

Lamm

The program allows to estimate sedimentation coefficient, diffusion coefficient, baseline, loading concentration (absorbance), meniscus radius, and at the whole boundary version N (R) also the bottom radius and a parameter for a possible small rotation of the concentration profiles. Additionally the molecular weight or friction ratio can be introduced if they known. With this the number of estimating parameters can be reduced and the numerical stability of the fit enhanced. With the function C (faxen type solution) and with the function N (whole boundary type) up to four independent species can be analyzed by using the sedimentation distribution plot (pressing y). The resolution can be enhanced by time extrapolation according to STAFFORD¹ slightly improved (pressing #). Furthermore time independent noise (TI) and radius independent noise (RI) can be eliminated. The best method to abolish TI noise is the subtraction of the TI-noise contour (baseline) derived from a trace stored by low rotation speed (p.e.3000 rpm). Alternatively, the first data trace stored in the shortest time possible can be used. In the last case only the plateau region may be taken without a small start and bottom region to cancel the influence of sedimentation. The radial derivation and the time extrapolation are now possible without falsification by TI noise. The next 4 pages demonstrate this possibility by example. The first trace subtracted was stored at 1 minute.

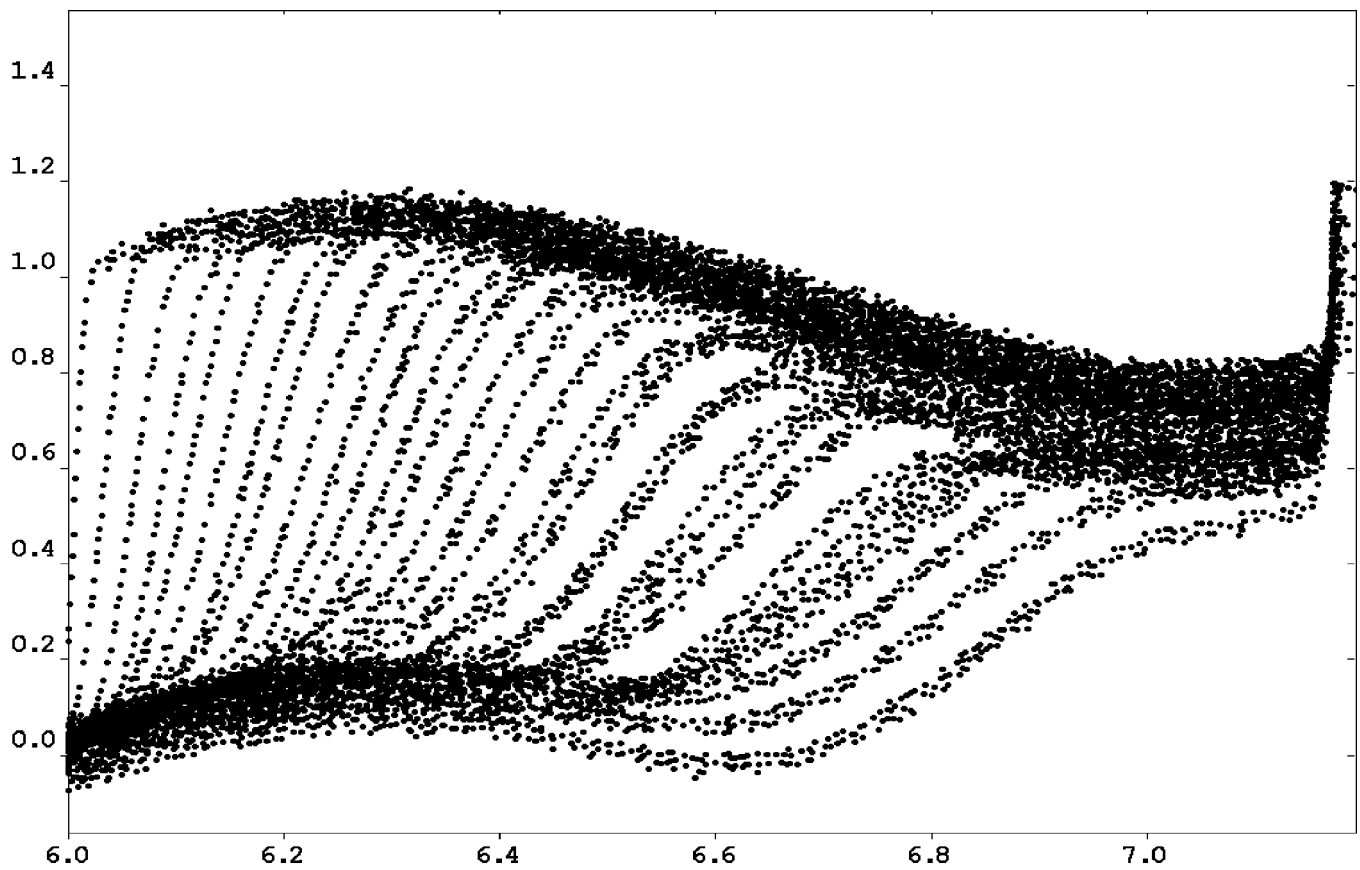
When the DC/DR plot (pressing y) is chosen additionally the weighted sedimentation coefficients are computed by use of the second moment approach. The plateau concentration (OD) necessary for this is once taken from the smoothed data trace and second won by integration of the differentiated same trace. The second approach is only useable if the meniscus is depleted but then the influence of a zero line (absorbance) is eliminated. The weighted sedimentation coefficients can be used for determination of selfassociation binding constants.

Three examples for fitting are shown. The simulation parameter is shown in the top rows. The three file sets are also given but with reduced title rows for generally use.

Attention:

To leave out the graphic in the DC/DT or time extrapolation mode it is necessary to give in at least one sedimentation coefficient true or dummy.

If typing any number of smooth cycles a new last file for the extrapolation procedure can be chosen. Otherwise by pressing key enter the sequence and the number of first file for extrapolation fit can be feed in. Now the extrapolation fit takes place. The first file number can now be enhanced by pressing key < or vice versa decreased again by pressing key >. If the smooth cycles number is typing negative a new not published extrapolation procedure is activated especially suitable for two species with narrow sedimentation coefficients.



The synthetic data set with time and radius independent noise

The data calculated with :

1. component, friction ratio 1.2, molecular mass 70 kDa

2. component, friction ratio 1.4, molecular mass 115 kDa

The corresponding sedimentation and diffusion coefficients are:

