JWMVS-529

CD Multivariate SSE Program

JASCO Spectra Manager™ for J-800/1000 series

P/N: 0302-7605C February 2014

Preface

This instruction manual is your guide for using this instrument. It instructs first-time users on how to use the instrument, and serves as a reference for experienced users.

Before using the instrument, please read this instruction manual carefully, and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. When not using the instrument, keep this manual in a safe place. If this instruction manual becomes lost, order a replacement from your local JASCO distributor.

Servicing

Contact your local JASCO distributor for instrument servicing. In addition, contact your JASCO distributor before moving the instrument to another location. Consumable parts should be ordered according to part number from your local JASCO distributor. If a part number is unknown, give your JASCO distributor the model name and serial number of your instrument.

Do not return contaminated products or parts that may constitute a health hazard to JASCO employees.

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- Transfer or licensing of the right to use software to a third party
- Disclosure of confidential information regarding software
- Modification of software
- Use of software on multiple workstations, network terminals, or by other methods (not applicable under a network licensing agreement concluded with JASCO)

(3) The contents of this manual are subject to change without notice for product improvement.

(4) This manual is considered complete and accurate at publication.

(5) This manual does not guarantee the validity of any patent rights or other rights.

(6) If a JASCO software program has failed causing an error or improper operation, this may be caused by a conflict from another program operating on the PC. In this case, take corrective action by uninstalling the conflicting product(s).

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(8) When disposing of the instrument, parts, and waste solvents, take appropriate measures in compliance with applicable regulations regarding waste disposal, and correctly dispose of them by yourself, or entrust disposal to a licensed industrial waste disposal company. In any case, comply with the regulations in your country, state, region or province to ensure they are disposed of legally and correctly.

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THIS WARRANTY DOES NOT APPLY TO DEFECTS RESULTING FROM THE FOLLOWING:

(1) IMPROPER OR INADEQUATE INSTALLATION
(2) IMPROPER OR INADEQUATE OPERATION, MAINTENANCE, ADJUSTMENT OR CALIBRATION
(3) UNAUTHORIZED MODIFICATION OR MISUSE
(4) USE OF CONSUMABLE PARTS NOT SUPPLIED BY AN AUTHORIZED JASCO DISTRIBUTOR
(5) CORROSION DUE TO THE USE OF IMPROPER SOLVENTS, SAMPLES, OR DUE TO SURROUNDING GASES
(6) ACCIDENTS BEYOND JASCO'S CONTROL, INCLUDING NATURAL DISASTERS

This warranty does not cover the consumable parts listed below:

(1) Tungsten lamp, and other light sources
(2) Mirrors in the light source section, and cell windows
(3) Fuses, batteries, glassware, chart paper and ink

THE WARRANTY FOR ALL PARTS SUPPLIED AND REPAIRS PROVIDED UNDER THIS WARRANTY EXPIRES ON THE WARRANTY EXPIRATION DATE OF THE ORIGINAL PRODUCT. FOR INQUIRIES CONCERNING REPAIR SERVICE, CONTACT YOUR JASCO DISTRIBUTOR AFTER CONFIRMING THE MODEL NAME AND SERIAL NUMBER OF YOUR INSTRUMENT.

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JAPAN

Notation Used

The following notational conventions are used throughout this manual:

Notation	Meaning
[Measurement] menu **[Parameters...] command**	Names of menus, commands, and text boxes are enclosed in square brackets '[ ]', followed by a description indicating whether the function is a menu, command, text box, etc. Shortcut keys used to select menus or commands are underlined.
<OK>, <Cancel>	Names of buttons are enclosed in angular brackets '< >'.

Keyboard Operations

Notation	Meaning
Shift **Ctrl**	The key is enclosed in a square and shown in boldface.
Alt , **F**	Keys that are to be pressed in succession are separated by commas. In the example shown on the left, the **Alt** key is to be pressed and released, followed by the **F** key.
Shift + →	Keys that are pressed simultaneously are separated by a "plus" sign. In the example shown on the left, press the → key while holding down the **Shift** key.

Mouse Operations

Notation	Meaning
Point	Move the mouse pointer to the specified item.
Click	Quickly press and release the mouse button.
Double-click	Click the mouse button twice in rapid succession.
Drag	Point to an item, click and hold down the mouse button. Move the mouse with the button held down, and release the button when the pointer is in the desired position.

Contents

1. Introduction

1.1 How to Use This Manual

This manual is for the JASCO [CD Multivariate SSE] program that runs on *Microsoft Windows*. The [CD Multivariate SSE] program consists of [Spectra Manager CFR-Compliant Program], which conforms to FDA regulation 21 CFR Part 11 concerning electronic records and electronic signatures, and [Spectra Manager v.2-Compliant Program], which does not conform to these standards. This instruction manual refers to [Spectra Manager CFR-Compliant Program] and [Spectra Manager v.2-Compliant Program] as [CFR Version Program] and [Non-CFR Version Program], respectively.

This instruction manual consists of four chapters including this chapter. An explanation of each chapter is given below.

1. Introduction

This chapter describes how to use this manual, provides an overview of the [CD Multivariate SSE] program, and describes the program's menus. Read this chapter first.

This program is for analyzing the secondary structure of proteins. In this manual, a standard model or a calibration model created with the [CD Multivariate Calibration Creation] program is used to enable simultaneous quantitation of multiple components.

- Two types of calibration models can be selected (a file created using the [CD Multivariate Calibration Creation] program or a standard model).
- Quantitation results can be saved in text or binary format (.jcdqt).
- An electronic signature is required for calibration models used in quantitation.

2. Introduction to the [CD Multivariate SSE] Program

This chapter describes in detail the procedures for starting the [CD Multivariate SSE] program, setting analysis parameters and loading data from files, and displaying, saving, and printing calculation results. This will ensure that the user is familiar with how the program works.

3. [CD Multivariate SSE] Program Reference

This chapter is a menu reference for the [CD Multivariate SSE] program. Refer to this chapter as required.

4. Appendix

This chapter contains the principles of multi-component analysis (PCR quantitative analysis and PLS quantitative analysis) and precautions concerning measurement and analysis. Refer to this chapter as required.

1.2 Overview of the [CD Multivariate SSE] Program

The [CD Multivariate SSE] program estimates the secondary structure of a sample from a spectrum obtained by using the multivariate analysis technique. It includes the CD spectra of 26 types of proteins measured by JASCO and the calibration models created from these data. The CD spectra of the 26 types of proteins have been converted and recorded in four different units on the vertical axis: Mol. Ellip., Mol. CD, Spc. Ellip. and g Value. In addition, calibration models for the four different units on the vertical axis that were created based on the data for the 26 types of proteins have been recorded. The standard calibration models that are included have been locked to prevent them from being overwritten, so ensure that another name is used when trying to save them after editing.

When analyzing the secondary structure of proteins, select a calibration model that has the same y-axis unit as the spectrum to be analyzed.

