SEGAL, a semi-automatic program for fitting sedimentation equilibrium patterns from analytical ultracentrifugation

Gia Machaidze* **and Ariel Lustig**

M. E. Mueller Institute of Structural Biology, Biozentrum, University of Basel, Klingelbergstrasse 70, 4056 Basel, Switzerland

The paper describes a computer program (available for PC and Mac) for the evaluation of molecular weights from sedimentation equilibrium centrifuge runs using the Beckman XL-A/ XL-I ultracentrifuge. The program can be used with both absorption and interference optical records, and requires no prior assumptions about the nature of the system under investigation (ideal, non-ideal, monodisperse, polydisperse, self-associating etc.). The user can simply choose and change the range of patterns for fitting to the data and simultaneously see the fitting result until the correlation is optimum. It is possible to produce fits to two parts of the same curve if more than one component is expected. The program can be used for the analysis of ultracentrifugation results for 2 and 6 sector centrifuge cells. The program supports the export of fitted data as text files, and can save the fitted images. The user-friendly interface, easy curve manipulation and simultaneous fitting make the program attractive for a wide range of users.

Keywords: analytical ultracentrifugation, curve fitting, molecular mass determination, sedimentation equilibrium

1. INTRODUCTION

The SEGAL computer program for the evaluation of molecular masses by sedimentation equilibrium is based on the concept of numerical fitting of a sedimentation equilibrium pattern to determine the molecular weight profile of mono- and polydisperse solutions. This procedure gives results with very good correlation between the data and the fit, and is especially valuable for determining the molecular weights of samples that cover a wide range, which are difficult to fit with other procedures.

The SEGAL program, with its relatively simple and friendly interface, may furthermore be of general benefit to users who have found it difficult to use sedimentation equilibrium fitting procedures. The program can directly read the files generated by the Beckman analytical ultracentrifuge with both absorption and interference optics.

We have tested this procedure for many nontrivial cases, such as:

- Non-classical and non-ideal solutions containing additives like sucrose, detergents and EDTA, that cannot be dialysed;
- Biological samples in which mass is lost during ultracentrifugation (due to simple precipitation);
- Low molecular weight samples, or samples with fast diffusion that cannot be depleted in normal filled cells to define their baseline levels;
- Multicomponent solutions, etc.

*Corresponding author. Fax: +41 61 267-2189. E-mail: Gia.Machaidze@unibas.ch

The user can choose and change any suitable part of the sedimentation equilibrium (SE) pattern and simultaneously see the appropriate result. Among the extant computer programs to fit SE patterns in order to establish the molecular masses (based upon ideal systems only), the determination of the real baseline level is essential. It can easily be obtained from the optical baseline, if a dialysed sample and its external dialysate is used. However, there are many cases where it is impossible or impracticable to dialyse the sample, so that the apparent baseline is not the true one. In that case, to achieve the true baseline it is possible to deplete the dissolved particles in the centrifuge cell, so that the depleted part indicates the true optical zero, i.e. the real baseline. Unfortunately, for several reasons there are many cases where we can not deplete the substances to create a free region and hence a true baseline:

- Fast back-diffusion occurs in a relatively short column filled analytical ultracentrifuge (AUC) cell;
- Certain AUC cells cannot be spun at the maximum rotor speed, so that depletion is ineffective;
- Substrates bound to enzymes are not at the same concentration in the solvent and in the solution cell sector;
- Detergent micelle solutions are pressure-dependent and give rise to speed-dependent effects (see [1]);
- Additives like DTT or ATP may influence the total optical absorption of the sample or reference solution.

The characterization of certain molecular complexes requires that the run conditions are varied; a single SE run cannot reveal the molecular weight of a complex, nor of the

Journal of Biological Physics and Chemistry **6** (2006) 91–102
Received 4 April 2006; *accepted* 6 April 2006

dissociated monomer. Each run represents only an intermediate part of the sedimentation equilibrium behaviour.

Changing run parameters such as rotor speed, probe concentration, cell filling height, wavelength, optical system or temperature may contribute to a better overall characterization, especially of complex systems.

2. THEORY AND OPERATION

The SEGAL program evaluates molecular masses using sedimentation equilibrium (SE) patterns, based on numerically fitting the SE pattern to one or two exponentials, and determines the molecular weight (M_r) of mono and polydisperse particles in solution.

For an ideal noninteracting single component system (i.e. the simplest possible system), the equilibrium distribution obtained is an exponential function of the buoyant mass of the macromolecule, $M(1-\bar{\nu}\rho)$, where M is the molecular mass of the macromolecule, $\bar{\nu}$ the partial specific volume of the macromolecule particle, and ρ the density of the solution, as described by eqn (1) [3]:

$$c(\mathbf{r}) = c_0(\mathbf{r}_0)\exp\left\{M(1-\bar{\nu}\rho)\omega^2/[RT(\mathbf{r}^2-\mathbf{r}_0^2)]\right\} \quad (1)$$

where $c(\mathbf{r})$ is the sample concentration at radial position $\mathbf{r}$, c_0 the sample concentration at a reference radial position $\mathbf{r}_0$ and ω^2 the angular velocity.

After differentiation of eqn (1):

$$\frac{(1/a)\partial a}{\omega^2\mathbf{r}\partial\mathbf{r}} = \frac{M(1-\bar{\nu}\rho)}{RT}(a_\mathbf{r} - a_{\mathbf{r}_0})$$

whence

$$\frac{\mathrm{d}(\ln c)}{\mathrm{d}(\mathbf{r}^2)} = \frac{2RT}{M(1-\bar{\nu}\rho)\omega^2}, \quad (2)$$

we can determine the component mass:

$$M = \frac{2RT}{(1-\bar{\nu}\rho)\omega^2}\frac{\mathrm{d}(\ln c)}{\mathrm{d}(\mathbf{r}^2)}. \quad (3)$$

Using this formula, conservation of mass is immaterial, so any loss of protein during centrifugation (as often occurs with biological substances) does not significantly affect the apparent molecular weight of the remaining part.

Eqn (2) shows that a plot of ln (concentration) versus (radius)^2 yields a straight line with a slope proportional to the molecular weight: this will be the case for a non-aggregated monomeric protein solution. Alternatively, one can fit eqn (2) to the data plotted as c versus $\mathbf{r}^2$ to find the least-squares best estimate of $M(1-\bar{\nu}\rho)$.

For interference or absorption optical systems, if the measurements are in the linear range we have:

$$c(\mathbf{r}) = \mathrm{const}(a_\mathbf{r} - a_{\mathbf{r}_0})$$

where $a_\mathbf{r}$ is the ordinate of an interference fringe or an absorption curve measured from an arbitrarily chosen origin of scale in the $\mathbf{r}$ distance from the centre of rotation, and $a_{\mathbf{r}_0}$ is the true but nonobservable absorption baseline [2]. Hence

$$a(\mathbf{r}) - a_{\mathbf{r}_0} = Ec(\mathbf{r}).$$

E is proportional to the extinction coefficient of a given substance. Eqn (2) can be rewritten with these substitutions:

$$\frac{(1/a)\partial a}{\omega^2\mathbf{r}\partial\mathbf{r}} = \frac{M(1-\bar{\nu}\rho)}{RT}(a_\mathbf{r} - a_{\mathbf{r}_0}) \quad (4)$$

If $a_{\mathbf{r}_0}$ and $\frac{M(1-\bar{\nu}\rho)}{RT}$ are assumed to be constant, the solution of eqn (3) after integration gives:

$$a = a_{\mathbf{r}_0} + d\exp\left(\frac{2RT}{M(1-\bar{\nu}\rho)\omega^2}\frac{\mathbf{r}^2}{2}\right) \quad (5)$$

where d is the constant of integration.

$$\frac{\mathrm{d}(\ln a)}{\mathrm{d}(\mathbf{r}^2)} = a_{\mathbf{r}_0} + \frac{2RT}{M(1-\bar{\nu}\rho)\omega^2}.$$

In terms of the variable $b = d\exp\left(\frac{2RT}{M(1-\bar{\nu}\rho)\omega^2}\frac{\mathbf{r}^2}{2}\right)$, eqn (4) becomes linear:

$$a = a_{\mathbf{r}_0} + b \quad (6)$$

with b as a nonlinear parameter.

The solution of this linear-nonlinear problem is given by Barham and Drane [4]. Using the straight-search method it is possible to vary $a_{\mathbf{r}_0}$ by adding equal increments to $a_{\mathbf{r}_0}$ and then finding the best fit having a minimal residual sum of squares $S(b)$ calculated using the linear least-squares method. The corresponding slope will then be determined. with the above procedure using the computer program SEGAL varies the baseline to obtain the best linear fit of the slope ln(c) versus $\mathbf{r}^2$, and hence determining the molecular mass.

