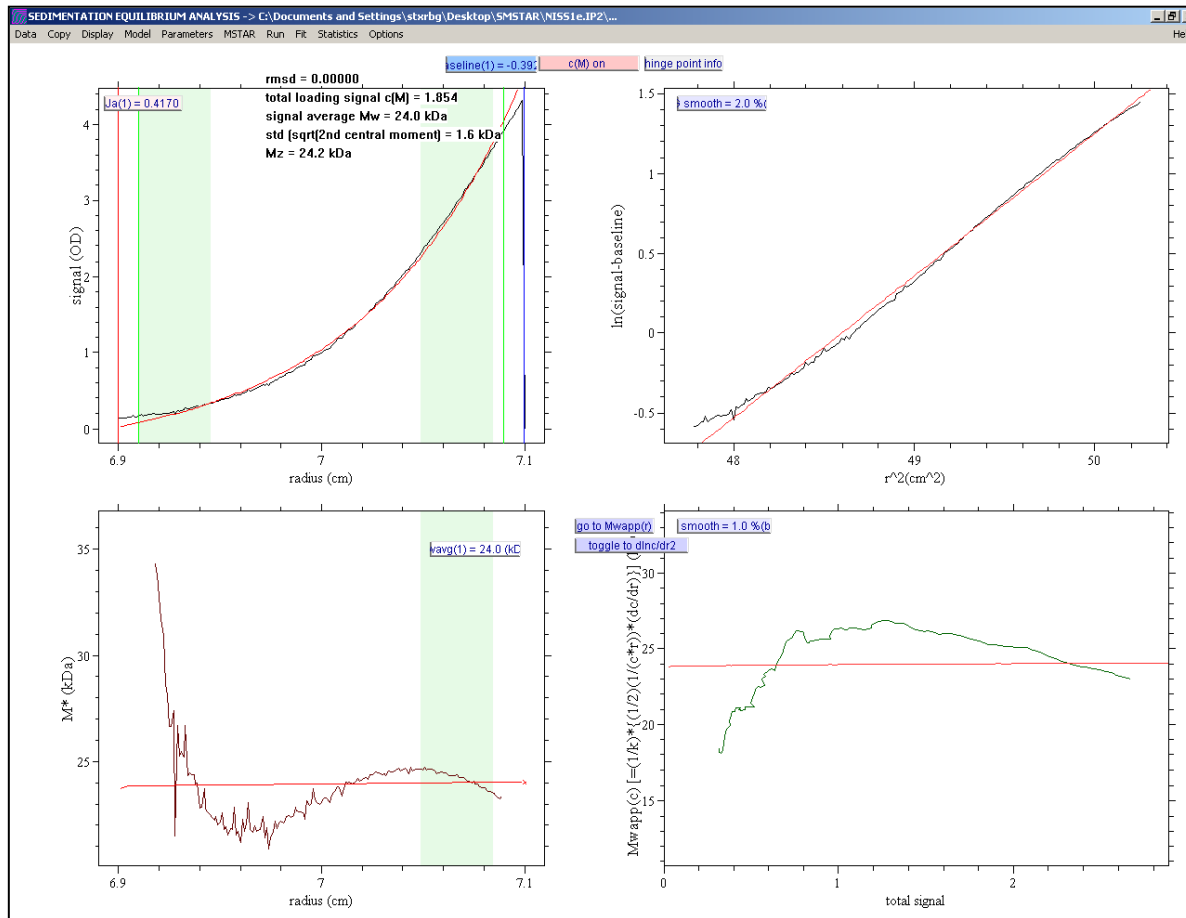# SEDFIT MSTAR

Significant non-ideality

As before, load sedimentation equilibrium data. For this tutorial use NISS1e. This is a simulated single solute with significant non-ideality, also with error.