For information on the procedure for using the calibration models and CD spectra that come standard with [CFR Version Program], refer to "Procedure for Creating Calibration Models from the Standard Models Included in the CFR Version Program" in the "CD Multivariate Calibration Creation Program Software [1.4 Using Calibration Models That Come Standard in CFR Version Programs]".

Note 1: In [CFR Version Program], an electronic signature is required to use calibration models.
Note 2: Refer to Chapter 4 "Appendix" for the principles behind multivariate analysis (PCR quantitative analysis and PLS quantitative analysis).

1.3 FDA 21CFR Part11

FDA 21 CFR Part 11 is a set of regulations regarding digital archiving of data and data records for GLP, GCP, and GMP procedures. These regulations cover a number of areas, including: 1) access control and electronic signature requirements for data recorded by any computer-controlled analytical system in which the results are digitally archived; 2) the provision of security functions that can only be accessed by authorized personnel to ensure the security and integrity of data; and 3) a data auditing mechanism with the automatic creation of an Audit Trail to maintain a record of any creation, modification or deletion of instrument data.

1.4 Features of [Spectra Manager] Compatible with FDA 21CFR Part 11

JASCO Spectra Manager™ is an integrated software package that acts as a common platform for the range of JASCO analytical instruments, including UV/Vis, near-infrared, infrared, fluorescence, Raman, polarimetry, and circular dichroism spectrometers. The Spectra Manager™ software provides functionality ranging from analytical instrument control and spectral data processing to sophisticated and specialized data analysis programs. JASCO Spectra Manager™ CFR provides security and auditing functionality that ensures the security, integrity and confidentiality of electronic records by enforcing the use of electronic signatures and other security measures as described in the regulations outlined in 21 CFR Part 11.

JASCO Spectra Manager™ CFR consists of three core modules: Administrative Tools and Security Manager modules; intrinsic components of the Spectra Manager™ CFR software; and a database, which is an external component to the Spectra Manager™ CFR software. In addition, there are two broad classes of add-on modules: instrument drivers, which are spectrometer-specific; and spectral analysis modules, which provide a range of sophisticated analytical and data processing tools. These add-on modules integrate seamlessly with the three core components of the Spectra Manager system to provide compliance with 21 CFR Part 11 regulations over the entire range of JASCO products. Figure 1.1 illustrates the system organization.

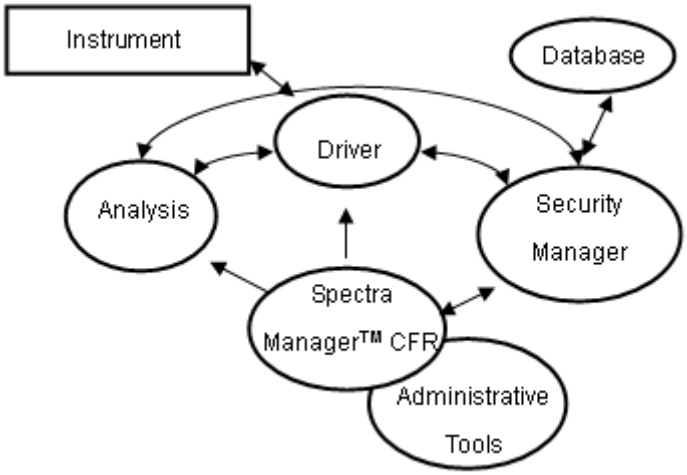


Figure 1.1 Structure of Spectra Manager™ CFR

All of the security management functions of JASCO Spectra Manager™ CFR are accessed through the Administrative Tools interface. The Security Manager components of the system are not directly accessible by a user, and thus the operation of these modules is not described. For secure management of data and data records, JASCO Spectra Manager™ CFR has two levels of security: system-level security and workgroup-level security.

When a user is registered in JASCO Spectra Manager™ CFR, the user must be assigned to one of four possible System Access Levels: “Administrators”, “Power Users”, “Users”, or

“Limited Users”. The level of control that a user has over the management functions of the software system is determined by the System Access Level assigned to that user. Table 1.1 outlines the different rights assigned to the different types of users depending on their System Access Levels.

Table 1.1 Rights of each System Access Level

	Administrators	Power Users	Users	Limited Users
Display/modify system policy	OK			
Add/delete users	OK			
Modify user properties (incl. System Access Level)	OK			
Add a workgroup	OK			
Delete a workgroup	Workgroup Managers only			
Modify workgroup users	Workgroup Managers only			
Add/delete instruments	OK			
Modify instrument configuration	OK			
Add/delete programs	OK			
Display log management	OK	OK		

Workgroups correspond to actual groupings of users within the organization and may represent divisions such as projects, departments, or research groups. Each workgroup has a set of users, instruments, accessories, and analysis programs associated with it; only users registered with a specific workgroup may access the instruments and programs of that workgroup. Within a workgroup, users are granted access as either a “Manager”, “Analyst”, or “Operator”, with authorities to modify instrument and analysis settings and use resources within the workgroup determined by the Workgroup User Rights granted. Selectable Workgroup User Rights vary depending on the system access level of the user. Table 1.2 outlines the relationship between the System Access Levels and Workgroup User Rights.

Table 1.2 Qualified access levels by Workgroup User Rights

	Administrators	Power Users	Users	Limited Users
Managers	OK	OK		
Analysts	OK	OK	OK	
Operators	OK	OK	OK	OK

By employing a two-level security model, global security authorization is independent of instrument, accessory, and application authorization, resulting in a highly flexible security system. Table 1.3 outlines the different rights assigned to the different types of users depending on their Workgroup User Rights.

Table 1.3 Rights of each Workgroup User Rights

	Managers	Analysts	Operators
Register/unregister workgroup users	OK		
Change Workgroup user authorities	OK		
Register/unregister instruments for the workgroup	OK		
Register/unregister programs for the workgroup	OK		
Use a registered instrument	OK	OK	OK
Use a registered instrument application	OK	OK	OK
Modify measurement parameters	OK	OK	
Use a registered analysis program	OK	OK	OK
Modify analysis parameters	OK	OK	
Electronically sign data	OK	OK	OK

Figure 1.2 shows the typical operational flow of the JASCO Spectra Manager™ CFR. First, a user is created (registered) and their System Access Level is assigned using Administrative Tools. A Workgroup is created (registered), and individual users and analytical instruments are assigned to the Workgroup. Detailed access to instrument control and analysis programs can be set at the User and Workgroup levels. Access control levels as required by FDA 21 CFR Part 11 regulations are determined by the Administrative Tools settings.

Routine analyses can then be conducted by the operator using the measurement/analysis programs in JASCO Spectra Manager™ CFR. Since all levels of access are managed by Security Manager, individual users are only able to execute procedures that are allowed by their assigned access level. Access control ranges from declaring measurement parameters to conducting measurements and performing data analysis.

Analytical data obtained using any of the procedures described above can be saved as an electronic record with an electronic signature.