A complete scan from meniscus to bottom reveals a single linear slope if we are dealing with a monodisperse sample, or a departure from a unique straight line if the system is polydisperse.

In the case of a two-component system we may be able to determine an average molecular weight of both components at a relatively low speed, but the correlation of the data points is not as good as with a monodisperse sample. Due to the absence of a true baseline for each part, the data points in one part have a concave and in the other part a convex shape.

This concave or convex distribution stems from the logarithmic function used. Only if the baseline is a true one for the used part is the correlation optimal.

For better visual evaluation of the fit the user can control the fitting accuracy using the 'residuals' window for each part of the sedimentation equilibrium pattern.

At a speed that reveals a good signal for the higher molecular masses, we observe the best possible correlation in the case of good fitting to the true baseline, but this does not hold for the the lower masses. As a next step we would spin the rotor at a reasonable speed for the lower molecular masses, in order to achieve a good correlation for this part as well.

More details are given in the practical companion to SEGAL, which includes examples and operational instructions for users [5]. A block diagram of the working principles of SEGAL is shown in Figure 1.

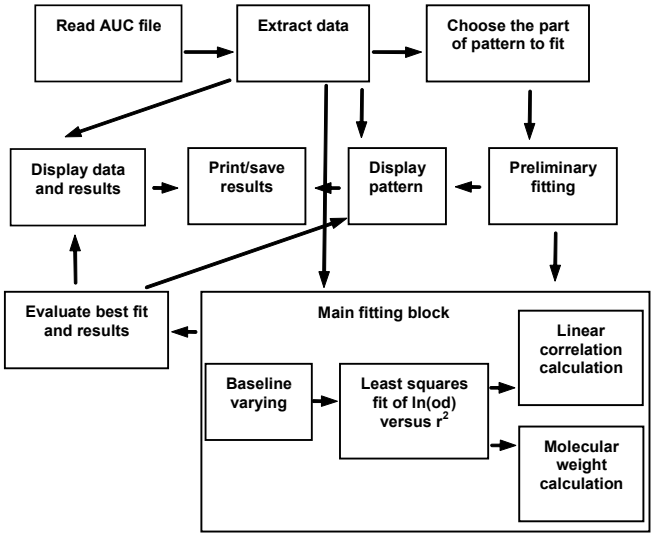


Figure 1. Block diagram of the working principle of SEGAL. The AUC file contains the primary data.

Minimum required computer configuration

A PC with PII Pentium Processor, RAM 64MB, Win 98 operating system, 8 MB free space on the hard drive and "LabView run-time engine 5" is required to run the software.

For Apple PPC computers: Os X 10.3 or higher, 8 MB free space on the hard drive and "LabView run-time engine 7.1" is required.

Operating procedure

After starting the program blank window for the molecular weight calculation appears. Clicking the READ FILE button enables the user to choose an appropriate sedimentation equilibrium data file to read from the opened file dialogue box. After selecting the file, the data pattern will be shown in the pattern window (see Figure 2, left upper window). It is necessary to fill the boxes labelled Partial vol with the partial specific volume of the sample, Density with the density of the solvent, and Conc with the sample concentration. The box labelled Baseline step usually should remain unchanged at 0.005. The Centrifuge type (XLA or XLI), Directory, file path, and Cell N° (The cell N° is that of the rotor hole where the cell was inserted) are automatically read from the data file.

The part of the pattern to fit can be chosen by moving the left and right borders using the mouse cursor and the left button to move the red and green vertical lines in the pattern window. The chosen part of the curve will be coloured in pink. It is possible to choose two parts to fit simultaneously using the mouse cursor as to move the blue and black vertical lines and the chosen part of curve will be coloured in red. The results (molecular weight, linear correlation coefficient, calculated baseline slope) will be displayed on the screen. The operator has to change the borders of the fitted part(s) of the pattern to get the best correlation, which then corresponds to the most accurate molecular weight.

Both parts of the distribution can be overlapped, as shown in the example (Figure 2) and the green line can be pushed to the right e.g. to 6.86 and the dark blue to 6.80. The display shows that there are some mixed species the cell. When the differences of the masses are larger, it becomes easier to separate. One of the most important functions is the "fine fit" button for polynomial prefitting. From our experience the "fine tune sensitivity" 4 (the range is from 2–5) is usually best, but there are also cases at low OD were the "fine fit" is better not used.

In many cases, we are unable to know whether certain measuring points are an important part of the function or only optical artefacts; the button Reduce reduces the number of measuring points.

It essential and worthwhile to look in the field Ln (OD) during the determination of the slope versus radius (one for each part) to confirm the property of each slope.

Some examples demonstrating the capabilities of the SEGAL program now follow, one using interference optics to show both normal and complicated cases.

3. EXAMPLES

Haemoglobin

As a first example for the SEGAL software, the well known protein human oxyhaemoglobin with molecular weight 64 kD is presented [6].

The experimental details of the sedimentation equilibrium run of the human oxyhaemoglobin at a concentration of 0.23 mg/mL in 50 mM tris-HCl, pH 7.4, including 100 mM NaCl, were recorded at 540 nm. The centrifugation speed was 13000 rpm, and the temperature was 20 °C, and a 12 mm double sector (DS) cell was used.

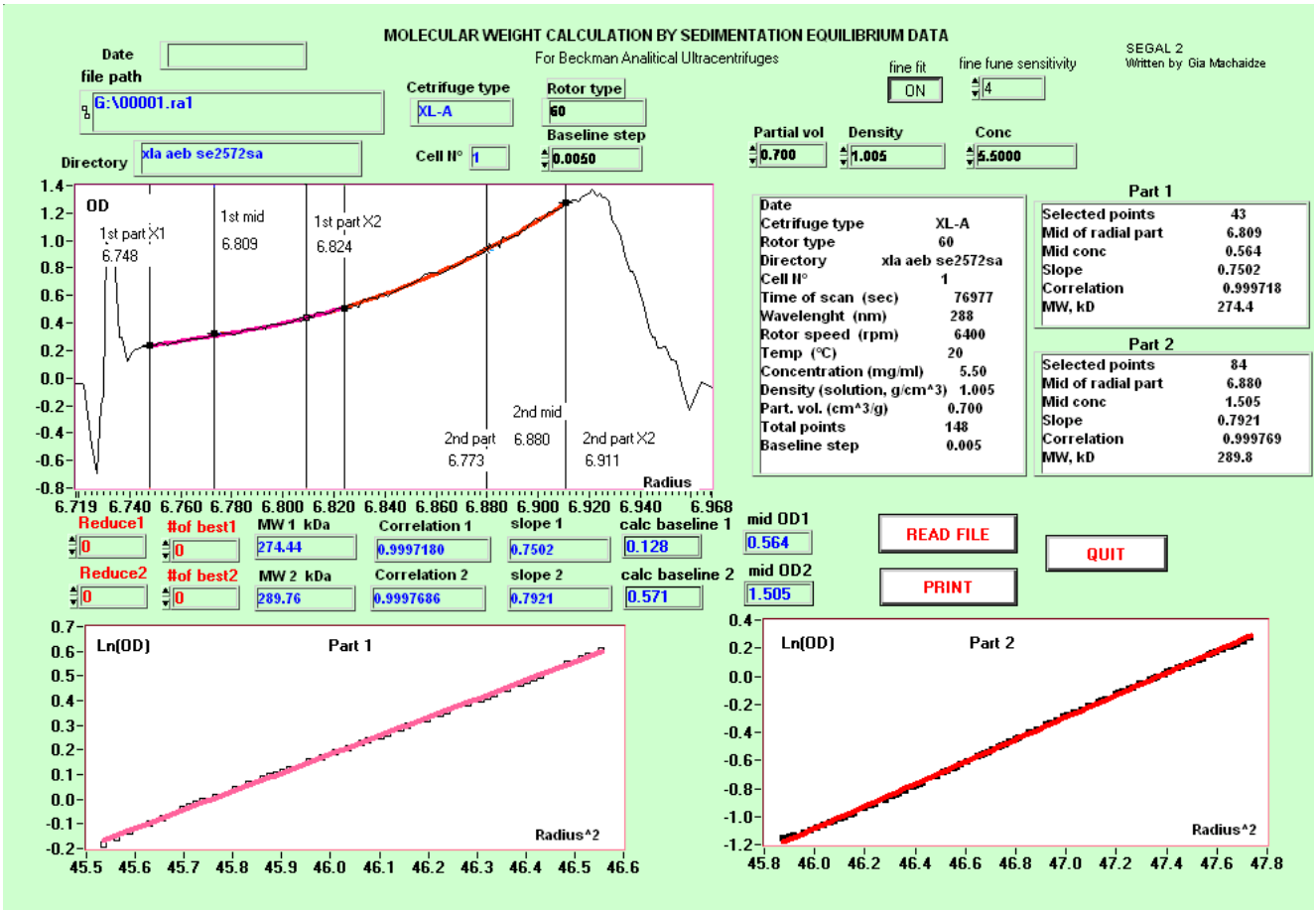


Figure 2. SEGAL software screen of the fitting layout. The window left upper is for choosing the parts of the run pattern. Two windows at the bottom are fits of the chosen parts of the pattern.