From the MSTAR screen, there are certain clues of non-ideality:
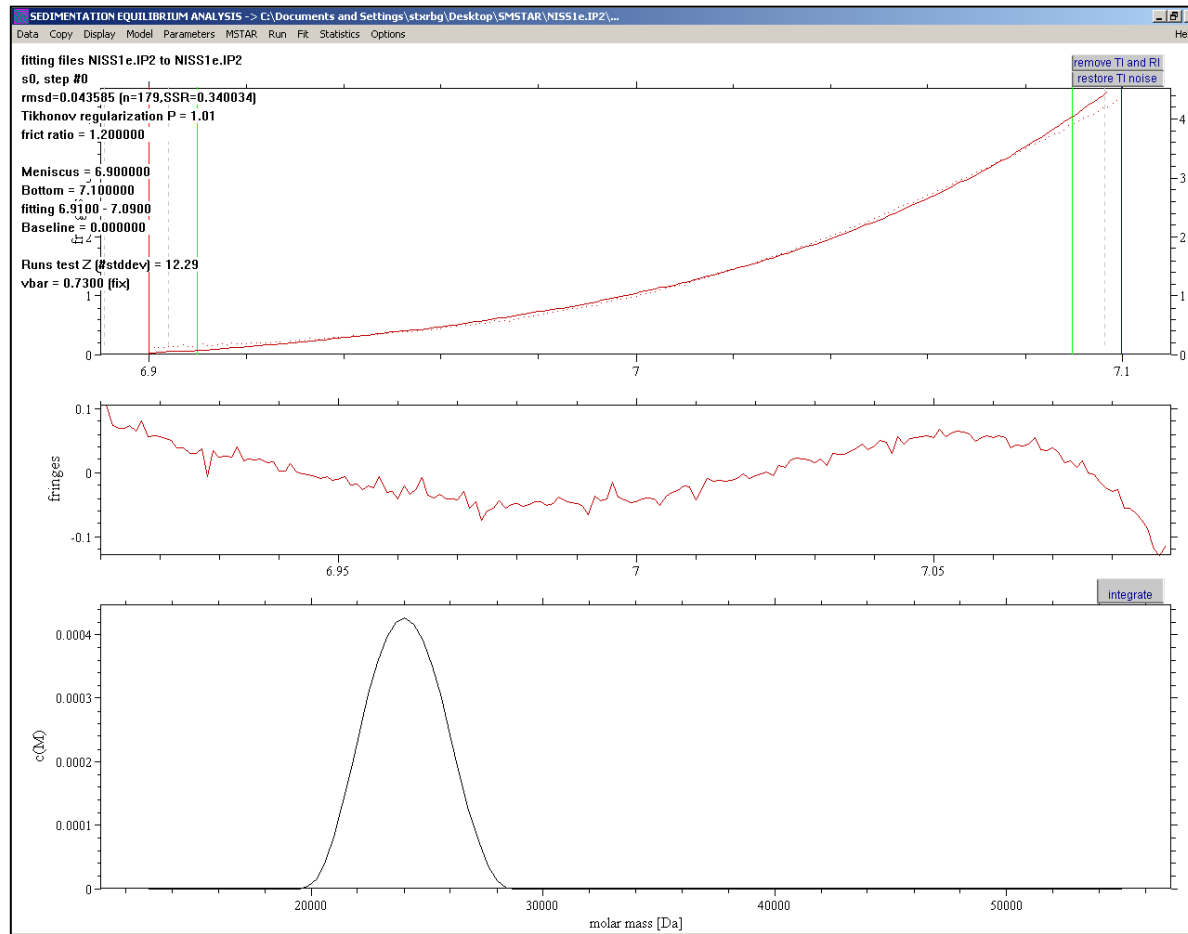- ln(c) vs. r^2 