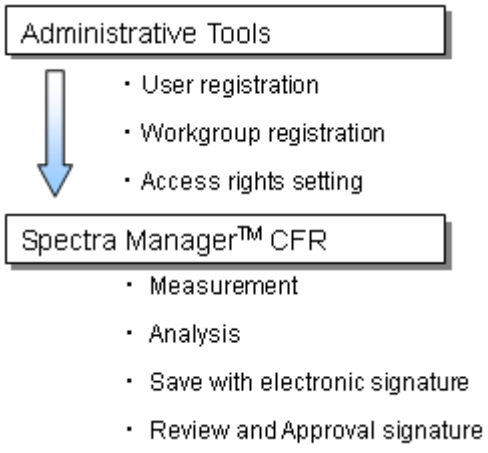


Figure 1.2 Operational flow

2. Introduction to the [CD Multivariate SSE] Program

This chapter describes in detail the procedures for starting the [CD Multivariate SSE] program, using the calibration models included with the program to estimate the secondary structure of sample spectra, and saving/printing the results. This will ensure that the user is familiar with how the program works. Refer to Section 2.4 and Chapter 3 for details about parameters.

Note:	*Calibration models created by using data from 26 types of proteins with known component ratios are registered in this program as standard data files (Refer to Section 1.2).*

2.1 Operation Overview

Starting the [CD Multivariate SSE] program.	Refer to Section 2.2
↓	
Loading protein CD spectra and performing analysis.	Refer to Section 2.3
↓	
Setting analysis parameters.	Refer to Section 2.4
↓	
Saving/printing quantitation results.	Refer to Section 2.5 and 2.6
↓	
Exiting the [CD Multivariate SSE] program.	Refer to Section 2.7

2.2 Starting the [CD Multivariate SSE] Program

Double-click [CD Multivariate SSE] in the [Spectra Manager] window. The [CD Multivariate SSE] program starts and the window in Fig. 2.1 is displayed.

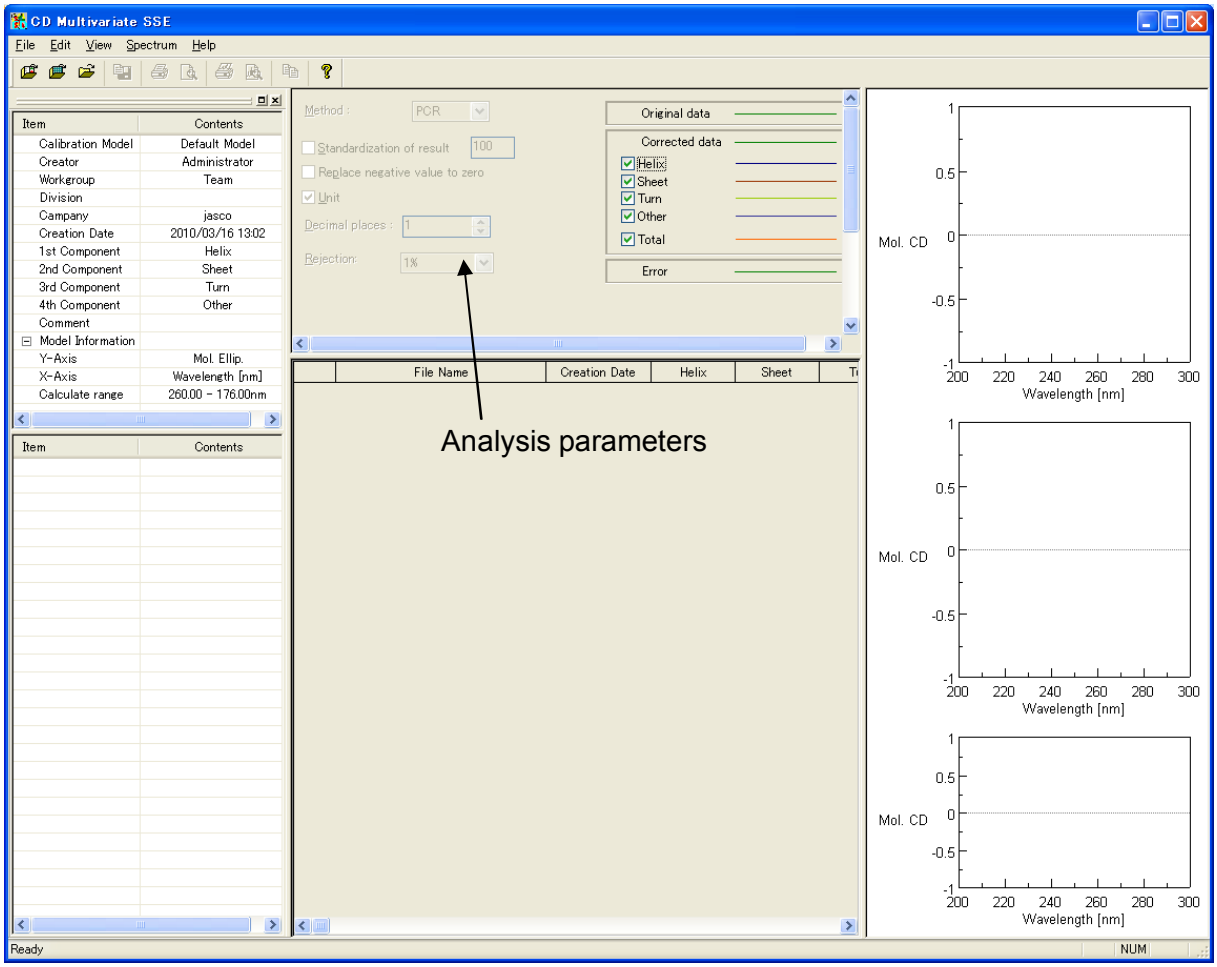


Figure 2.1 [CD Multivariate SSE] window (CFR version)