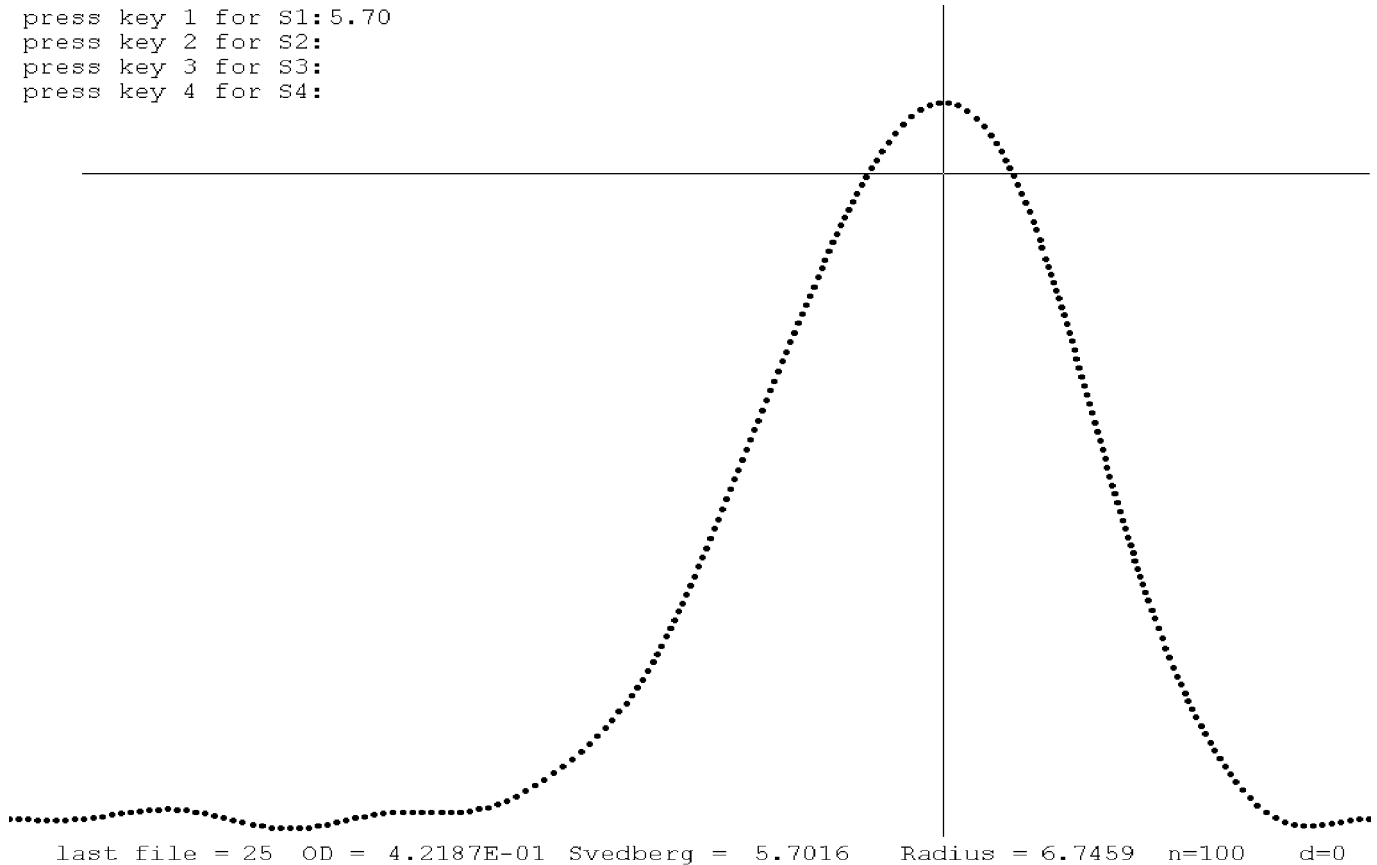
1. component, 5.04 S and 4.507 F

2. component, 6.02 S and 4.727 F

Concentration of both components 0.5

Temperature 20° C, and psv=0.73

press key 1 for S1:5.70
press key 2 for S2:
press key 3 for S3:
press key 4 for S4:



Sedimentation distribution plot of the radial derivative
according to Bridgman

The left top corner show the s-values accepted from the program LAMM obtained by pressing the designated keys

The data points are equidistant by interpolation, but the original data remain unchanged.

The data were then differentiated by fitting a sliding 26 point centered, parabel. The smoothing takes place again with a sliding 26 point parabel fitting. This may be valid also for the time difference method.

The number of smoothing repeats can be chosen (see below).

The bottom border line show from left to right

The file used for the distribution plot, can be chosen by its number

The $g^*(s)$ value in dependence of the horizontal line of cross hairs

The value of sedimentation coefficient in dependence of vertical line

The corresponding radius in the Bridgman relation

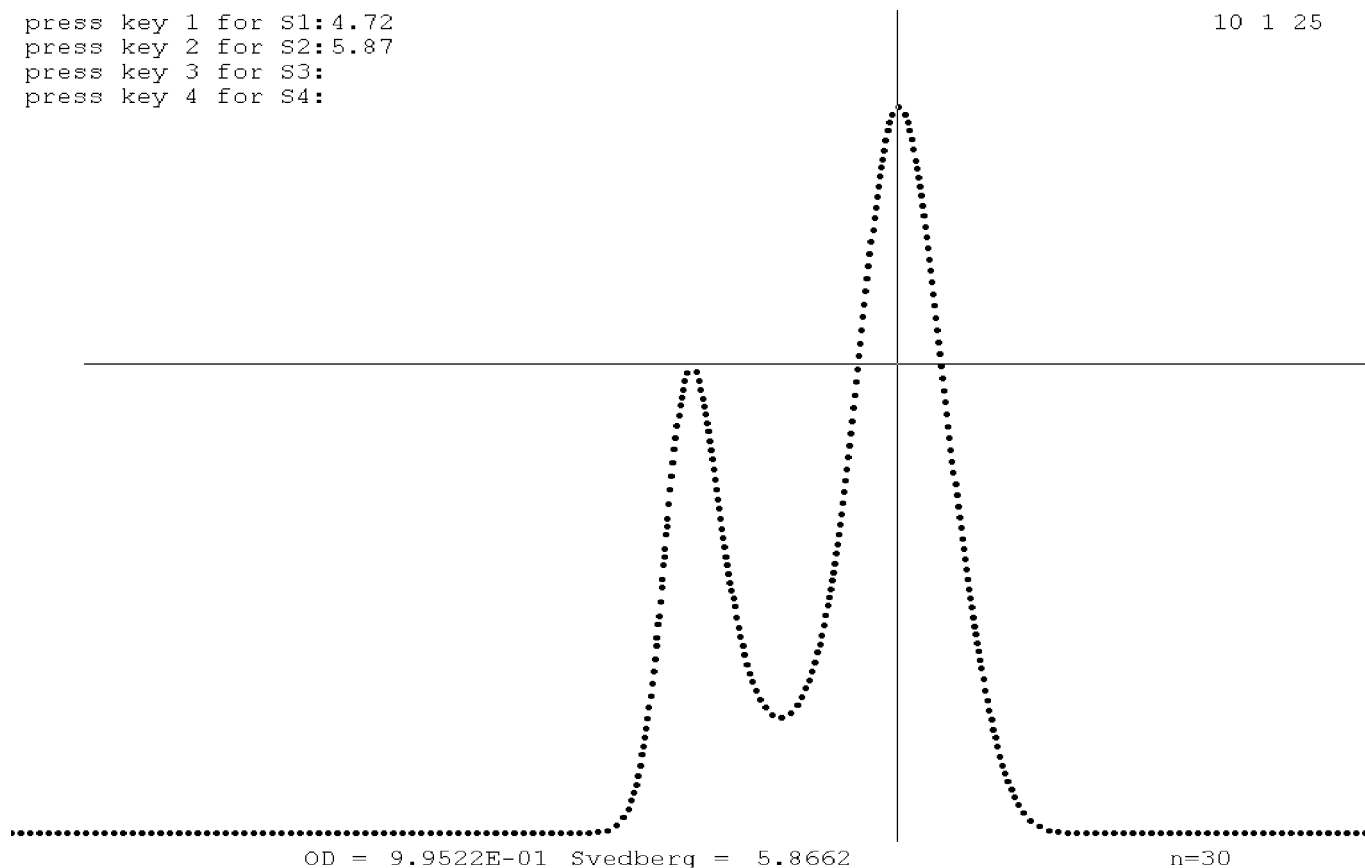
The number of repeats for smoothing can be chosen, standard $n=100$

The two substracted files in the time variant are neighbours, $d=1$

The corresponding starting values for diffusion coefficients and amplitudes were calculated by LAMM

press key 1 for S1:4.72
press key 2 for S2:5.87
press key 3 for S3:
press key 4 for S4:

10 1 25



Sedimentation distribution plot of the radial derivative,
extrapolated to infinite time according to STAFFORD improved

The left top corner show the from program LAMM accepted s-values
obtained by pressing the designated keys

The right top corner show the number of start and last data trace used
for the extrapolation procedure. The middle number gives the quantity
of extrapolation procedures produced with different starting traces.