bends down towards the base
- Mw,app vs c negatively slopes
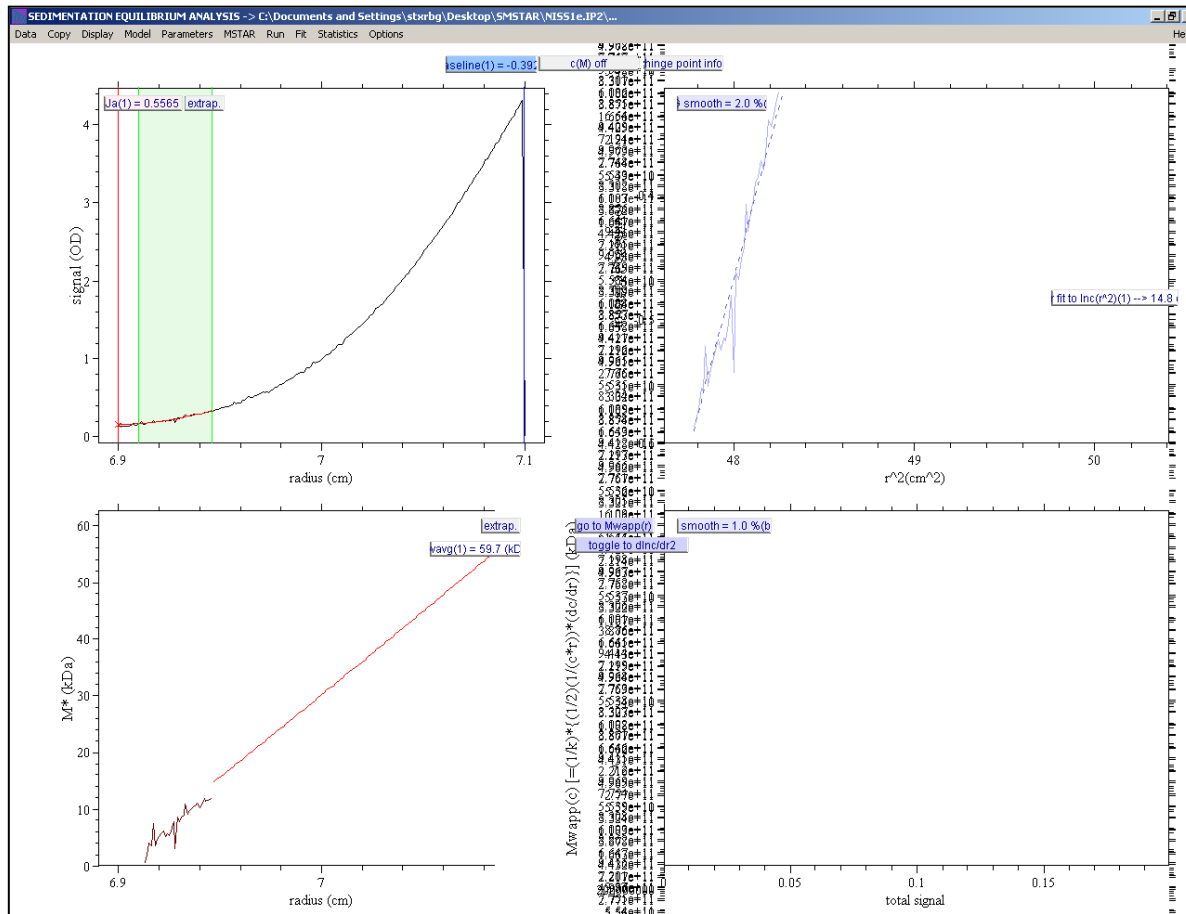
Turn c(M) on.