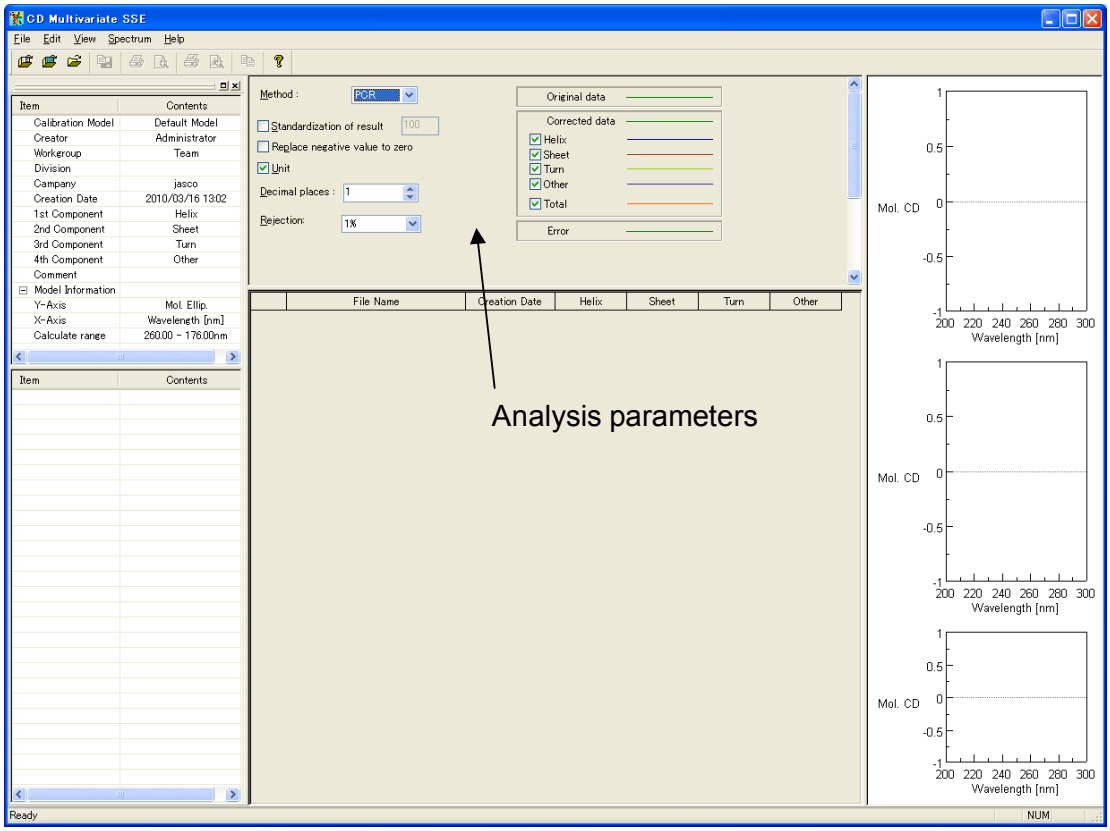


Figure 2.2 [CD Multivariate SSE] window (Non-CFR version)

(1) The [CD Multivariate SSE] program automatically loads the standard calibration models and their analysis parameters at startup. Figures 2.1 and 2.2 show the program windows once the calibration models have been loaded and their analysis parameters have been displayed. Select [Select Model] - [Open Model...] from the [File] menu to open a separately created calibration model (or click the  tool button). The [Open Model] dialog box is displayed. Analysis parameters cannot be changed in the CFR version of the [CD Multivariate SSE] program. A new calibration model and analysis parameters must be created in the [CD Multivariate Calibration Creation] program and then opened in this program.

2.3 Loading Protein CD Spectra and Performing Analysis

(1) Select [Open Spectra...] from the [File] menu or click the  tool button). The [Open Spectrum] dialog box in Fig. 2.3 is displayed.

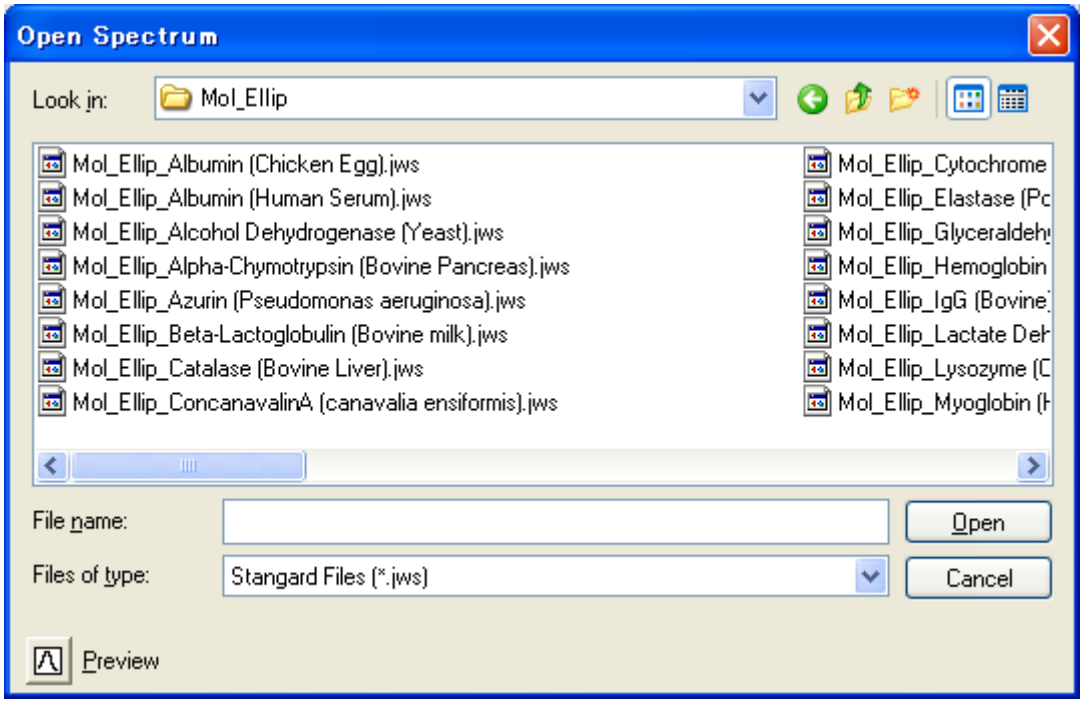


Figure 2.3 [Open Spectrum] dialog box

Note 1: Multiple files can be selected at once by holding down Ctrl or Shift while clicking the file names.
Note 2: Refer to Section 2.5 for details about setting analysis parameters.

(2) Select the protein spectrum file from the file list and then click the <Open> button. Analysis automatically begins as soon as the file opens. The spectra data opened, the spectra for each secondary structure (Helix, Sheet, Turn, and Other) and their total spectrum, and the residual spectrum are displayed, respectively at the top right, middle right, and bottom right. Quantitation results are displayed in the data sheet.