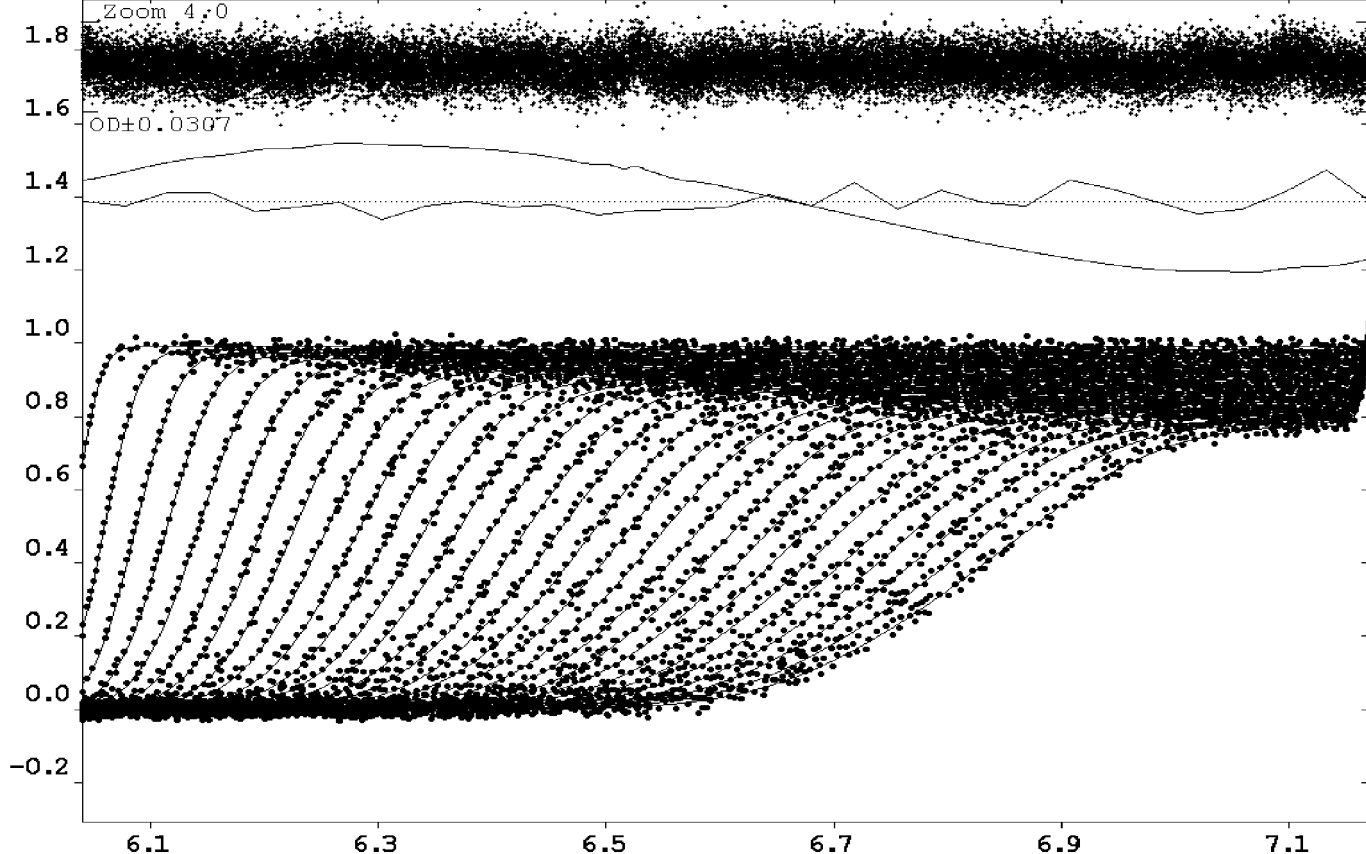
The right number n in the border line designate the smoothing cycles
The starting trace number can be enhanced by pressing the key <,
vice versa by pressing > the number can reduced again.

This feature is suitable to find the best resolution

The parameter are: first component friction ratio 1.2 and Mw. 70 kDa,
second component friction ratio 1.4 and Mw. 115 kDa, psv=0.73, 20° C
Concentration of both components are 0.5

Radius independent and time independent noise were also added.

Time independent noise were eliminated by subtraction of the contour
at the first data trace as baseline from all other traces. The influence
of sedimentation was abolished by cutting off the corresponding file
pieces at the begin and at the end of the trace.



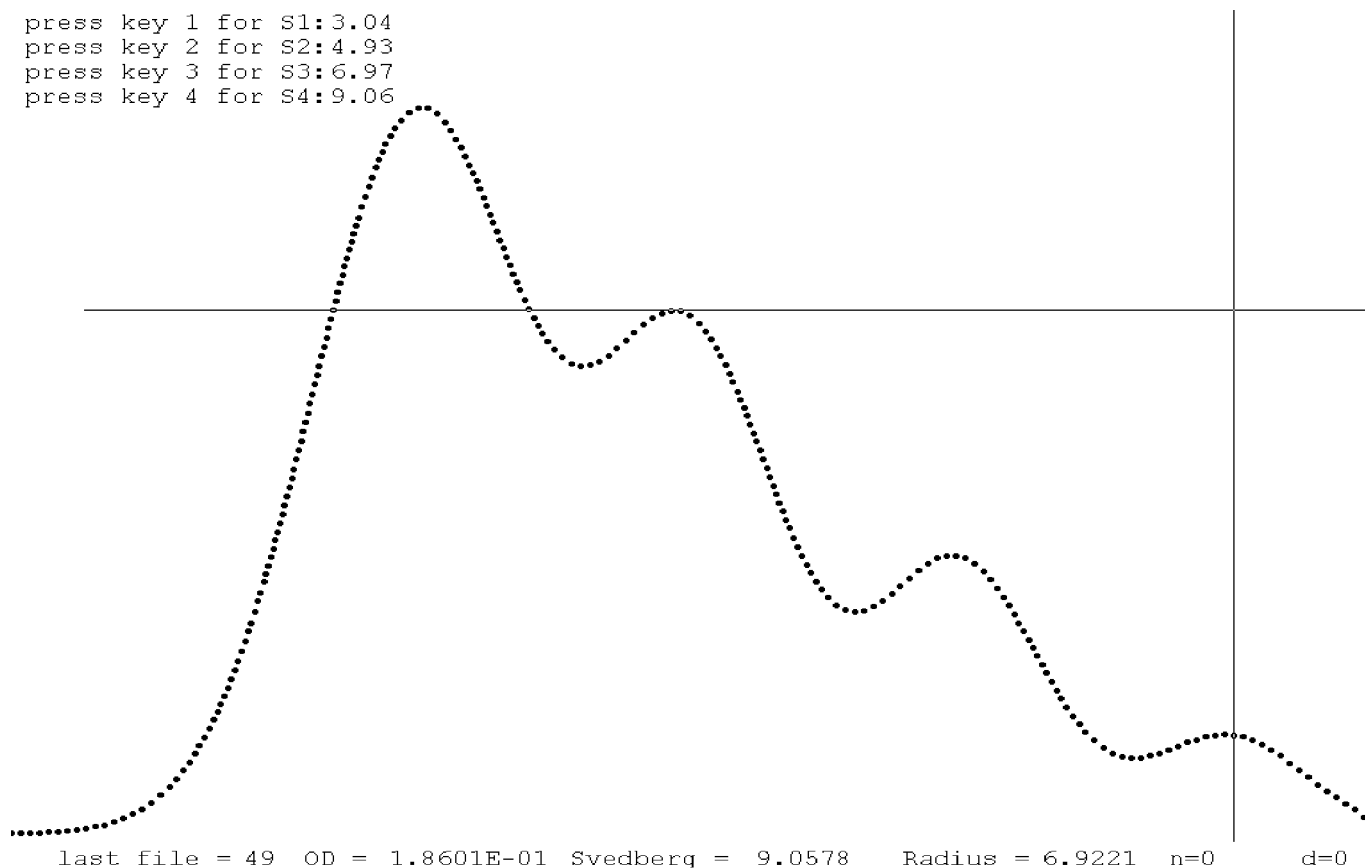
LAMM (N = correct air + bottom boundary) (interference) Date: 26:3:2008
 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
 29 30

S=5.04 6.02 0.00 0.00 D= 6.507 4.727 0.000 0.000 Rm=6.0 Rb=7.2 TD: 240
 F:\210000\210002\00031

Parameter(total/estimated)/tau(last set)	10/ 8	2.3389E-01
Data points / files / TI-noise	32799/(2- 30)	FALSE
Cell,temperature,wavelength,rotor:rpm,rpc	1 20.0 220	55000 1
Iterations/faulted/part.spec.vol./load.conc	6/ 0 0.7300	FALSE
Maximal iterations/OD-max/slit speed(mm/sec)	200 1.02	0.0000
Radius range / rpm / outliers corrected	6.0410-7.1710	55000 N
Test for validity<1/no.of data file/RI-noise	0.140 29	TRUE
Cancel outliers/start time(s)/TI-noise subtract. N	300	TRUE
Standard deviation(rmsd)/time diff./SSR	1.0229E-02 240	3.4310E+00
Damping parameter,start / epsilon	4.5415E-01 5.00	2.1867E-03