Very poor fit on the c vs. r plot.

Little correlation between fit and data on Mw,app vs. c and M* vs. r plots.
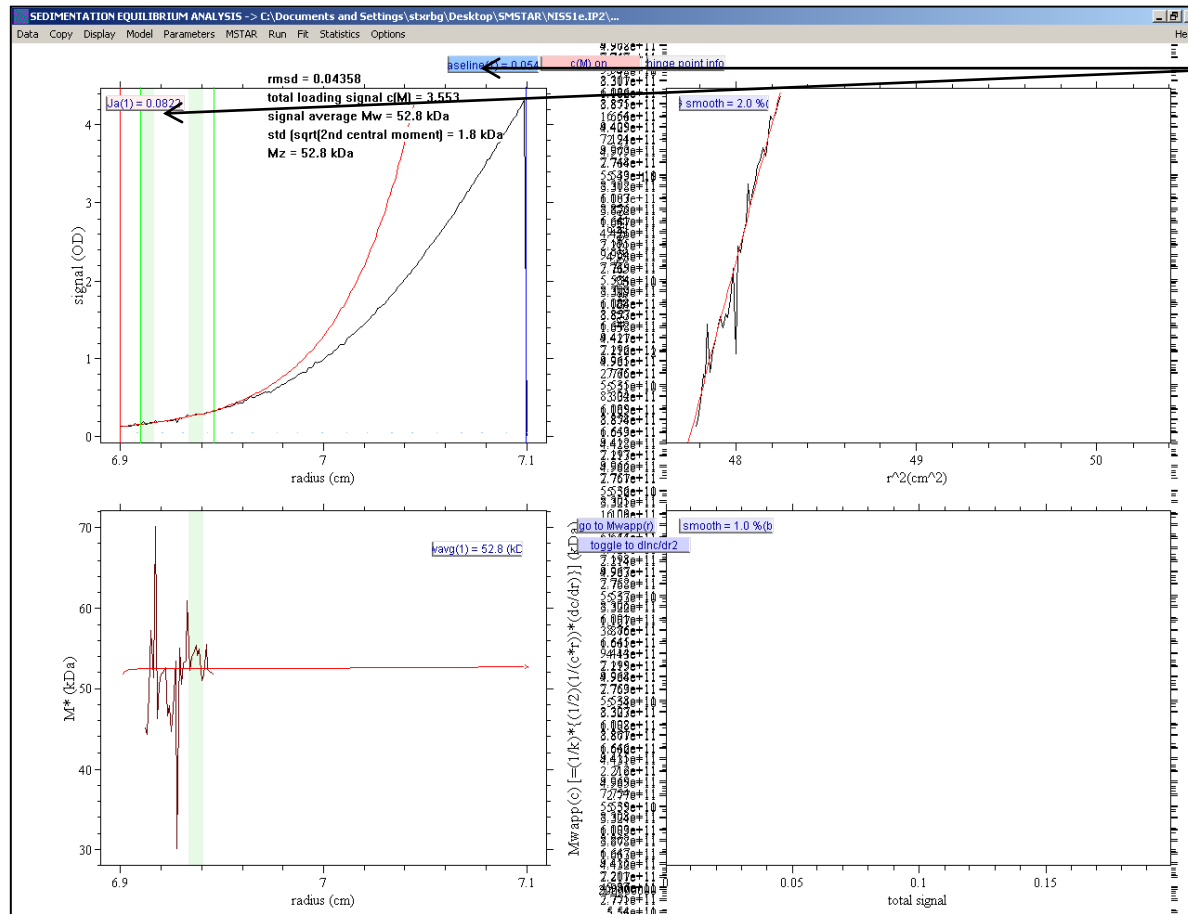
Press Esc on keyboard.

Very large residuals with an overall wavey pattern.

Go back to MSTAR and turn c(M) off.