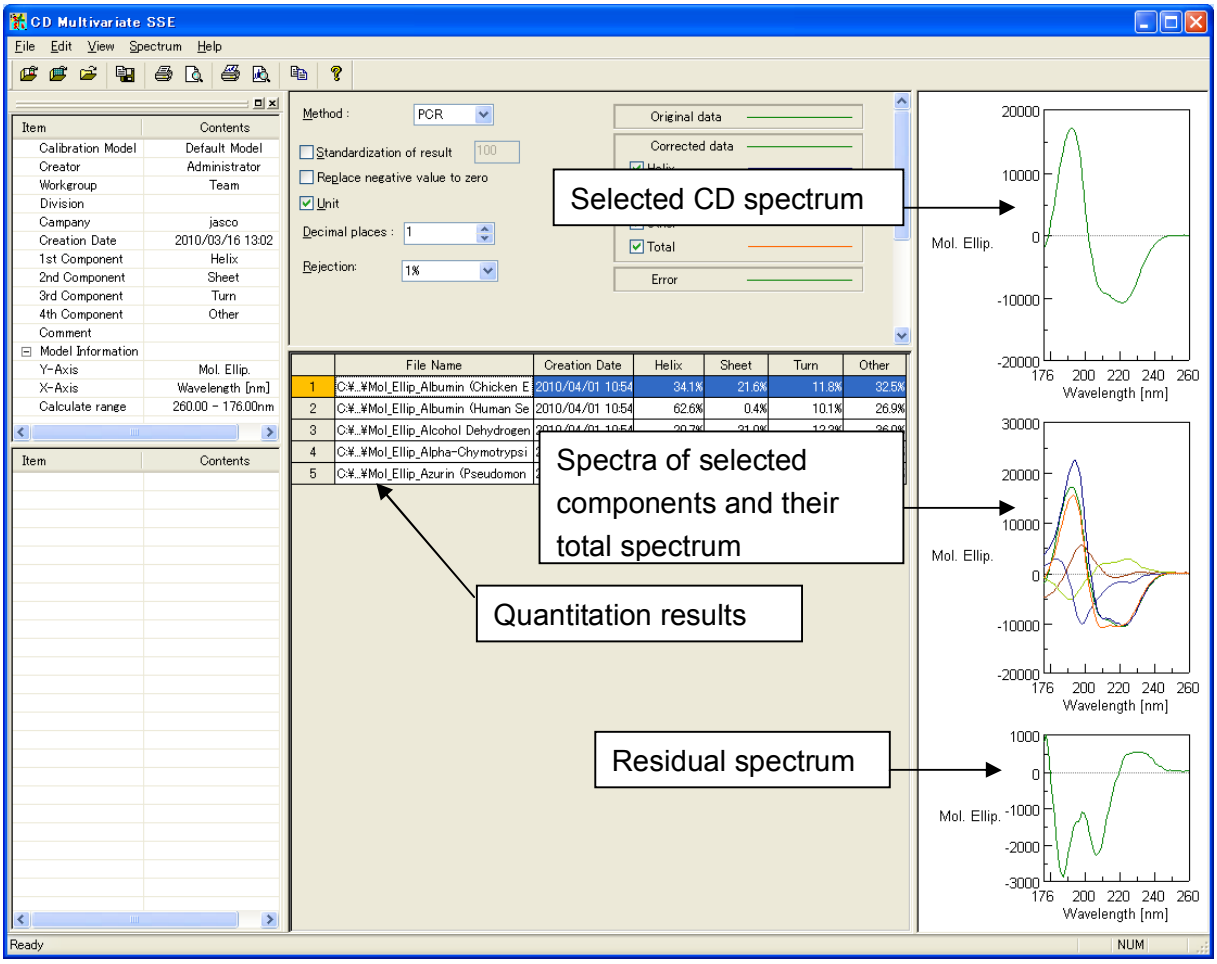


Figure 2.4 [CD Multivariate SSE] window after loading spectra

(3) The [CD Multivariate SSE] program can quantitate a total of five types of vertical unit data, specifically measurement data (mdeg) and four types of post-optical constant calculation data (Mol. Ellip, Mol. CD, Spc. Ellip, and g Value). The data to quantitate must be converted to one of these four vertical units. In addition, the vertical unit of the calibration model and data to quantitate must be identical. When pre-optical constant calculation data (mdeg) is loaded, the [Optical Constant] dialog box in Figure 2.5 is automatically displayed. Enter values in [Path-length] and [Concentration] and then click the <Apply> button. The data will be converted to the same vertical unit as the calibration model, and the quantitation results will be displayed.

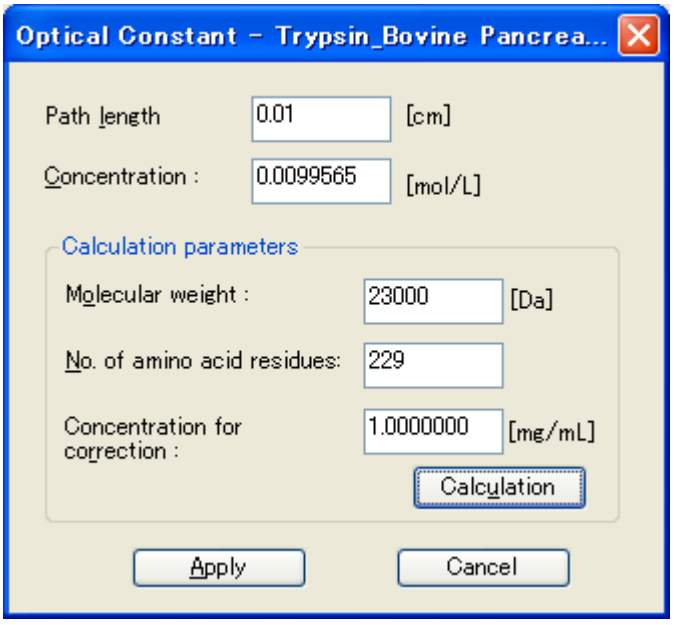


Figure 2.5 [Optical Constant] dialog box

(4) The parameters under [Calculation parameters] are for calculating the molar concentration of protein in [Concentration] (mol/L). Enter values in [Molecular weight], [No. of amino acid residues], and [Concentration for correction], and then click the <Calculation> button. The calculation results are displayed in [Concentration] (mol/L). In the case of polymers, such as proteins, their molar concentration is multiplied by the number of amino acid residues to calculate the concentration.

Even if the vertical units of the calibration model and quantitation data differ between Mol. Ellip. and Mol. CD, they will be automatically converted. When CD spectra with the Mol. CD unit is loaded into a calibration model with the Mol. Ellip. unit, or vice versa, quantitation will be automatically performed without the [Optical Constant] window being displayed.

2.4 Setting Analysis Parameters

The window in Fig. 2.6 is where analysis parameters are set. As soon as these parameters are changed, they will be applied to quantitation results. Change these parameters when performing operations, such as comparing the results of PCR and PLS quantitative analysis. Analysis parameters cannot be changed in the CFR version.