PARAMETER	VALUE	STD.DEVIATION	START VALUES
Initial absorbance	4.8427E-01	8.5609E-03	5.5542E-01
Svedberg coeff. (Svedb.units)	5.0219E+00	1.0141E-02	4.7006E+00
Base line	-1.0132E-01		-2.1161E-01
Meniscus radius	6.0002E+00	6.2585E-05	6.0000E+00
Bottom radius	7.2003E+00	1.5201E-04	7.1880E+00
Slope correction(turn)	0.0000E+00		0.0000E+00
Diffusion coefficient	6.5576E-07	3.7498E-09	6.9171E-07
Molecular mass/stdv/(psv*rho)	6.9150E+04	4.1936E+02	7.3000E-01
Stokes radius(water) (m)/excluded volume(L/g)	3.2459E-09		4.9922E-03
Water thicknis from stokes radius(m)//-Vol/g	5.3133E-10		5.1804E-01
Friction ratio/viscosity/rho	1.1958E+00	1.0086E+00	1.0000E+00
2.Initial absorbance	5.1674E-01	8.5757E-03	5.5542E-01
2.Svedberg coeff. (Svedb.units)	6.0011E+00	7.5794E-03	5.8758E+00
2.Diffusion coefficient	4.8222E-07	4.4103E-09	6.1868E-07
Molecular mass/stdv/(psv*rho)	1.1237E+05	1.0375E+03	7.3000E-01
Stokes radius(water) (m)/excluded volume(L/g)	4.4140E-09		7.7257E-03
Water thicknis from stokes radius(m)//-Vol/g	1.2226E-09		1.2014E+00
Friction ratio/viscosity/rho	1.3831E+00	1.0086E+00	1.0000E+00

press key 1 for S1:3.04
 press key 2 for S2:4.93
 press key 3 for S3:6.97
 press key 4 for S4:9.06



Sedimentation distribution plot of the radial derivative according to Bridgman

The left top corner show the s-values accepted from the program LAMM obtained by pressing the designated keys

The data points are equidistant by interpolation, but the original data remain unchanged.

The data were then differentiated by fitting a sliding 26 point centered, parabel. The smoothing takes place again with a sliding 26 point parabel fitting. This may be valid also for the time difference method.

The number of smoothing repeats can be chosen (see below).

The bottom border line show from left to right

The file used for the distribution plot, can be chosen by its number

The $g^*(s)$ value in dependence of the horizontal line of cross hairs

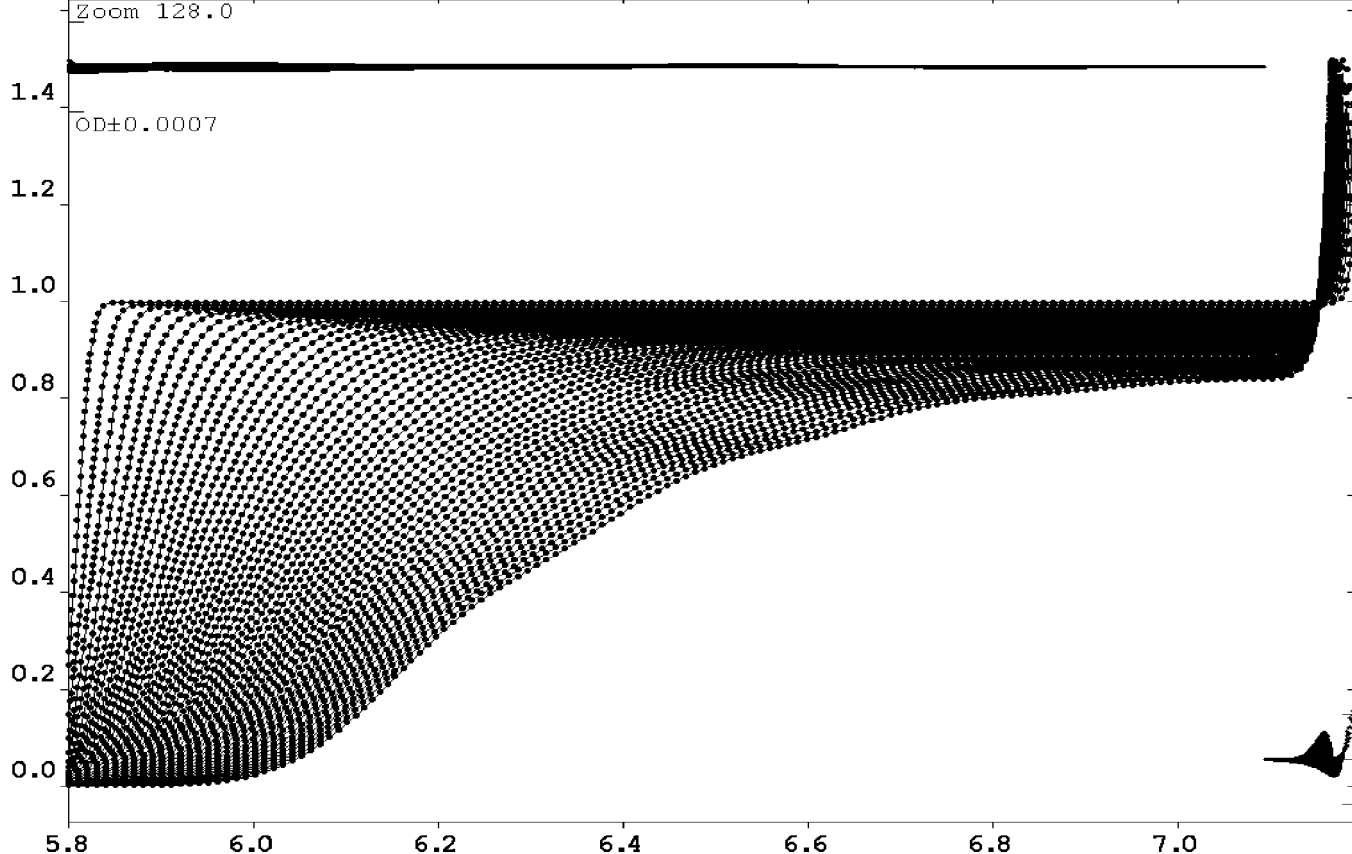
The value of sedimentation coefficient in dependence of vertical line

The corresponding radius in the Bridgman relation

The number of repeats for smoothing can be chosen, standard $n=100$

The two substracted files in the time variant are neighbours, $d=1$

The corresponding starting values for diffusion coefficients and amplitudes were calculated by LAMM