The calculated single slope reveals a monodisperse molecular weight of 64 kDa (Figure 3a). Using the option to divide the SE pattern into two parts, each of them showed the same value as the full pattern. Such a test is recommended, for the first estimation of monodispersity, see Figure 3b.

Vimentin

To determine the molecular weight of elongated or filamentous molecules with the AUC is usually a big challenge. The reason for this is the non-ideal hydrodynamic behaviour affecting concentration dependence, self-association, hydration and the influence of electrostatic charge.

Usually in the case of a strong polyelectrolyte, we can reduce the charge effect by raising the ionic strength of the solution, but in the case of vimentin [7], salt induces the formation of huge filaments, and their sizes can be determined only by electron microscopy.

The influence of this effect is presented with a single SE run of 0.8 mg/mL frog vimentin at 11 000 rpm, 20 °C, in the low salt buffer consisting of 10 mM tris-HCl, pH 8.0. At this very low protein concentration the salt effect is minimized, but the optical signal of the experiment is very small, so that only concentrations extrapolated to zero can be used. Therefore we need to determine additionally the molecular weight at higher vimentin concentrations to determine the concentration dependence.

The filling of the centrifuge cell was 2 mm. The lower concentration part of the SE pattern reveals a molecular weight of 177 kDa and the higher concentration part, just 122 kDa (Figure 4a). It is self-evident that the slope values from the middle to the bottom are less steep (slope = 0.9) than from the meniscus to the middle (slope = 1.3).

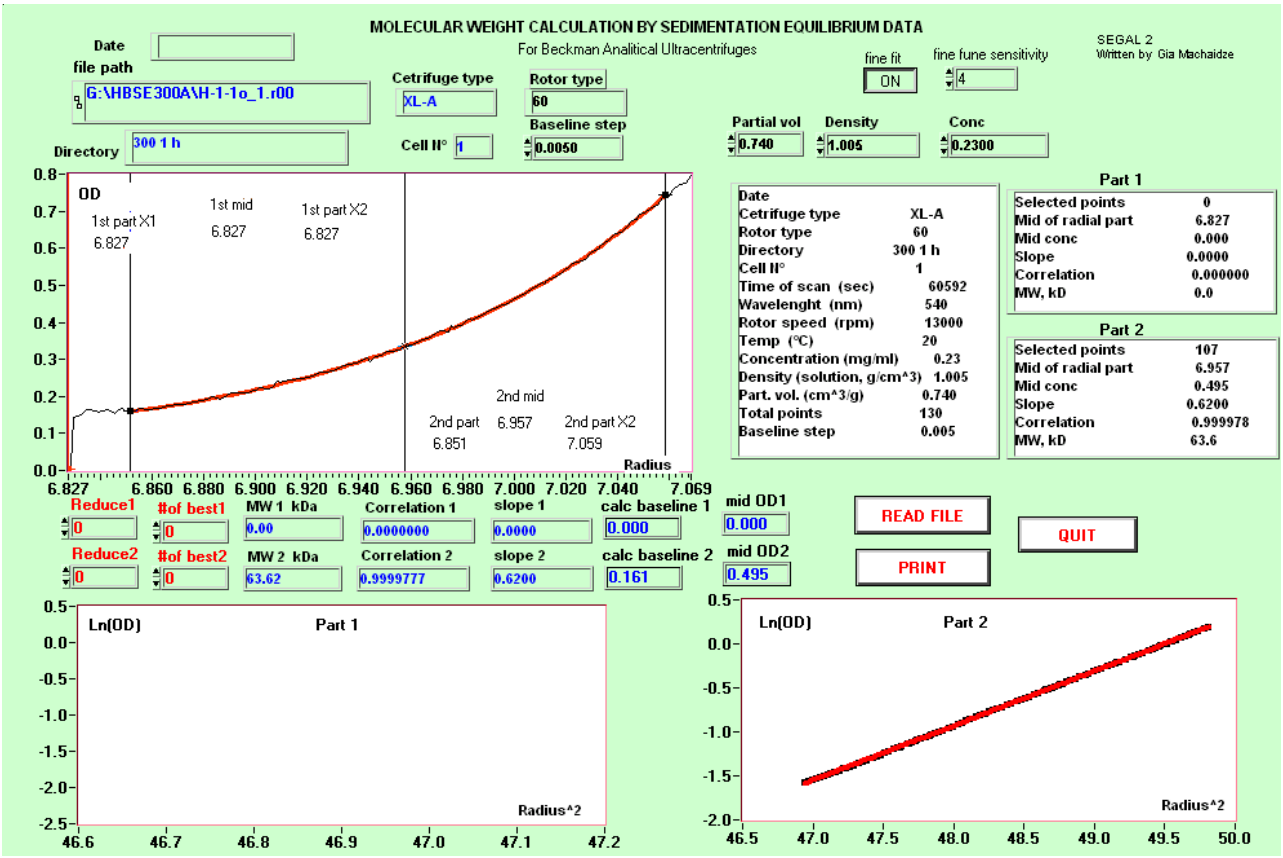
The rather poorly fitted straight-line (yet still with correlation coefficient 0.99624) reveals an average value of 153 kDa and a slope of 1.1. (Figure 4b). This is the average apparent molecular weight from a whole series of runs. Extrapolating to zero concentration reveals the true molecular weight of 220 kDa.

IIA subunit of the mannose transporter

The IIA subunit of the mannose transporter shows a sharp transition from dimer to monomer in guanidine HCl (GuHCl) solution [8].

A single run was conducted with the IIA isolated subunit at 0.08 mg/mL, in 20 mM sodium phosphate