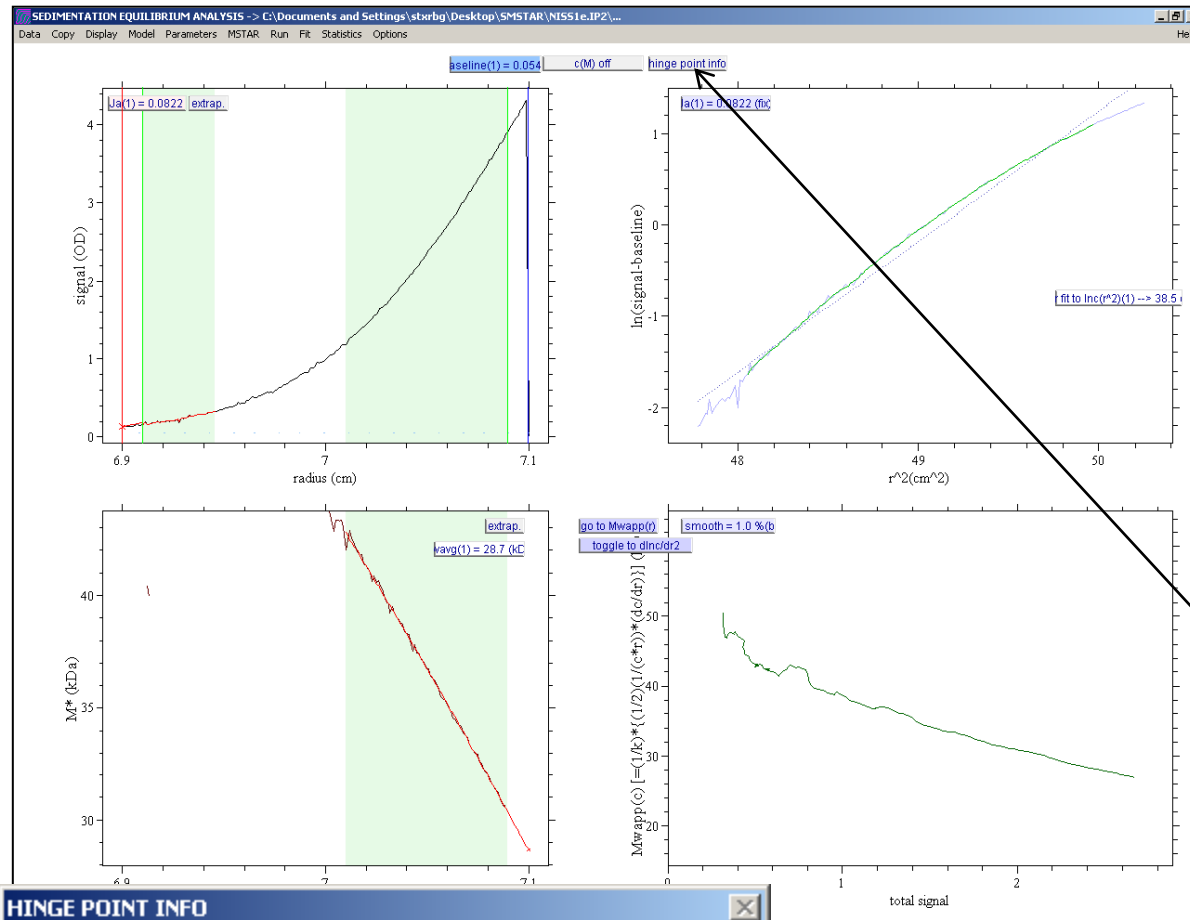Move the lower green line close to the meniscus as shown.

Turn c(M) back on, and say Yes to determining baseline.

Make a note of the Ja and the baseline.
(in this case: 0.0822, 0.0541)

The rationale behind this is that the non-ideality will be at its lowest near the meniscus as the concentration is lowest.

Turn c(M) back off. The Ja will revert back to extrapolation mode.

The baseline should stay the same, however manually type in the Ja
(in this case: 0.0822).

Click the extrap. button on the M* plot and select a wider area on the c vs. r plot.

Now click "hinge point info"
- Unless you know the loading concentration more precisely, do not manually overide – 'No'.
- 'OK'

- NB The Mw,app from the hingepoint is less affected by non-ideality than Mw,app from the M* extrapolation.

Summary