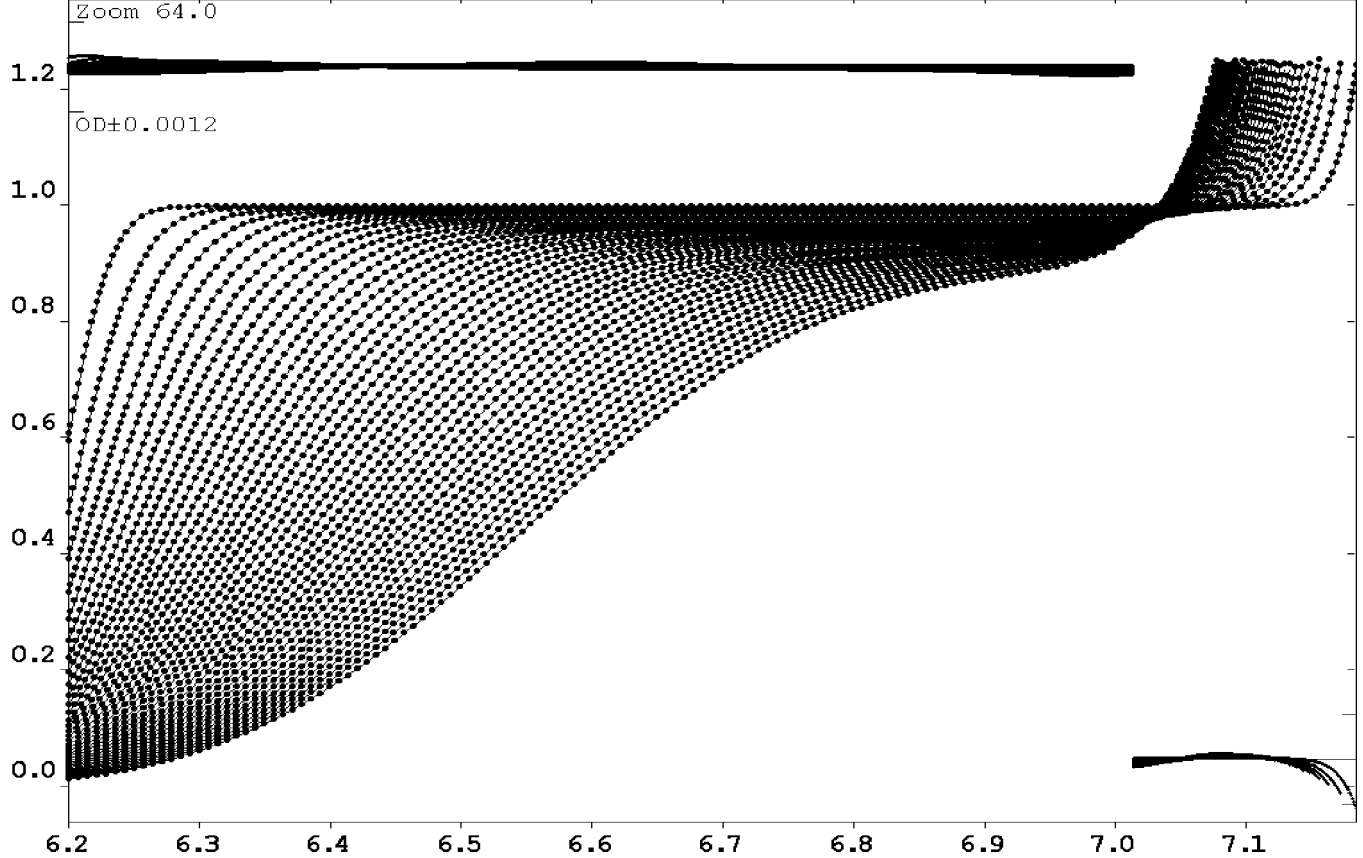


LAMM (N = correct air + bottom boundary) (absorption) Date: 1:2:2006
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50
 Ns:0.000 Amp:0.50 0.30 0.15 0.05 PSV:0.730 R-R:0.0010 T-R:0.100 ts: 100
 S=3.00 5.00 7.00 9.00 D= 8.44 6.54 5.53 4.87 Rv:1.20 M=5.8 B=7.2 T: 100
 D:\daten\Lamm04\163000\00051

Parameter(total/estimated)/tau(last set)	16/15	1.1840E-01
Data points / files / TI-noise	68696/(1- 50)	FALSE
Cell,temperature,wavelength,rotor:rpm,rpc	9 20.0 220	60000 1
Iterations/faulted/part.spec.vol./load.conc	5/ 0 0.7300	FALSE
Maximal iterations/OD-max/slit speed(mm/sec)	200 1.50	0.0000
Radius range / rpm / outliers corrected	5.8000-7.1950	60000 N
Test for validity<1/no.of data file/RI-noise	0.453 50	FALSE
Cancel outliers/start time(s)/TI-noise subtract. N	100	FALSE
Standard deviation(rmsd)/time diff./SSR	2.9658E-05 100	6.0413E-05
Damping parameter,start / epsilon	5.1993E-01 5.00	4.2323E-03

PARAMETER	VALUE	STD.DEVIATION	START VALUES
Initial absorbance	4.9994E-01	2.9506E-06	4.4748E-01
Svedberg coeff. (Svedb.units)	2.9993E+00	7.6903E-06	3.0540E+00
Base line	-9.9120E-05	4.6560E-07	0.0000E+00
Meniscus radius	5.8000E+00	1.0881E-07	5.8000E+00
Bottom radius	7.2001E+00	1.0351E-07	7.1950E+00
Rotation parameter	0.0000E+00		0.0000E+00
Diffusion coefficient	8.4291E-07	3.2127E-12	7.6107E-07
Molecular mass/stdv/(psv*rho)	3.2130E+04	1.4759E-01	7.3000E-01
Stokes radius(water)(m)/excluded volume(L/g)	2.5252E-09		5.0590E-03
Water thicknis from stokes radius(m)//-Vol/g	4.2271E-10		5.3475E-01
Friction ratio/viscosity/rho	1.2011E+00	1.0086E+00	1.0000E+00
2.Initial absorbance	3.0035E-01	3.9703E-06	3.2050E-01
2.Svedberg coeff.(Svedb.units)	5.0000E+00	1.7252E-05	4.9149E+00
2.Diffusion coefficient	6.5536E-07	1.4320E-11	5.9993E-07
Molecular mass/stdv/(psv*rho)	6.8891E+04	1.5240E+00	7.3000E-01
Stokes radius(water)(m)/excluded volume(L/g)	3.2479E-09		5.0203E-03
Water thicknis from stokes radius(m)//-Vol/g	5.3675E-10		5.2507E-01
Friction ratio/viscosity/rho	1.1980E+00	1.0086E+00	1.0000E+00
3.Initial absorbance	1.4978E-01	4.2659E-06	1.6866E-01
3.Svedberg coeff.(Svedb.units)	7.0006E+00	2.9557E-05	7.0004E+00

3.Diffusion coefficient	5.5248E-07	2.9951E-11	5.0269E-07
Molecular mass/stdv/(psv*rho)	1.1441E+05	6.2216E+00	7.3000E-01
Stokes radius(water) (m)/excluded volume(L/g)	3.8527E-09	3.8527E-09	5.0453E-03
Water thicknis from stokes radius(m)//-Vol/g	6.4201E-10	6.4201E-10	5.3132E-01
Friction ratio/viscosity/rho	1.2000E+00	1.0086E+00	1.0000E+00
4.Initial absorbance	5.0034E-02	2.9549E-06	5.9817E-02
4.Svedberg coeff.(Svedb.units)	8.9997E+00	5.8028E-05	9.0577E+00
4.Diffusion coefficient	4.8941E-07	6.2089E-11	4.4193E-07
Molecular mass/stdv/(psv*rho)	1.6605E+05	2.1093E+01	7.3000E-01
Stokes radius(water) (m)/excluded volume(L/g)	4.3492E-09	4.3492E-09	5.0014E-03
Water thicknis from stokes radius(m)//-Vol/g	7.1418E-10	7.1418E-10	5.2034E-01
Friction ratio/viscosity/rho	1.1965E+00	1.0086E+00	1.0000E+00



LAMM (N = correct air + bottom boundary) (absorption) Date: 1:2:2006
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
 28 29 30 31 32 33 34 35 36 37 38 39 40
 Ext=1.000;Ampl:1.000 R-RASTER: 1.00E-03 T-RASTER:0.10 start time: 400
 Noise=0.0000 S=2.00 D=10.000 Rm=6.2000 Rb=7.2000 time differ.: 400
 C:\temp\091503\085340\00041
 Parameter(total/estimated)/tau(last set) 7/ 6 1.1213E-01
 Data points / files / TI-noise 36381/(1- 40) FALSE
 Cell,temperature,wavelength,rotor:rpm,rpc 9 20.0 220 40000 1
 Iterations/faulted/part.spec.vol./load.conc 4/ 0 0.7300 FALSE
 Maximal iterations/OD-max/slit speed(mm/sec) 200 1.25 0.0000
 Radius range / rpm / outliers corrected 6.2000-7.2000 40000 N
 Test for validity<1/no.of data file/RI-noise 0.138 40 FALSE
 Cancel outliers/start time(s)/TI-noise subtract. N 400 FALSE
 Standard deviation(rmsd)/time diff./SSR 6.9692E-05 400 1.7667E-04
 Damping parameter,start / epsilon 8.3855E-01 5.00 1.4826E-02

PARAMETER	VALUE	STD.DEVIATION	START VALUES
Initial absorbance	1.0010E+00	2.0784E-06	9.9720E-01
Svedberg coeff. (Svedb.units)	1.9971E+00	5.2469E-06	2.0162E+00
Base line	-1.0148E-03	2.0190E-06	0.0000E+00
Meniscus radius	6.2000E+00	4.2343E-07	6.2000E+00
Bottom radius	7.2001E+00	5.6899E-07	7.2000E+00
Rotation parameter	0.0000E+00		0.0000E+00
Diffusion coefficient	9.9856E-07	6.7102E-12	7.5450E-07
Molecular mass/stdv/(psv*rho)	1.8060E+04	1.3030E-01	7.3000E-01
Stokes radius(water)(m)/excluded volume(L/g)	2.1316E-09		5.4137E-03
Water thicknis from stokes radius(m)//-Vol/g	3.9646E-10		6.2343E-01
Friction ratio/viscosity/rho	1.2285E+00	1.0086E+00	1.0000E+00
Archibald mass at meniscus/bottom (1.File)	1.8052E+04		1.8071E+04

The program LAMM

The program LAMM can fit maximal 500 concentration files simultaneously. One file can contain maximal 2200 data points. Some windows variants need the system option "border line automatically in the back ground" else the border line can appear on the graphic screen. The program reads XLA-files in the old or new version and also the interference files of the XLI.