a



b

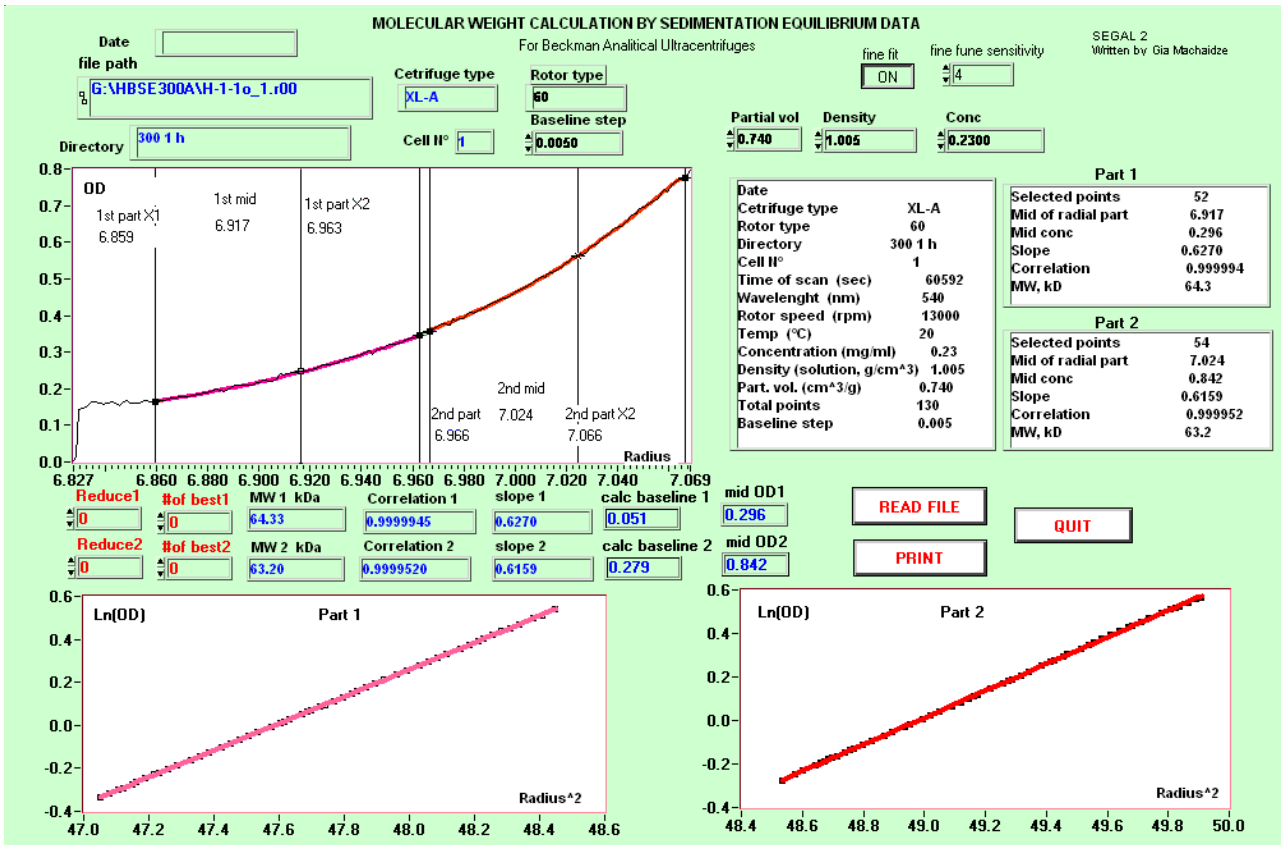
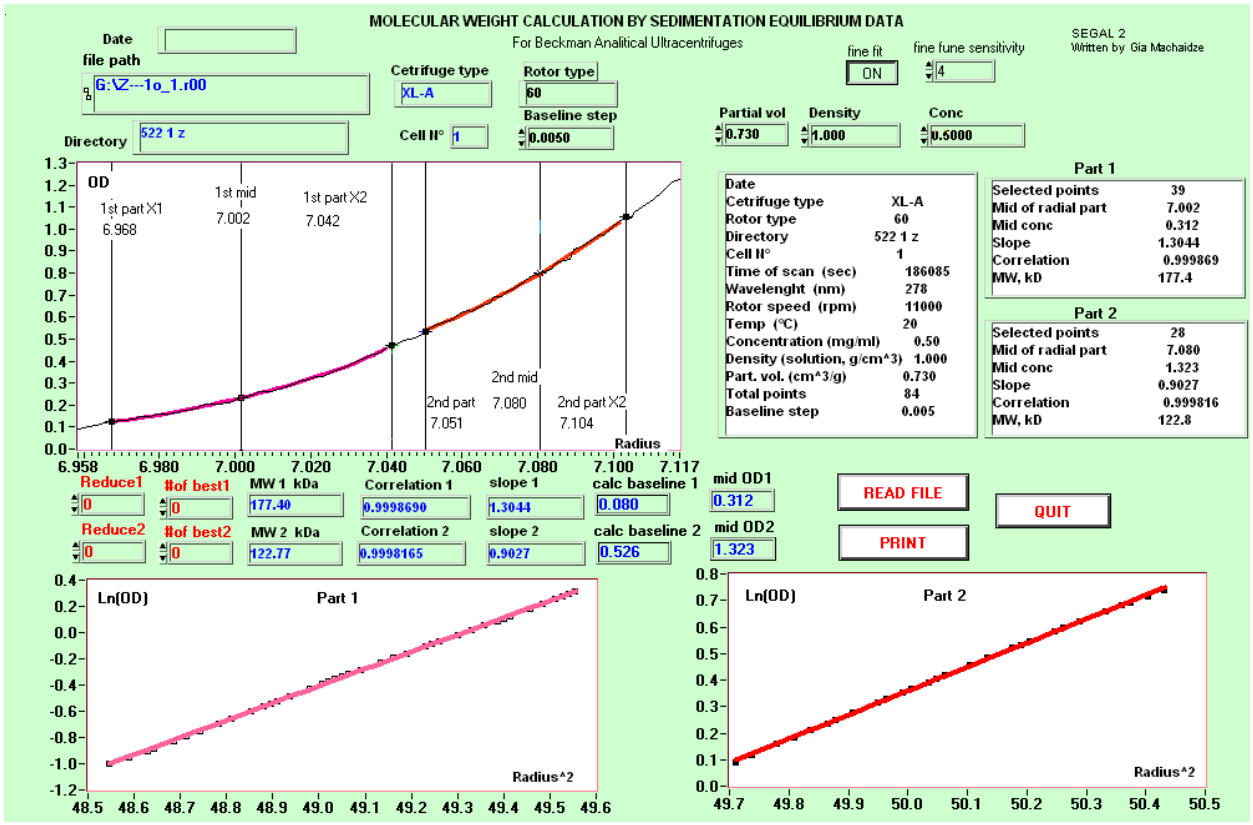


Figure 3. SEGAL window of fitting of 0.23 mg/mL human oxyhaemoglobin in 50mM Tris-HCl, pH 7.4, plus 100 mM NaCl. Absorption wavelength was 540 nm and centrifugation speed 14000 rpm, at 20 °C. a. The fit of the chosen part of the pattern gave a molecular weight 63.67 kDa with a very good linear correlation coefficient (0.9999777). b. The fitting of two parts of the pattern showed similar molecular weights of 64.33 kDa and 63.20 kDa with very good linear correlation coefficients (better than 0.9999).

a



b

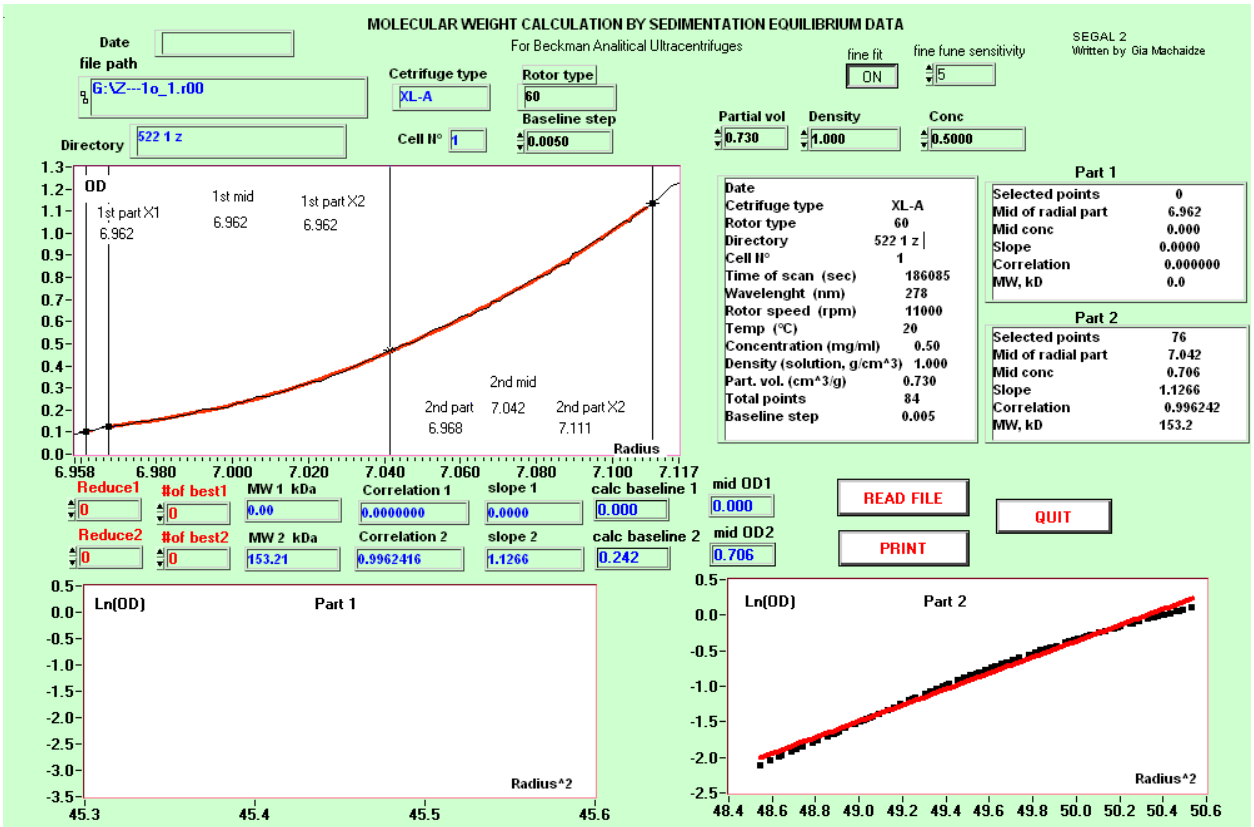


Figure 4. SEGAL window of fitting of 0.8 mg/mL frog vimentin in low salt buffer of 10 mM Tris-HCl, pH 8.0. Absorption wavelength was 278 nm and centrifugation speed 11 000 rpm, at 20 °C. a. The fit of two parts of the pattern showed the molecular weight 177 kDa and with very good linear correlation coefficients (0.999869 and 0.9998165). b. The fitting of the full range pattern had the average value of molecular weight 153 kDa with pure linear correlation coefficient (0.99624).