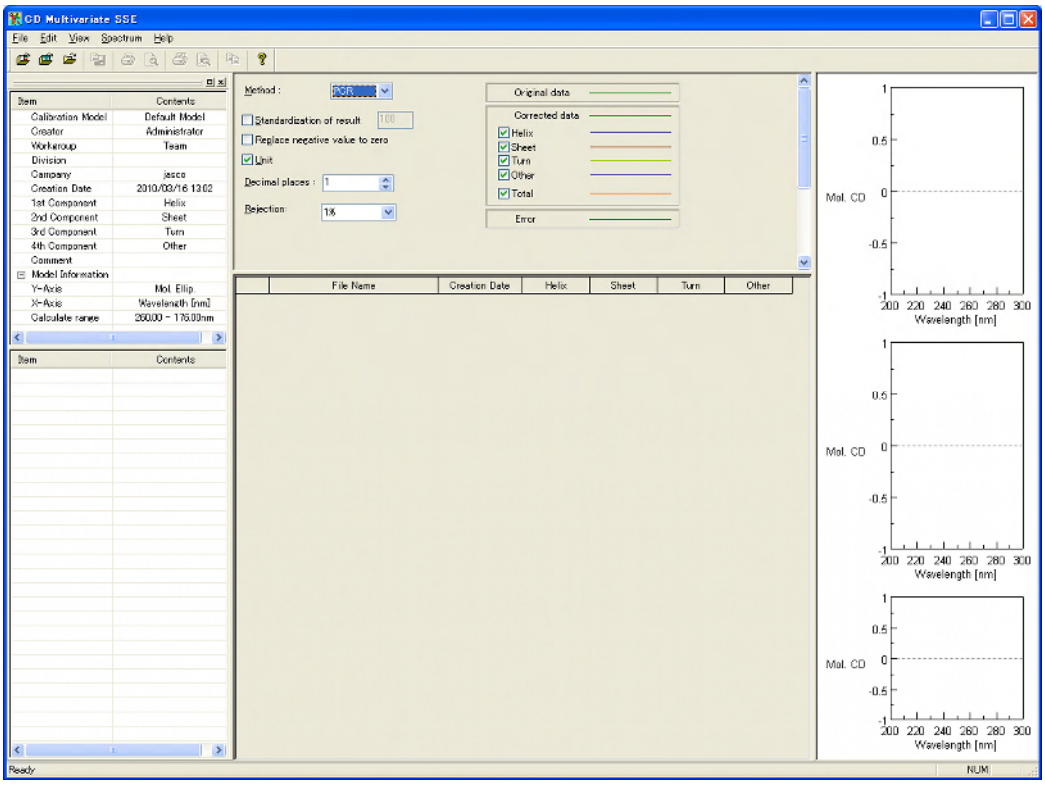


Figure 2.6 [CD Multivariate SSE] window (Non-CFR version)

[Method] Selects the PCR or PLS method.

Note: Refer to Chapter 4 "Appendix" for details about the PCR and PLS methods.

[Standardization of result] Adjusts the total of the quantitation value to the selected value.

[Replace negative value to zero] Displays negative quantitation results as zero.

[Unit] Adds the percentage symbol (%) to the analysis results.

[Decimal places] Specifies the number of decimal places for quantitation results.

[Rejection] Sets the rejection percentage for quantitation results. Data are rejected when they contain lots of errors and are therefore unreliable. Quantitation results that are outside the rejection percentage are displayed in parentheses in the data sheets. A higher rejection rate results in a stricter evaluation of the data. A value of 2% is normally set.

2.5 Printing Quantitation Results

2.5.1 Printing Result List

A list of quantitation results can be printed by selecting [Print] - [List...] from the [File] menu. All the results displayed in the data table are printed.

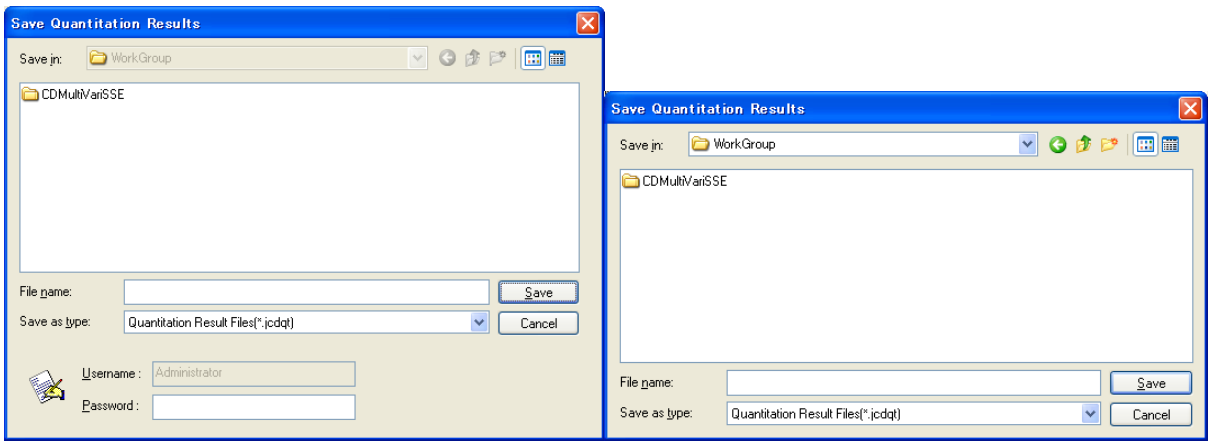
2.5.2 Printing a Spectrum

A spectrum can be printed by selecting [Print] - [Spectrum...] from the [File] menu. The currently displayed spectrum and its quantitation results are printed.

2.6 Saving Quantitation Results

2.6.1 Saving Analysis Result Files

Select [Save Quantitation Results...] from the [File] menu to save the currently displayed results in the special file format used by the [CD Multivariate SSE] program (.jcdqt).



(CFR version) (Non-CFR version)

Figure 2.7 [Save Quantitation Results] dialog box

2.6.2 Exporting Quantitation Results in Text Format

Select [Export Results] from the [File] to save the currently displayed results in text format.

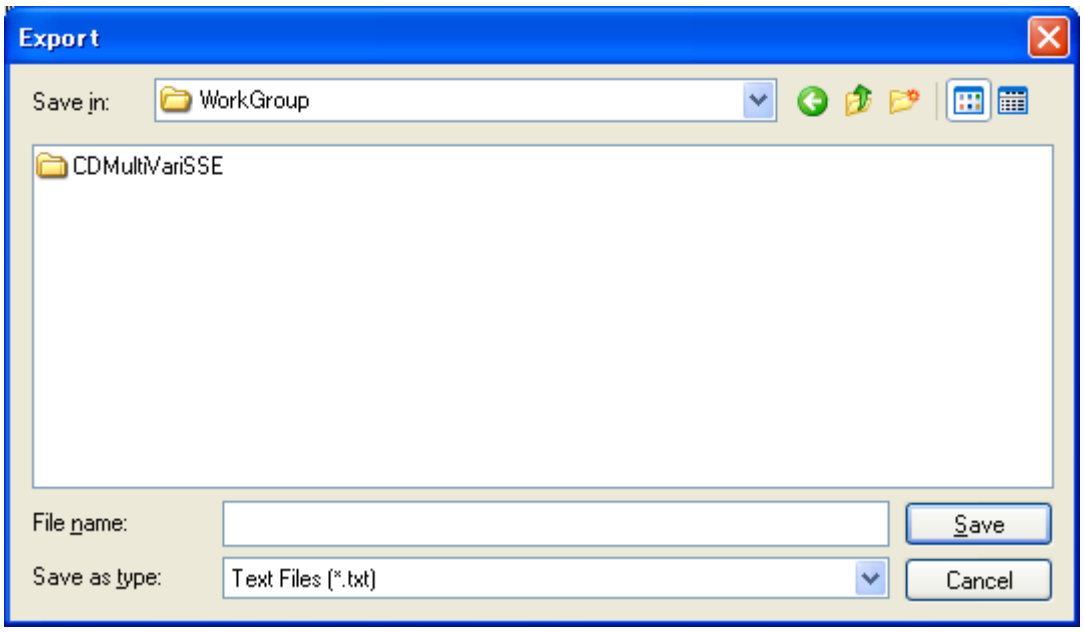


Figure 2.8 [Export] dialog box

2.6.3 Exporting Calculated Spectra in Text Format

Calculated spectra can be saved in text format. Selecting [Export Calculated Spectra] from the [File] menu saves the currently selected spectra calculation results in text format.