The organization of the file input is oriented on the output of the XLA/I. That means at first the program reads the date of the experiment as subdirectory and then the subdirectory named by the time of experiment in which the actual data files are stored. Therefore after the file type is chosen (absorption or interference) and some useful program options are passed the **device** (with pathway) and the name of the folder which contain the date subdirectories must be given. The wanted one is chosen by a mouse click. A list with the time directories is now presented and the wanted is again selected by a mouse click. The data files can be loaded all simultaneously or step by step. They are shown in a graphic on the monitor screen. In the first mode the wanted part of the files and the meniscus and bottom radii are given in for all together whereas in the second mode the meniscus and bottom radius is given in at the first concentration profile loaded but the wanted part for fit can be chosen separately for each data file. Moving the cross hairs to the wanted radial value chooses the corresponding radii. The mouse with simultaneously pressing the left button will move the crosshairs. The cross-hairs can alternatively moved with the arrow keys on the keyboard. By simultaneously pressing the shift key you can accelerate the moving. Press now the key **A** for the wanted start radius and **E** for the end radius of the fitting region, respectively. Additionally the meniscus position and the bottom position have to inspire by pressing the key **M** (meniscus) and **B** (bottom). By other model functions as N the bottom position is a dummy parameter. In the one by one mode the program loads successively all files but the meniscus and bottom radius must be inspired only at the first trace. Furthermore you can finish untimely the further loading of data files by pressing the keys A and E at the same radius position. For the fit of Faxen type model functions it is possible to cut up the traces by input of the wanted radius position. The data points cut off are stored and can reactivated.

To enable the reading of data sets with more than one wavelength it is possible to choose the start file and the following sequence. If p.e. records are made at three wavelengths each 3. data file has the same wavelength. The start file determines the wavelength for the following files.

If a trace is stored at low rotation speed (p.e.3000 rpm) it is possible to determine the loading concentration (absorption). Alternatively also the first nearly flat concentration profile recorded at very short time can be used. If the data are very noisy and additionally the meniscus is not depleted it is of advantage to know the loading concentration and held this parameter constant during the fit. At the begin of the program there is an option to load the low rpm file in the absorption mode to get this information (only for absorption data). Again with the cross hairs the wanted part of the low-rpm trace can be determined (pressing key A and E). In the interference

mode the program select from the chosen part the important skyline of the trace and subtracted this contour (baseline) from all data traces.

Additional it is possible to load two data sets with different rotor speeds but identical meniscus, bottom radius and loading concentration. After loading the last data file of a set you can stop the loading process or you can load one new data set with a new name with other rotor speed.

The **sedimentation distribution** (see later) $g^*(s)_t$ will be calculated with the first loaded data set alone.

Optional it is possible to correct pull outs by interpolation.

A special point is the choice of the slit velocity of the XLA. For molecules with quick sedimentation velocity this parameter has some influence especially in the first concentration profiles. The recording time for one trace takes some minutes depending dominantly on the length of the liquid column in the cell and some other parameters p.e. repeat rate by absorption measurements. Accurate details to this problem fail in the XLA-file header or in the manual of the XLA. The program allows to consider or to simulate the influence of this technical parameter. A slit velocity of 0.025 cm/sec is the usual value for radial space of 0.003 cm and no replication of measurements. For smaller space the speed is adequate slower. By input of 'zero slit speed' this correction is switched off. This is automatically done by interference data files.

The program allows to eliminate radial independence errors (**RI-noise**) for all 9 model functions. This means the estimation of local baseline offsets for each data trace.

For **interference** data it is possible to eliminate additionally the time independent (**TI-noise**) errors. Both errors are eliminated according to Schuck and Demeler³ adapted to our iteration method according to Levenberg/Marquardt, which works with derivatives of the model functions in contrast to the simplex method used by Schuck or Demeler. To avoid the linear dependence of the two parameters (TI and RI) contrary to Schuck/Demeler the sum of the local base lines (RI noise) was set to zero. Therefore our procedure allows the use of data sets with different length typical for concentration profiles with higher molecular mass. For **interference data** the subtraction of the sky line (contour, baseline) of a trace stored at low rotation speed (p.e. 3000 rpm) is the better method, which works with more accuracy (especially at low number of data traces) and more over somewhat faster. Alternatively, the first data trace stored in the shortest time possible may be used. In this case only the plateau region may be taken without a small part in the meniscus and bottom region to cancel the influence of sedimentation. The radial derivation and the time extrapolation are now possible without falsification by TI or RI noise. The loading concentration is altered by this procedure and must be estimated as usual.

By using of the model function **N** and **C** up to **four independent species** can be fitted. The start values will be find out by use of the $g^*(s)$ plot (sedimentation coefficient distribution) offered by the program. It can be chosen between radial and time derivative version. The radial version is of advantage when the TI-noise was abolished by the subtraction method. In this case the existence of RI-noise does not affect the quality of the derivatives. In the boarder line the following information are given (from left to right). The number of the last file included in the derivative calculation, the intensity of the signal, the Svedberg coefficient (both connected with the cross-hairs), the value of radius used in the Bridgman² relation, the number of smoothing cycles, and at least the file distance of the two traces used for time derivation. By means of the cross hairs and the number keys (1-4) the peaks (s-

values) are selected and stored. The number of independent species is simultaneously determined by this. The resolution can be enhanced by time extrapolation according to STAFFORD¹.

Now the program asks for the partial specific volume and the density of the solution to calculate the molecular weight by the Svedberg formula. Additionally the program ask for the viscosity of the solution to calculate the friction ratio and the stoke radius. As default the viscosity of water temperature dependent is implemented in the program.

The wanted model function will be selected by pressing the designated key. After this the multitude of **not** to estimating parameters is to feed in and then to choice what parameters you mean. This will be done by the number (sequence) of the parameters shown just before separated by space. Instead of the **diffusion** parameter also the **molecular weight** can be presented. The diffusion parameter will then calculated by the program by use of the Svedberg equation with the currently estimated sedimentation coefficient. In this case it is also possible to give in additionally the **friction ratio**. The program then calculates the sedimentation and diffusion coefficient by use of the molecular weight, viscosity and specific volume. When the sedimentation parameter is also held constant during the fit only the loading concentration (absorbance) and the meniscus and bottom radius are to estimate. This possibility can be of advantage by fitting more than one independent species for a first attempt. If instead of the **diffusion coefficient** a zero is given in the program ask later for the **friction ratio**. The program held then this ratio constant during the fit and calculates the diffusion coefficient from the current estimated sedimentation coefficient with the help of the given friction ratio.

Now the program tries to fit the data. The fit progress is shown graphically.

On the bottom of the screen appears a border, which contain 7 numbers.

1. Number of files
2. The square sum of the residuals (SSR)
3. The last improvement of SSR
4. The last improvement of SSR which is necessary to finish the iteration
5. The square sum of the scaled parameter improvements (the solutions of the scaled information matrix) which is additional necessary to finish the iteration
6. The last improvement of this square sum
7. The current damping parameter λ which must be <1 to finish the iteration

On the top of the screen the result of every iteration is given out. The sequence of the parameters is the same as in the parameter input or output (like result printing) and designated by the following letters:

a = amplitude

s = sedimentation parameter

z = zero line

m= meniscus radius

b = bottom radius

r = rotation parameter

D = diffusion parameter

μ = parameter in $s_c = s_0 - \mu \cdot C / C_{load}$

To avoid endless iterations two other criteria are enclosed. The iteration is also finished either the last improvement of SSR is 0.01 times smaller as mentioned

above or the last improvement of the parameter square sum is 0.1 times smaller as in the triple condition mentioned above (4, 5 and 7).

After finishing the fit press ESCAPE to continue the program. The fit procedure can alternatively **finished by pressing key 'I'**.

Now the results are shown on the monitor. By use of the function **N** it is taken into account that one restriction holds for the approximate whole boundary solution of the LAMM equation. The solution is composed by two independent solutions consider either meniscus or bottom boundary. This restriction means that meniscus trace and bottom trace should not be influence mutual the corresponding boundary condition markedly. Therefore in the results the actual value of a test parameter is printed out. If this value exceeds the value 1 one or some of the last data sets has to be omitted. The number of the data file, which exceeds firstly the limit value, is also printed out. It is favorable to use high rotor speed (at the same column length) to obtain lower ε and therefore lower mutual influence.