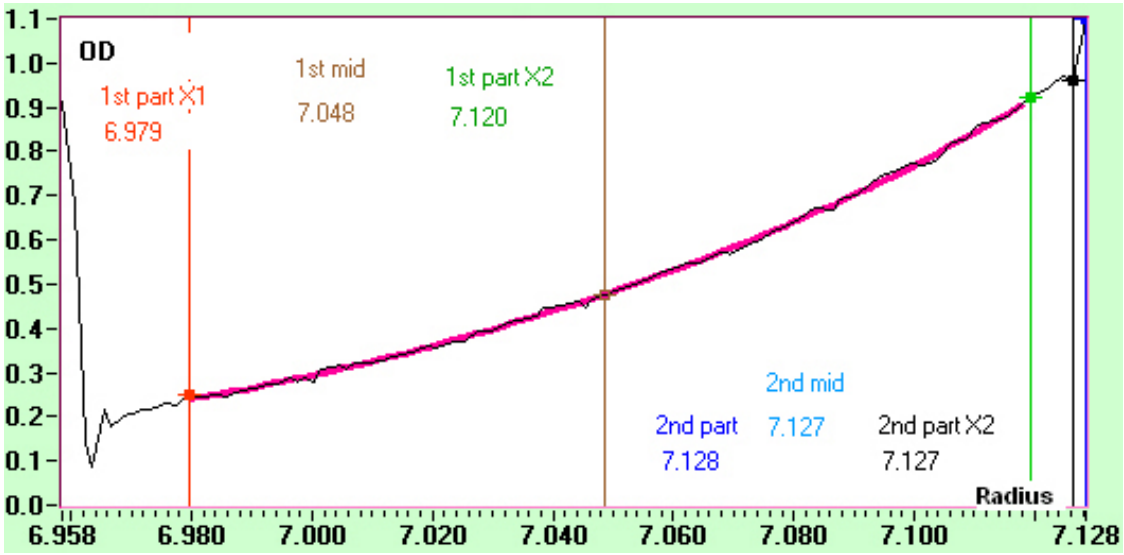
buffer, pH 7.4, 6 M GuHCl in a 12 mm DS (nonmetallic) charcoal-filled Epon cell, at 20 °C, 36000 rpm.

A series of runs was then conducted where each sample contains the same protein concentration but a different GuHCl concentration, and the transition from dimer to monomer is clearly exhibited.

According to Cassas and Eisenberg [9] the samples should be dialysed for each concentration of GuHCl separately. Practically, due to the small amounts of sample available, all samples were diluted from a stock solution of 8 M GuHCl where the protein is completely refolded to a constant concentration at various GuHCl molarities. The given example confirms a monomer.

The non-dialysed, poorly matched system was no obstacle for the SEGAL program, which found the absorption baseline level, and yielded the expected molecular weight of 14 kDa as the best correlation, which corresponds to the monomer of the IIA subunit of the mannose transporter, see ref. [8] (Figure 5).

a



b

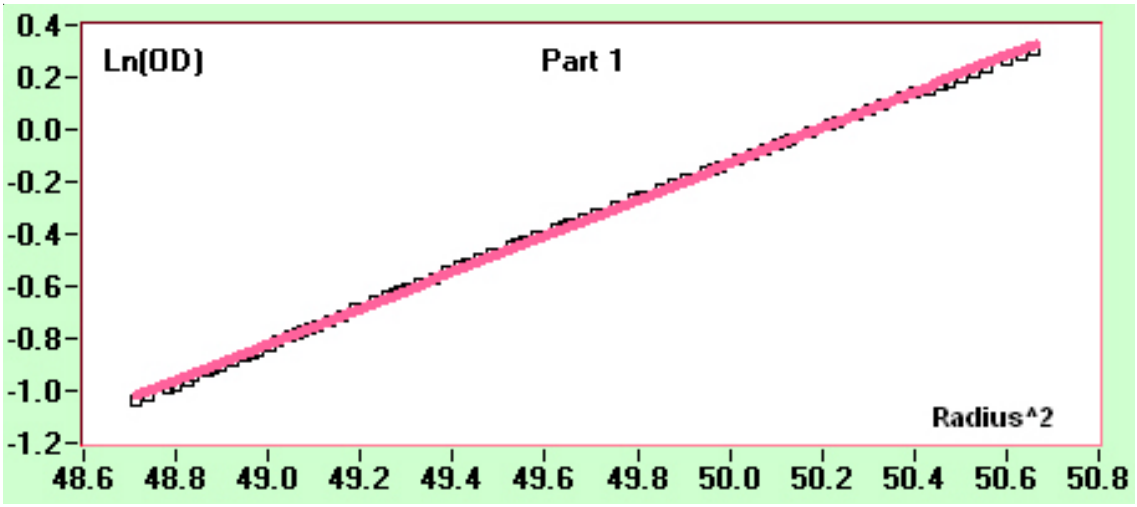


Figure 5. SEGAL fitting of 0.08 mg/mL mannose transporter in 20 mM sodium phosphate buffer, pH 7.4, 6 M GuHCl. Absorption wavelength was 228 nm and centrifugation speed 36000 rpm, at 20 °C. a. SEGAL pattern window of the part used for fitting. b. SEGAL fitting window of the chosen pattern with average molecular weight 14 kDa and linear correlation coefficient 0.999755.

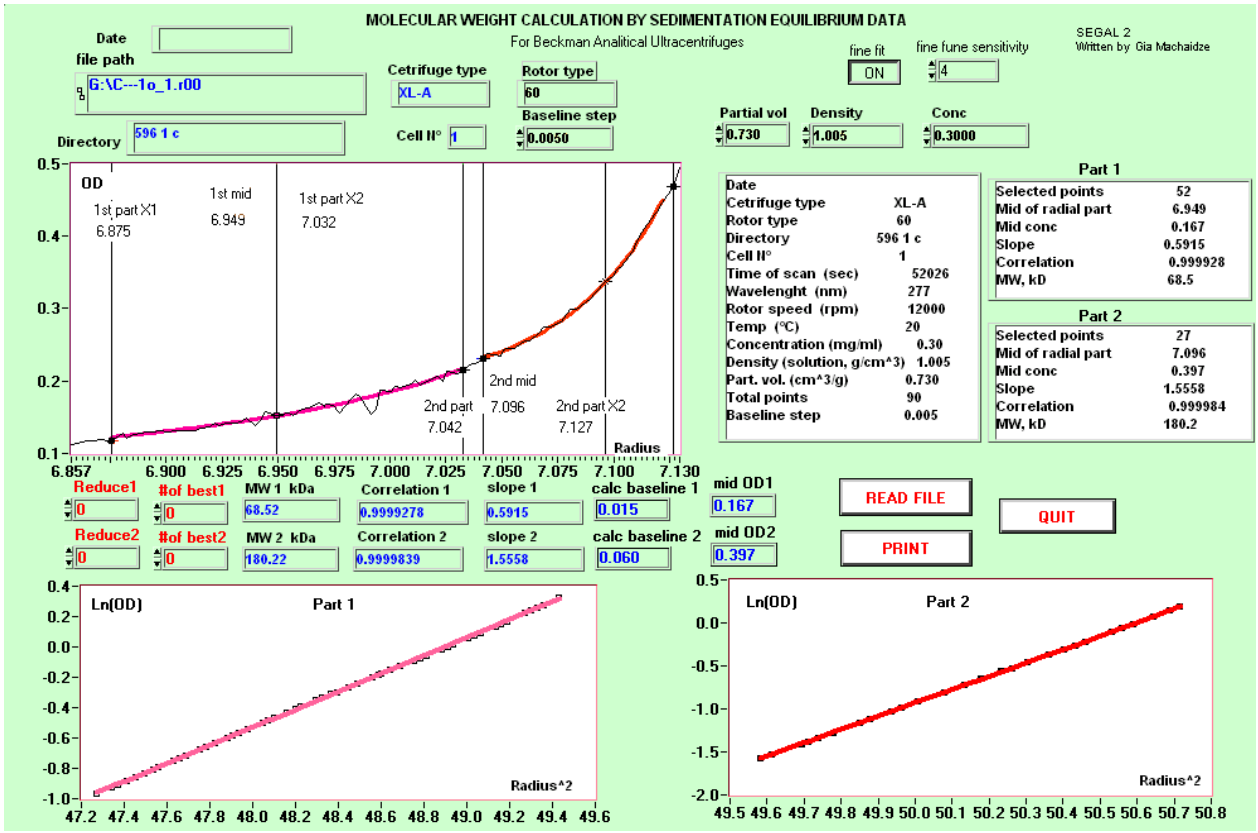
NC1 domain in Type IV collagen

The NC1 domain of Type IV collagen contains monomers and larger complexes like dimers and hexamers.