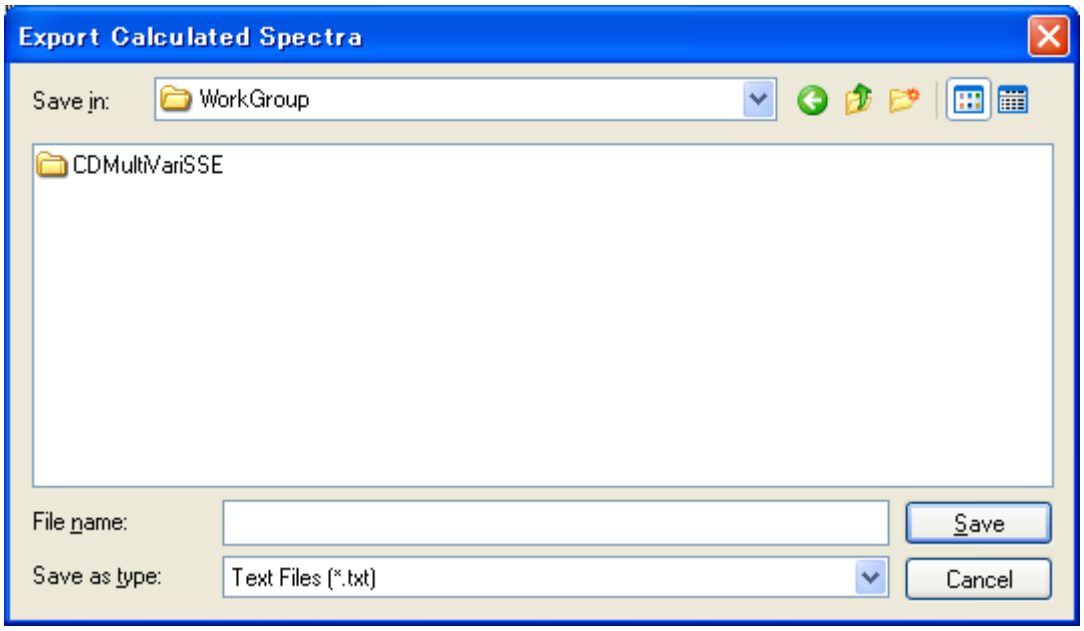


Figure 2.9 [Export Calculated Spectra] dialog box

2.7 Exiting [CD Multivariate SSE] Program

(1) Exiting the [CD Multivariate SSE] program.
In the [CD Multivariate SSE] window, select [Exit] from the [File] menu to return to the [Spectra Manager] window.

(2) Exiting the [Spectra Manager] program.
Select [Exit] from the [Program] menu.

3. [CD Multivariate SSE] Program Reference

The window in Fig. 3.1 is displayed when the [CD Multivariate SSE] program is started from the [Spectra Manager] window. The calibration models that come standard and their analysis parameters are loaded automatically when the [CD Multivariate SSE] program starts. Select [Select Model] - [Open Model...] from the [File] menu to open a separately created calibration model (or click the  tool button). The [Open Model] dialog box is displayed.

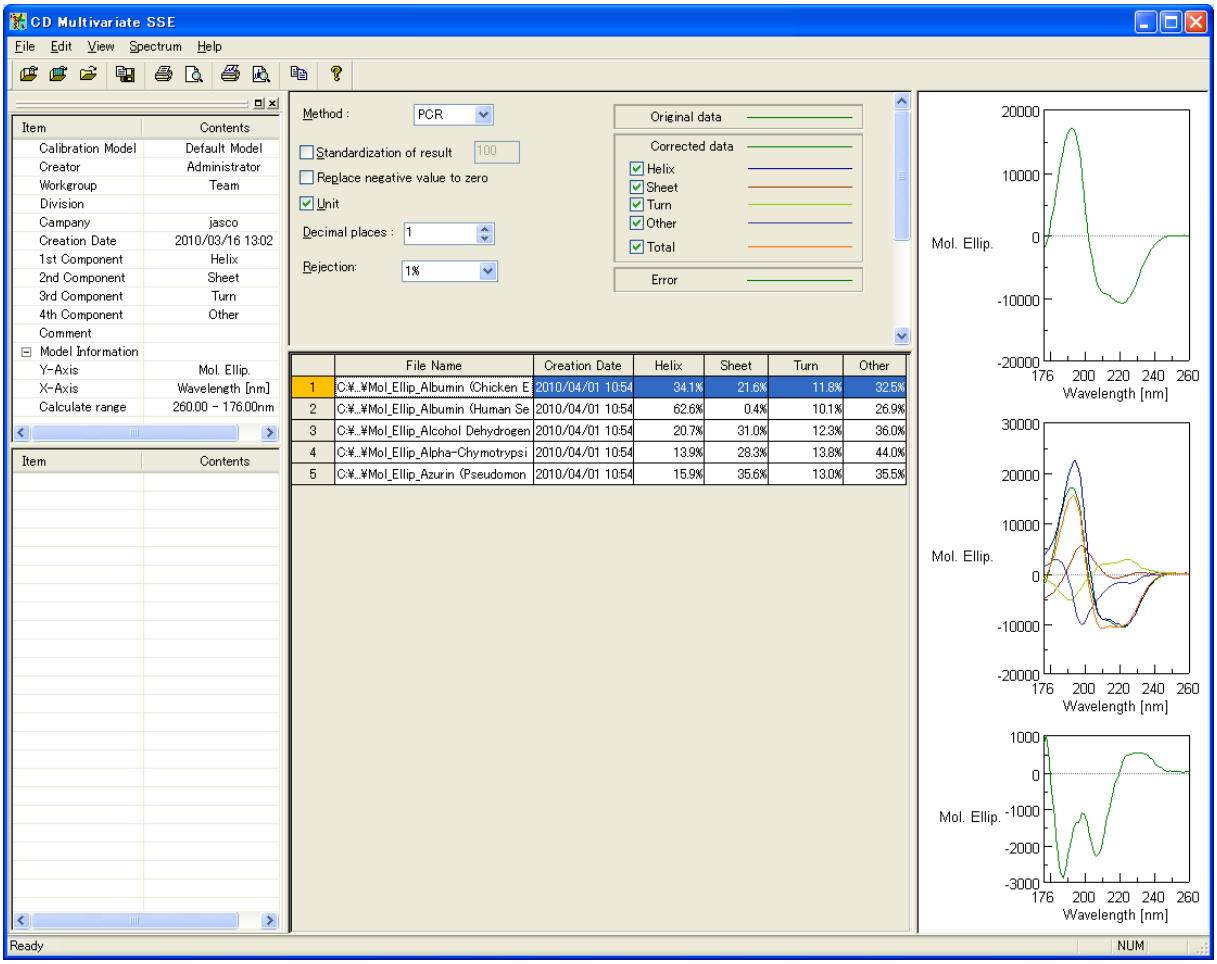


Figure 3.1 [CD Multivariate SSE] window

(1) Menu

[File] menu	Selects calibration models to use in the [CD Multivariate SSE] program and prints/saves quantitation results.
[Edit] menu	Copies quantitation results to the Clipboard.
[View] menu	Changes the scale, font, gridlines, pattern, style of spectra, and changes the font of data sheets and the color/style of input cells.
[Spectrum] menu	Selects and deletes spectra for performing quantitation.
[Help] menu	Displays version information for the program.