After showing the values of the estimated parameters the final graphic is presented (press enter). By pressing the keys CTRL+H you can write additionally text horizontally in the graphic beginning at the cross hairs. By pressing the keys CTRL+V this can be done vertically. The graphic can be printed out by **pressing the keys CTRL + L (hard copy)**. In the left corner of the screen are shown (i) the amplifying factor for the residues relative to the concentration profiles and (ii) the OD scale belonging to the two yellow tixes.

After pressing ESCAPE a list of some possibilities (options) is shown. By **pressing the key D** the results will be printed out including the graphic (if CTRL+L was pressed before) and the paper sheet should be ejected. By pressing key **X** only the picture will be printed out. This possibility is of advantage when more than two species are included because the data print plus graphic do not suit on one page.

A complete output is shown at the begin of this instruction. A file set simulated according to Claverie was fitted with the whole boundary function **N**. The parameter chosen for simulation are shown at the top of the output. The residuals are plotted with 64 fold amplification compared to the data files.

At the end an example for a fit of **4 independent species** is shown together with a hardcopy of the sedimentation distribution plot shown on the screen.

The **option T** represents the possibility to show the two traces, which constitute the whole boundary trace. These two curves are the meniscus part, which represent a Faxen type solution of the Lamm equation, which fulfills the meniscus boundary condition and the bottom part, which fulfills the bottom boundary condition. In the approximate solution used here both curves are taken as independent from another. That means the Faxen solution may be already in the plateau region when the bottom part is already nearly zero.

With the **options S, H and A** it is possible to leave out some data sets from the parameter estimation procedure. The numbers of the files are the numbers on the left hand side of the screen. The option **L** allowed to define a maximal amplitude (OD or fringes) for all date files. Additionally it recommends a maximal amplitude calculated from the greatest slope acceptable for the used model functions **N or R**. With the **option O** all omitted files and data points are restorable.

Additional to the calculation of the mole mass by the Svedberg relation the mass is additionally calculated by the Archibald method. This is done by use of the estimated parameter of the used model function and its differentiated form. Corresponding to

the used model function the Archibald mass is calculated for the meniscus or bottom radius or both but only for the first concentration profile.

The stoke radius and from this the excluded volume are calculated. The friction of the solvent water is taken into account temperature dependent. But the true friction can be given in. Furthermore the friction ratio is calculated.

An additionally possibility is the generation of a graphic (plot) file in the HPGL language (vector graphic) with **option Z**. The position of the plot can be chosen by offset x and y values (in pixels). By choice the additional text in the screen graphic can be drawn within the plot. Additional residual plots can be generated. Up to nine residual plots can be drawn in one picture with four different extensions. The options 'C' and 'G' (gradient curves) ,residual graphics, RI and TI-noise are also included in this possibility. Each HP-Laser-printer accepts such HGL-files.

1. Stafford WF (1992) Methods for obtaining sedimentation coefficient distributions.
In: Harding SE, Rowe AJ, Horton JC (eds) Analytical Ultracentrifugation in Biochemistry and Polymer Science p 359-393
2. Bridgeman WB (1942) J Am Chem Soc 64: 2349
3. Schuck P, and B Demeler Biophys (1999) Biophys. J. 76: 2288-2296
4. Fujita H Foundations of Ultracentrifugal Analysis
John Wiley, New York 1975
5. Fujita H Mathematical Theory of Sedimentation Analysis
Academic Press, New York 1962
6. Hiester NK and T Vermeulen (1952) Chem. Eng. Progr. 48: 505-516
7. Philo JS (1997) Biophys. J. 72: 435-444
8. Holladay LA (1979) Biophys. Chem. 10: 187-190
9. Behlke J and O Ristau (2002) Biophys. Chem. 95: 59-68

The program **LAMM** contains 9 model functions. The first four equations (**P**, **S**, **H**, **R**) are suitable for synthetic boundary experiments. The so-called diffusion time τ_d implemented in the XLA (I) output can be used by choice. The diffusion time works in the divisors of the error functions containing the diffusion coefficient (by function R only in the first error function),

$$\sqrt{\varepsilon\tau} = \frac{2}{r_m} \sqrt{Dt} \quad \text{and} \quad \sqrt{\varepsilon(e^{\tau_d} - 1)}$$

but not in the transport terms, the numerator of the error functions. Tests with synthetic traces (Claverie) have shown that this statement can well describe a pure diffusion process.

Function P. Fujita⁴ 1975, Eq.2.127

$$c_r = \frac{c_0 e^{-\tau}}{2} \operatorname{erfc}\left(\frac{z - \tau}{2\sqrt{\varepsilon\tau}}\right), \quad z = 2\ln\left(\frac{r}{r_m}\right), \quad \tau = 2\omega^2 s t, \quad \varepsilon = \frac{2D}{s\omega^2 r_m^2} \quad (1)$$

Function S. Fujita⁵ 1962, Eq.2.191 With consideration of concentration dependent sedimentation.

$$x = \frac{r^2}{r_m^2}, \quad g = \sqrt{1 - \alpha(1 - e^{-\tau})}, \quad \varepsilon = \frac{2D}{s_0 \omega^2 r_m^2}, \quad s_c = s_0 \left(1 - \alpha \frac{c}{c_0}\right)$$

$$c_r = \frac{c_0 e^{-\tau} \left(\sqrt{x} - 0.25\varepsilon(e^{\tau/2} - e^{-\tau/2}) \right) \operatorname{erfc}(p) e^{p^2} + g\sqrt{\varepsilon(e^{\tau} - 1)}/\pi}{g^2 \sqrt{x} e^{p^2} (2 - \operatorname{erfc}(p)) + g \operatorname{erfc}(\xi) e^{\xi^2}} \quad (2)$$

$$\xi = \frac{e^{\tau/2} - \sqrt{x} + 0.25\varepsilon(e^{\tau/2} - e^{-\tau/2})}{\sqrt{\varepsilon(e^{\tau} - 1)}}, \quad p = \frac{g^2 e^{\tau/2} - \sqrt{x} + 0.25\varepsilon(e^{\tau/2} - e^{-\tau/2})}{g\sqrt{\varepsilon(e^{\tau} - 1)}}$$

Function H. According to Hiester and Vermeulen⁶ (Fujita 1975, Eq. 2.167). This is a very accurate formula.

$$c_r = \frac{c_0 e^{-\tau}}{2} \left\{ \operatorname{erfc}(\xi) + \frac{\sqrt{2\varepsilon \sinh(\tau/2)}}{x^{1/4} \left[1 + (xe^{-\tau})^{1/4} \right] \sqrt{\pi}} e^{-\xi^2} \right\} \quad (3)$$

The symbols have the same meaning as in Eq.2.

The next function is also suitable for synthetic boundary but with consideration of **bottom boundary**. It follows from function **N** (see later) without terms 2 and 3 responsible for the meniscus boundary.

Function R⁹

$$\begin{aligned} \frac{2ce^\tau}{c_0} = \operatorname{erfc} & \left(\frac{e^{\tau/2} - 0.5w + 0.25\varepsilon(e^{-\tau/2} - e^{\tau/2})}{\sqrt{\varepsilon(e^\tau - 1)}} \right) \\ & - \operatorname{erfc} \left(\frac{-z + d\tau}{2\sqrt{\varepsilon_b \tau}} \right) - \frac{1}{1-d} \exp \left(\frac{dz}{\varepsilon_b} \right) \operatorname{erfc} \left(\frac{-z - d\tau}{2\sqrt{\varepsilon_b \tau}} \right) \\ & + \frac{2-d}{1-d} \exp \left(\frac{(1-d)\tau + z}{\varepsilon_b} \right) \operatorname{erfc} \left(\frac{-z - (2-d)\tau}{2\sqrt{\varepsilon_b \tau}} \right) \end{aligned} \quad (4)$$

For definition of the symbols see function N.

The next four model functions (**D**, **O**, **C**, **M**) are suitable for standard double sector cells with consideration of meniscus boundary. The last function (**M**) considers additionally the concentration dependence of sedimentation coefficient.