At the concentration of 0.3 mg/mL and rotor speeds of 12000 and 20000 rpm, it was possible to detect the monomers and oligomer of the NC1 domain of Type IV collagen in the same run by varying the running speed as described in the pervious publication [10].

The determined molecular weights using SEGAL software were: at 12000 rpm, 68 kDa and 180 kDa; and 33 kDa and 68 kDa at 20000 rpm (Figure 6).

a



b

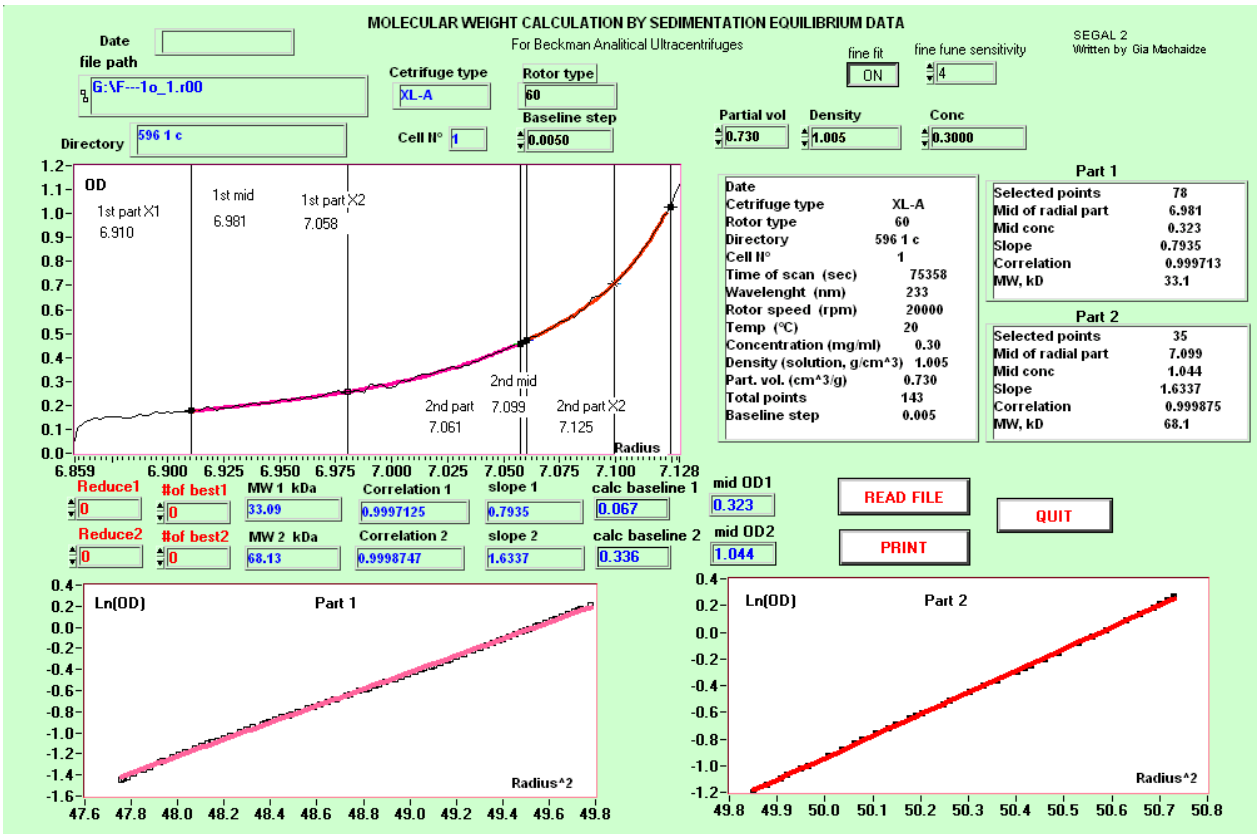


Figure 6. SEGAL fitting of 0.3 mg/mL NC1 domain of Type IV collagen in the buffer of containing of 50 mM sodium phosphate and 100 mM NaCl with pH 7.4. Absorption wavelength was 228 nm. a. Fitting the sedimentation pattern at centrifugation speed 12000 rpm, at 20 °C. Molecular weights were 68 kDa and 180 kDa with very good linear correlation coefficients (up to 0.99999) for both parts. b. Fitting the sedimentation pattern at centrifugation speed 12000 rpm, at 20 °C. Molecular weights were 33 and 68 kDa with linear correlation coefficients 0.9997425 and 0.9998747 respectively.

The repeated run showed the presence of dimer with a molecular weight of 68 kDa, demonstrating the credibility of the software in this mode. More runs using different rotor speeds and greater cell filling height could yield more precise results.

Membrane proteins

The determination of the molecular weight of membrane proteins using only SE runs is not trivial, especially when the density of the detergent micelles is over the critical micelle concentration (CMC) and higher than D_2O (which also has a specific gravity of 1.1). At the gravitational transparency (matching) level, the molecular weight of the protein can only be determined without the attached detergent micelle. As described in ref. [10], it is not possible to dialyse such solutions and thus determine their real baselines.

Two experiments with membrane proteins are presented as examples.

The first experiment, with 1 mg/mL photosystem II complex (PS II) in MES buffer contained 0.03% dodecylmaltoside at 20 °C; wavelength was 436 nm and rotation speed 9000 rpm. The solution density 1.21 g/cm^3 produced a negative slope of – 0.53 [11] (Figure 7).

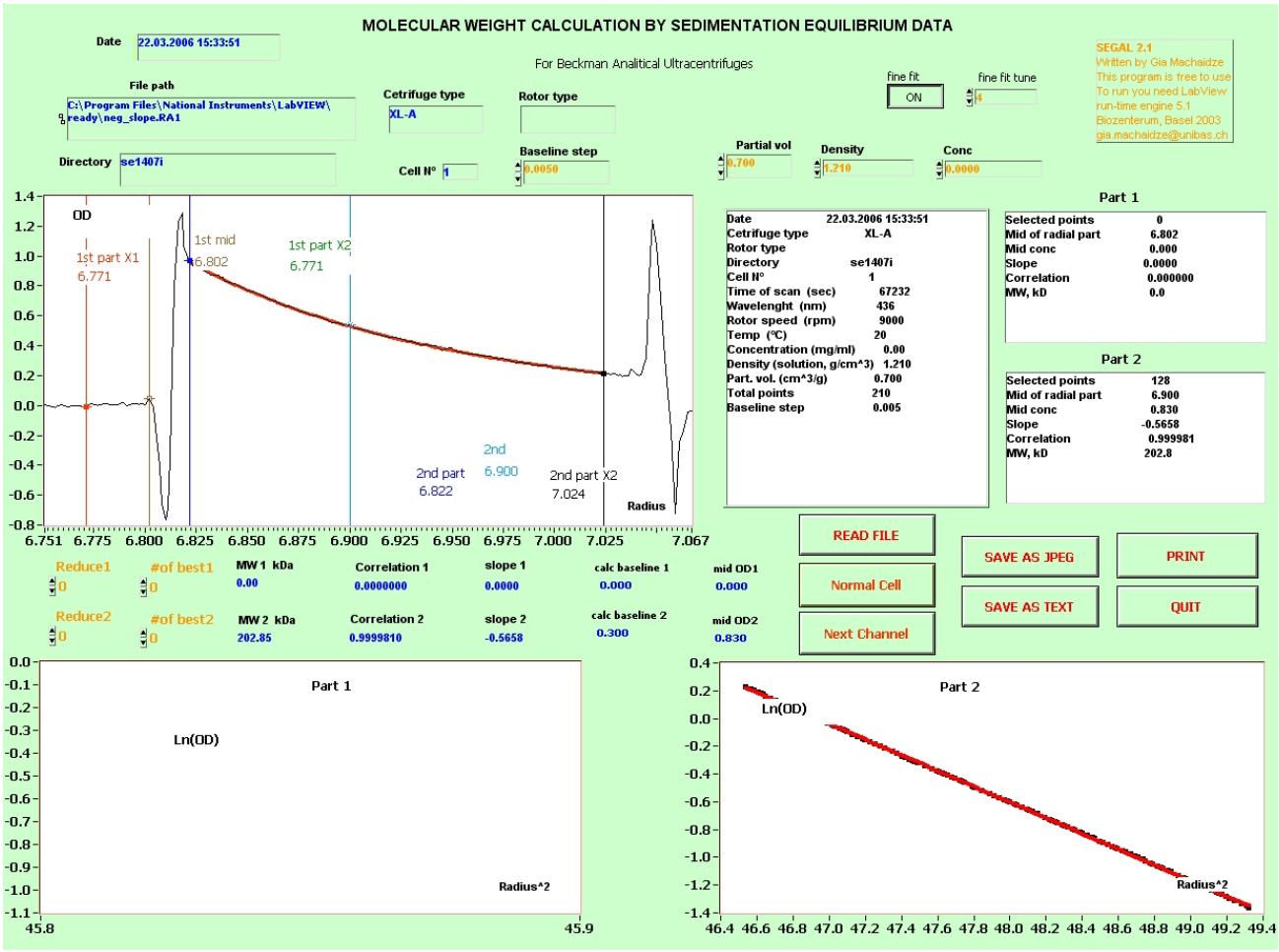


Figure 7. SEGAL fitting window of the sedimentation pattern from 1 mg/mL photosystem II complex (PS II) MES buffer contained 0.03% dodecylmaltoside at 20 °C. The solution density was 1.085 g/cm^3, sedimentation speed was 9000 rpm. Slope was negative (–0.53), due to flotation of the sample.

The second experiment, the sedimentation of OmpF-porin is presented in Figure 8. The protein concentration was 0.7 mg/mL in PBS in the presence of 1.2% octylglucoside and sucrose, solution density was 1.085 g/cm^3 at 11 000 rpm, 20 °C. The slope was 0.51. The calculated molecular weight using the SEGAL software was 186 KDa, the same as that previously published in ref. [11].

During the determination of the gravitational transparencies in the experiments with membrane proteins different positive and negative slopes occurred, and are reflected in the SEGAL representation (Figures 7 and 8).

The use of interference optics: polysaccharides

As a last example, the hitherto unpublished results of a non-fractionated polysaccharide sample are presented. The concentration of the non-fractionated polysaccharides

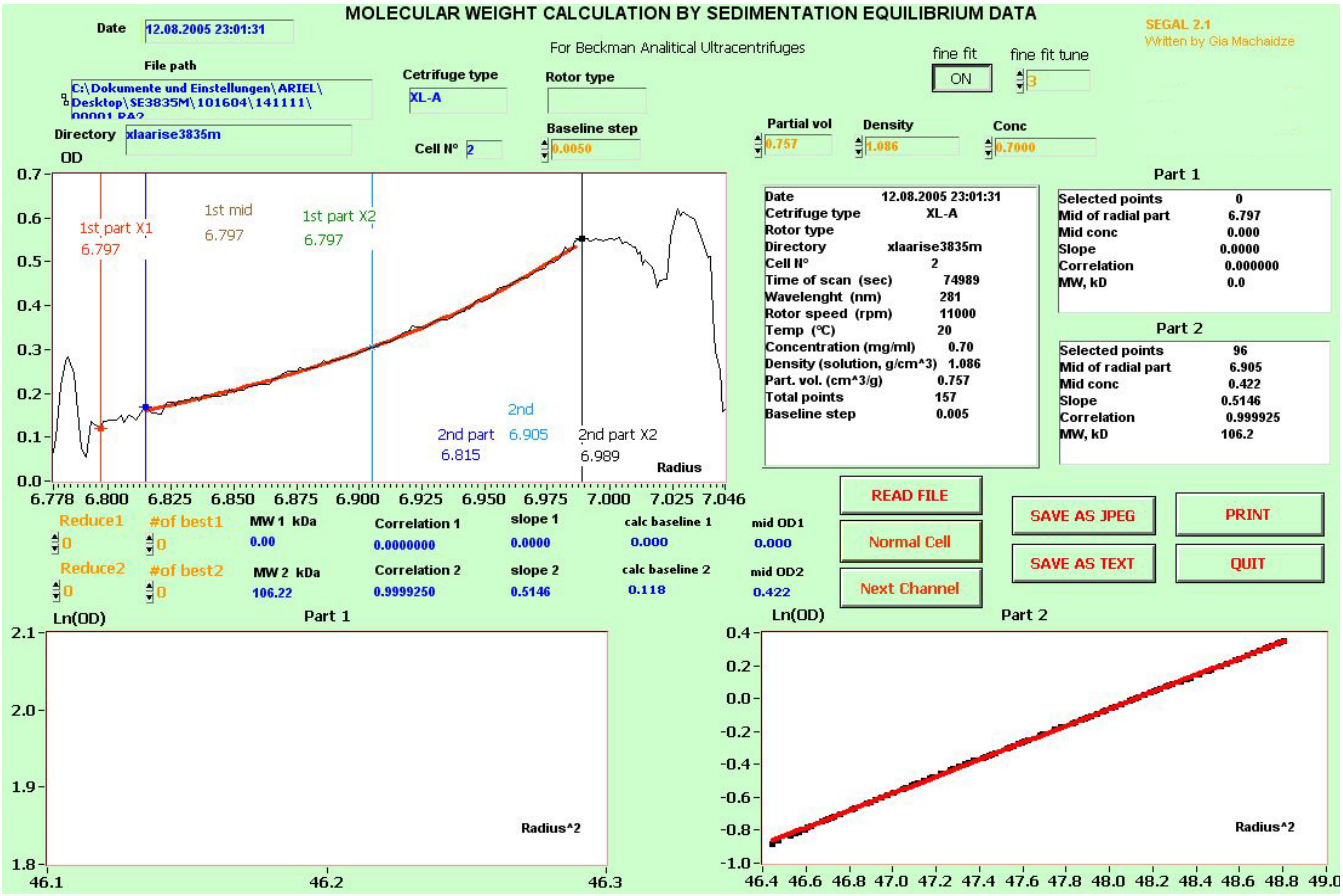


Figure 8. SEGAL fitting window of the sedimentation pattern from 0.7 mg/mL OmpF-porin in PBS in the presence of 1.2% octylglucoside and sucrose. Solution density was 1.085 g/cm^3, sedimented at 11 000 rpm, 20 °C. The slope was 0.51.

in the sedimentation equilibrium run was 1.37 mg/mL in PBS buffer pH 7.2. The centrifugation was performed at 4800 rpm centrifugation speed, and at 20 °C.

Pure polysaccharides do not reveal a specific absorption in the UV and the visible regions, thus the SE experiments were conducted using a Beckman XL-I analytical ultracentrifuge equipped with an interference optical system.

The SEGAL program is able to automatically recognize the type of run and instead of optical density versus radius uses fringes versus radius.

The total one part scan appears to show a curved line (correlation coefficient 0.9992) (Figure 9).

The two separated parts correlate better (correlation coefficients 0.9996 and 0.9998). The mixture of two molecular masses has a molecular weight of about 400 kDa. After a crude separation into two parts we achieve 280 kDa and 550 kDa. We would like to emphasise 'crude separation', because each part can be separated again, using different rotor speeds. To pre-operatively fractionate such a sample is better, but for the first guess and choice of the right columns it is advantageous to know the sizes of the different ranges.

4. OUTLOOK

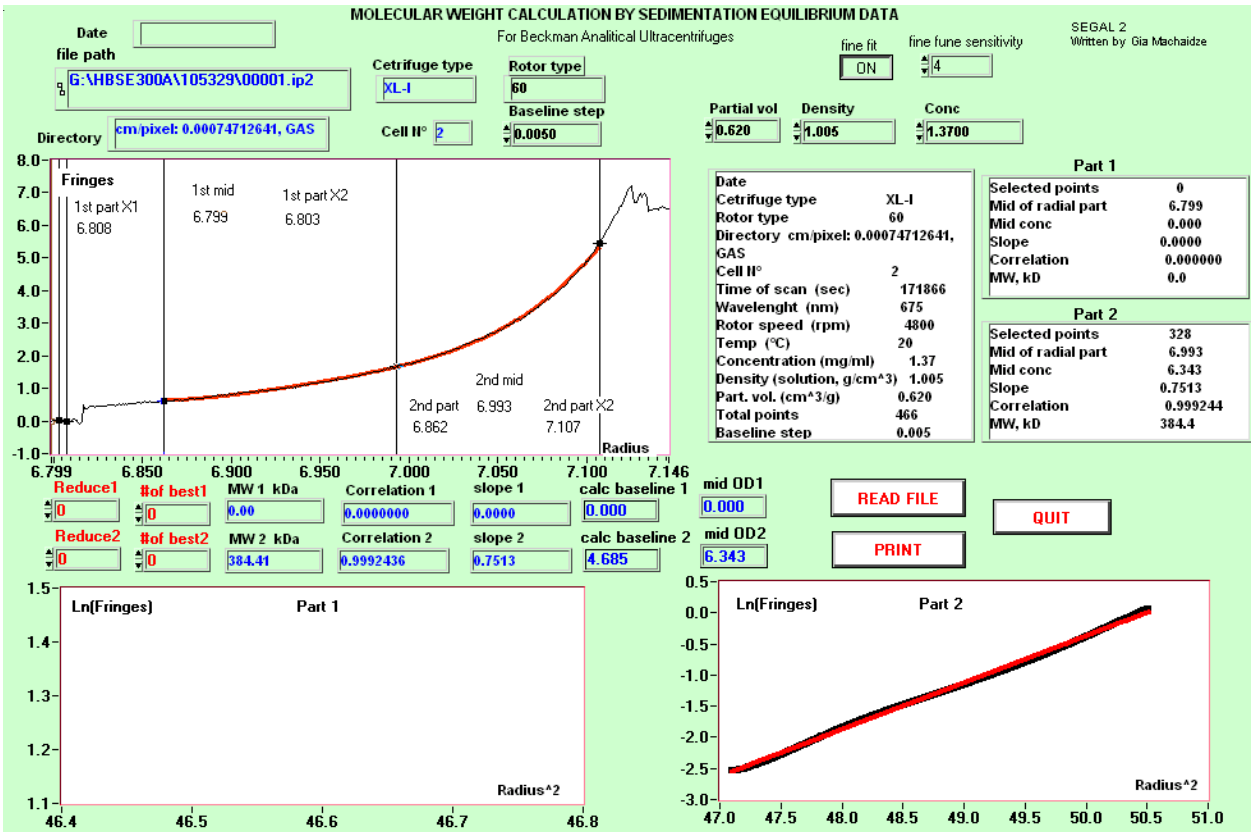
In the latest version of SEGAL, windows for fitting residuals for each part of the pattern have been added. A version of SEGAL with an auto search function for scanning a chosen part of the pattern and a facility for automatically sorting the fitted results for evaluation of the best estimation of molecular weight based on the best linear correlation is under development.