Function D. Philo⁷ 1997 developed an improved form of the Fujita-MacCosham equation.

$$\begin{aligned} c_r = \frac{c_0 e^{-\tau}}{2} & \left\{ \operatorname{erfc}(p(1 + \alpha\tau)) - 2\sqrt{\frac{\tau}{\pi\varepsilon}} e^{-p^2(1+\beta\varepsilon\tau)} + \left(1 + \frac{\tau + \ln x}{\varepsilon} \right) \operatorname{erfc} \left(\frac{\tau + \ln x}{\sqrt{4\varepsilon\tau}} \right) e^{\left(\frac{\ln x}{\varepsilon} \right)} \right\} \\ p = \frac{\tau - \ln x}{\sqrt{4\varepsilon\tau}}, \quad \alpha = 0.2487, \quad \beta = 2, \quad \varepsilon = \frac{2D}{s\omega^2 r_m^2} \end{aligned} \quad (5)$$

Function O. This is an equation developed by Holladay⁸ 1979.

$$c_r = \frac{c_0}{2} e^{-\tau} \left\{ \operatorname{erfc} \left(\frac{\tau\alpha - z}{2\sqrt{a\varepsilon\tau}} \right) - \frac{a}{e} \exp \left(\frac{z\alpha}{a\varepsilon} \right) \operatorname{erfc} \left(\frac{\tau\alpha + z}{2\sqrt{a\varepsilon\tau}} \right) + \frac{\gamma}{\varepsilon} \exp \left(\tau + \frac{z}{\varepsilon} \right) \operatorname{erfc} \left(\frac{\tau\gamma + z}{2\sqrt{a\varepsilon\tau}} \right) \right\} \quad (6)$$

$$\alpha = a - \varepsilon, \quad \gamma = a + \varepsilon, \quad z = \left(\frac{r}{r_m} \right)^2 - 1, \quad a = \frac{e^\tau - 1}{\tau}$$

Function C⁹: This is the best function to analyze experiments obtained in standard double sector cells and considering the meniscus boundary condition only (Behlke and Ristau 2002).

$$\begin{aligned} \frac{2ce^\tau}{c_0} = & \operatorname{erfc} \left(\frac{e^{\tau/2} - 0.5w - 1 + 0.25\varepsilon(e^{-\tau/2} - e^{\tau/2})}{\sqrt{\varepsilon(e^\tau - 1)}} \right) \\ & - \frac{1}{(1-b)} \exp \left(\frac{bw}{\varepsilon} \right) \operatorname{erfc} \left(\frac{w + 2b(e^{\tau/2} - 1)}{2\sqrt{2\varepsilon(e^{\tau/2} - 1)}} \right) \\ & + \frac{(2-b)}{(1-b)} \exp \left(\frac{w + 2(e^{\tau/2} - 1)(1-b)}{\varepsilon} \right) \operatorname{erfc} \left(\frac{w + 2(2-b)(e^{\tau/2} - 1)}{2\sqrt{2\varepsilon(e^{\tau/2} - 1)}} \right) \end{aligned} \quad (7)$$

$$w = 2 \left(\frac{r}{r_m} - 1 \right), \quad b = 1 - \varepsilon/2$$

Function M⁹. This variant is developed for concentration dependent sedimentation. It combines the Fujita-formula S with the last two terms of function C but with slightly changed time terms (only τ instate of $2(\exp(\tau/2)-1)$). This model function is suitable to study the concentration dependence of sedimentation coefficient from concentration profiles with sufficient plateau region.

$$\begin{aligned} c_r = & \frac{c_0 e^{-\tau}}{g^2 \sqrt{x}} \frac{(\sqrt{x} - 0.25\varepsilon(e^{\tau/2} - e^{-\tau/2})) \operatorname{erfc}(p) e^{p^2} + g \sqrt{\varepsilon(e^\tau - 1)} / \pi}{e^{p^2} (2 - \operatorname{erfc}(p)) + g \operatorname{erfc}(\xi) e^{\xi^2}} \\ & + \frac{c_0 e^{-\tau(1-\alpha)}}{2} \left\{ - \frac{1}{(1-b)} \exp \left(\frac{bw}{\varepsilon} \right) \operatorname{erfc} \left(\frac{w + 2b(e^{\tau/2} - 1)}{2\sqrt{2\varepsilon(e^{\tau/2} - 1)}} \right) \right. \\ & \left. + \frac{(2-b)}{(1-b)} \exp \left(\frac{w + 2(e^{\tau/2} - 1)(1-b)}{\varepsilon} \right) \operatorname{erfc} \left(\frac{w + 2(2-b)(e^{\tau/2} - 1)}{2\sqrt{2\varepsilon(e^{\tau/2} - 1)}} \right) \right\} \end{aligned} \quad (8)$$

$$\begin{aligned}
x &= \frac{r^2}{r_m^2}, \quad \varepsilon = \frac{2D}{s_0 \omega^2 r_m^2}, \quad w = 2 \left(\frac{r}{r_m} - 1 \right) (1 + 0.475\alpha) \\
s_c &= s_0 \left(1 - \alpha \frac{c}{c_0} \right), \quad g = \sqrt{1 - \alpha(1 - e^{-\tau})}, \quad b = 1 - \frac{\varepsilon}{2} \\
\xi &= \frac{e^{\tau/2} - \sqrt{x} + 0.25\varepsilon(e^{\tau/2} - e^{-\tau/2})}{\sqrt{\varepsilon(e^\tau - 1)}}, \quad p = \frac{g^2 e^{\tau/2} - \sqrt{x} + 0.25\varepsilon(e^{\tau/2} - e^{-\tau/2})}{g\sqrt{\varepsilon(e^\tau - 1)}}
\end{aligned}$$

The supplement factor to w (pure α -relations) is an empirical improvement.

Function N^9 is a whole boundary solution for normal standard sector cells according to Behlke and Ristau 2002.

$$\begin{aligned}
\frac{2ce^\tau}{c_0} &= \operatorname{erfc} \left(\frac{e^{\tau/2} - 0.5w - 1 + 0.25\varepsilon(e^{-\tau/2} - e^{\tau/2})}{\sqrt{\varepsilon(e^\tau - 1)}} \right) \\
&\quad - \frac{1}{(1-b)} \exp \left(\frac{bw}{\varepsilon} \right) \operatorname{erfc} \left(\frac{w + 2b(e^{\tau/2} - 1)}{2\sqrt{2\varepsilon(e^{\tau/2} - 1)}} \right) \\
&\quad + \frac{(2-b)}{(1-b)} \exp \left(\frac{w + 2(e^{\tau/2} - 1)(1-b)}{\varepsilon} \right) \operatorname{erfc} \left(\frac{w + 2(2-b)(e^{\tau/2} - 1)}{2\sqrt{2\varepsilon(e^{\tau/2} - 1)}} \right) \\
&\quad - \operatorname{erfc} \left(\frac{-z + d\tau}{2\sqrt{\varepsilon_b \tau}} \right) - \frac{1}{1-d} \exp \left(\frac{dz}{\varepsilon_b} \right) \operatorname{erfc} \left(\frac{-z - d\tau}{2\sqrt{\varepsilon_b \tau}} \right) \\
&\quad + \frac{2-d}{1-d} \exp \left(\frac{(1-d)\tau + z}{\varepsilon_b} \right) \operatorname{erfc} \left(\frac{-z - (2-d)\tau}{2\sqrt{\varepsilon_b \tau}} \right)
\end{aligned} \tag{9}$$

$$b = 1 - \varepsilon/2, \quad w = 2 \left(\frac{r}{r_m} - 1 \right), \quad d = 1 - \varepsilon_b/2$$

$$z = 2 \frac{r}{r_b} \left(1 + a\varepsilon(e^{\tau/2} - 1) \right) - 2$$

$$\varepsilon_b = \frac{2D}{r_b^2 s \omega^2} \left(1 + c\varepsilon(e^{\tau/2} - 1) \right)$$

The first 3 terms are identical with function **C**. The quantities a and c are pure empirical improvements for the bottom part.

$$a = \frac{9.107}{p} \exp(-\ln(p)(0.1783 \ln(p) - 1)) + 2.4917$$

$$p = 1 + 84.08(\varepsilon - 0.01234)$$

$$c = \frac{0.2713}{h} \exp(-\ln(h)(0.02147 \ln(h) - 1)) - 0.091$$

$$h = 1 + 455.75(\varepsilon - 0.002539)$$