SEGAL is freely available for use and distribution. Download and installation instructions for PC and Mac may be found under ref. [12].

ACKNOWLEDGMENTS

Many years of collaboration with Prof. U. Aebi from the Biozentrum of the University of Basel, and many others too numerous to mention here, have given us a rich working experience with many different biological and other samples. As a result of this collaboration we have published more then 70 papers. We would like to thank Mr E. Kusznir and Dr F. Müller from Hoffman-La Roche AG, Basel, for running some samples with their Beckman XL-I centrifuge, and for their kind technical assistance on many occasions.

a



b

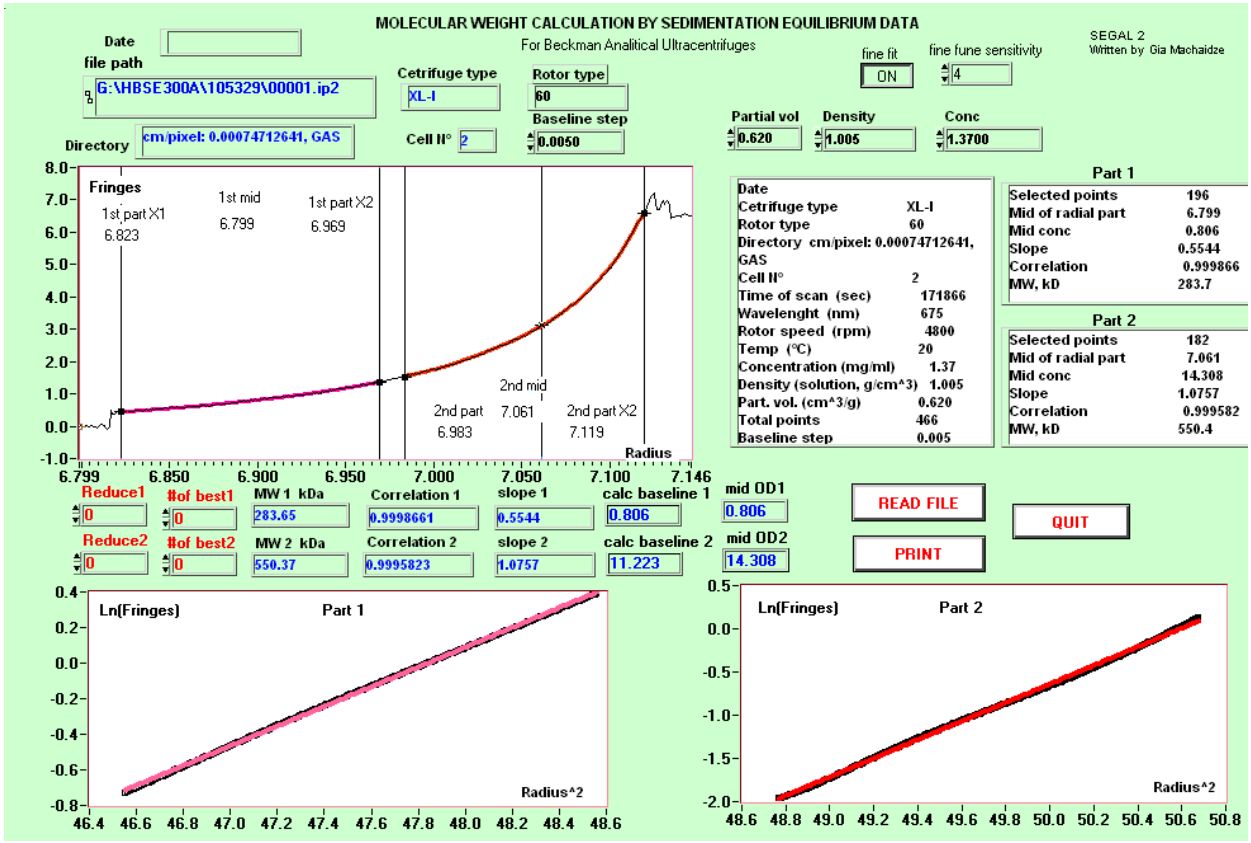


Figure 9. SEGAL window of fitting of 1.37 mg/mL non-fractionated polysaccharides in PBS buffer, pH 7.2, at 4800 rpm centrifugation speed and at 20 °C, using the interference optics of the analytical ultracentrifuge Beckman XL-I. a. The fit of the chosen part gave a molecular weight 384.43 kDa with weak linear correlation coefficient (0.9992), the curvature of the stretched fit line is visible. b. The fitting of two parts of the pattern showed the ability of two "crude" separated fractions with molecular weights 280 kDa and 550 kDa. The linear correlation coefficients were better (0.9996 to 0.9998).

REFERENCES

1. J.P. Rosenbusch, A. Lustig, M. Grabo, M. Zulauf & M. Regenass. Approaches to determining membrane protein structures to high resolution: do selections of subpopulations occur? *Micron* **32** (2001) 75–90.
2. V.Ya. Chernyak & N.N. Magretova. An "all-speed" autocalibration method for sedimentation equilibrium in dilute homogeneous and multicomponent solutions (Part I+II), *Anal. Biochem.* **123** (1982) 101–119.
3. H.K. Schachman. *Ultracentrifugation in Biochemistry*. New York: Academic Press (1959).
4. R.H. Barham & W. Drane. An algorithm for least squares estimation of nonlinear parameters when some of the parameters are linear. *Technometrics* **14** (1972) 757–766.
5. http://www.biozentrum.unibas.ch/auc/.
6. G.L. Kellett & H.K. Schachman. Dissociation of haemoglobin into subunits—monomer formation and the influence of ligands. *J. Mol. Biol.* **59** (1971) 387–399
7. H. Herrmann, M. Haener, M. Brette, S.A. Mueller, K.N. Goldie, B. Fedke, A. Lustig, W. Franke & U. Aebi. Structure and assembly properties of the intermediate filament protein vimentin: the rôle of its head and tail domains. *J. Mol. Biol.* **264** (1996) 933–953.
8. Z. Markovic-Housley, A. Cooper, A. Lustig, K. Flukiger, B. Stolz & B. Erni. Independent folding of the domains in the hydrophilic subunit IIABman of the mannose transporter of *Escherichia coli*. *Biochemistry* **33** (1994) 10977–10984.
9. E. Casassa & H. Eisenberg. Thermodynamic analysis of multicomponent solutions. *Adv. Protein Chem.* **19** (1964) 287–395.
10. A. Ries, J. Engel, A. Lustig & K. Kuehn. The function of the NC1 domains in type IV collagen. *J. Biol. Chem.* **270** (1995) 23790–23794.
11. A. Lustig, A. Engel, G. Tsiotis, E.M. Landau & W. Baschong. Molecular weight determination of membrane proteins by sedimentation equilibrium at the sucrose or nycodenz–adjusted density of the hydrated detergent micelle. *Biochim. Biophys. Acta.* **1464** (2000) 199–206.
12. http://www.biozentrum.unibas.ch/auc/download.html.