3.1 [File] Menu

[Select Model]	Selects a calibration model to use in the analysis. Select a model that comes standard or one created using the [CD Multivariate Calibration Creation] program.
[Open Quantitation Results...]	Opens a quantitation results file that was saved in .jcdqt format.
[Save Quantitation Results...]	Saves quantitation results in .jcdqt format.
[Open Spectra...]	Opens the selected spectra and performs quantitation.
[Export Results...]	Saves quantitation results in text format.
[Export Calculated Spectra...]	Saves the CD spectra of the selected sample and the CD spectra of each secondary structure in text format.
[Print]-[List...]	Prints all quantitation results displayed in the data sheet.
[Print]-[Spectrum...]	Prints the currently displayed spectrum and its quantitation results.
[Print Preview]-[List...]	Displays a print preview of the result list.
[Print Preview]-[Spectrum...]	Displays a print preview of the spectrum.
[Print Items]-[List...]	Selects the items to print.
[Print Items]-[Spectrum...]	Selects the items to print.
[Print Setup]	Selects and sets up the printer.
[Exit]	Exits the program.

3.2 [Edit] Menu

[Copy Result]	Copies the currently selected data to the Clipboard.

3.3 [View] Menu

[Data List...]	Shows/hides the items in the data sheet. [File Name], [Creation Date], [Sample Name], [Component name; Helix, Sheet, Turn and Other], [Comment], [Vessel No.] can be set.
[Toolbar]	Shows/hides the toolbar.
[Status Bar]	Shows/hides the status bar.
[Work Space]	Shows/hides the workspace.
[Scales...]	Changes the display range of the spectrum.
[Patterns...]	Sets the display color and line type of spectra.
[Gridlines...]	Shows/hides gridlines for spectra.
[Styles...]	Sets the display style for spectra.
[Fonts...]	Specifies the display font for spectra.

[Model Info...]	Displays information on the calibration model.

3.4 [Spectrum] Menu

[Open...]	Loads the selected spectrum files into the data sheet.
[Open All...]	For the spectrum files in the selected folder, all files that can be analyzed are displayed in the data sheet. Only files containing spectra with a wavelength range (horizontal axis of graph) equal to the calibration model or more, or with vertical units identical to the calibration model or mdeg, can be analyzed.
[Delete]	Deletes the selected spectra data from the data sheet.
[Delete All]	Deletes all the spectra data displayed in the data sheet.

3.5 [Help] Menu

[Contents...]	Displays the help window with the contents tab selected.
[Search Topic...]	Displays the help window with the index tab selected.
[About...]	Displays version information for the program.

4. Appendix

4.1 Principle of PLS Quantitative Analysis

PLS quantitative analysis is the quantitative analysis of a spectrum using the partial least squares (PLS) method. To perform PLS quantitative analysis, PLS calibration models, which contain correlation curves for quantifying the normal absorbance of the components to be estimated, need to be prepared first. Creating a PLS calibration model requires that there be at least the same number of standard spectra with known concentrations of the target components as there are components to quantitate. Twenty to thirty spectra are required to obtain a practical PLS calibration model. This means that the number of standard samples can be one-third to one-fifth of the number used in multiple regression determination (ILS). A description of the PLS calibration model and procedure is given here.

A PLS calibration model uses the least squares method to extract the spectral component that is dependant on concentration from multiple standard spectra, and it then uses the least squares method to determine the contribution of that spectral component to each standard spectrum and the regression equation of the concentration. As such, the spectra are the factor vectors, and the loading of these factor vectors is referred to as the score.

In very simple cases, such as when multiple samples of water in which pigment has been dissolved are measured in the visible region, the factor vector is the absorption spectrum of the pigment itself and the regression curve between the score and concentration will be a linear. In more complex cases, however, such as when the fat content of food is measured in the NIR region, the factor vector is almost a reflection of the absorption of the fat, and the relationship between the score and concentration is not linear, even though it is correlated. In other words, standard spectra and component concentration cannot be related with high accuracy using only a single factor vector.

PLS calibration involves performing the process described above, but additional factors are introduced to relate the spectrum residual and concentration residual, which are not accounted for by a single factor vector. Regression analysis is performed on this second factor vector over the residuals of score and concentration. The processing is repeated until errors become sufficiently small.

A correlation curve is created from n standard spectra by using n-1 samples with a single sample removed. A quantitative value is calculated using the above-mentioned correlation curve created using the spectrum that was not included, and the difference between the predicted value (evaluated concentration) and the true value (entered concentration) is obtained. These steps are repeated, with a different sample excluded each time. The root-mean-square error of prediction (RMSEP) is obtained by taking the sum of the squares of the concentration differences, dividing this by the number of standard samples used and then taking the square root of the result.

$$RMSEP = \sqrt{\frac{\sum \Delta^2}{N}}$$

Another method is to calculate the correlation coefficient between a known concentration value and a calculated value instead of the RMSEP value to determine the value H that maximizes the correlation coefficient. The PLS calibration model is made up of H factor vectors and H regression curves. PLS quantitative analysis obtains the score of the first quantitative analysis factor with respect to a spectrum of unknown concentration to obtain the spectrum residual. This is repeated H times to obtain the scores for H factor vectors. The concentration of the unknown spectrum is then calculated from these scores and the corresponding regression curves.

4.2 Principle of PCR Quantitative Analysis

PCR quantitative analysis is the quantitative analysis of a spectrum using the partial least squares (PCR) method. PCR calibration models need to be prepared first before performing PCR quantitative analysis. These PCR calibration models contain correlation curves for quantifying the normal absorbance of the components to be estimated. Creating a PCR calibration model requires that there be at least the same number of standard spectra with known concentrations of the target components as there are components to quantitate. Twenty to thirty spectra are required to obtain a practical PCR calibration model. This means that the number of standard samples can be one-third to one-fifth that required in multiple regression determination (ILS).

A PCR calibration model is a quantitation method that performs principal component analysis for multiple standard spectra and then performs multiple regression analysis for the results and concentrations. The spectral components obtained by principal component analysis are referred to as principal component spectra, and calibration models can be created by assuming that there are a number of principal components. The maximum number of principal components is (number of standard spectra - 1).

The number of principal components to use varies depending on the target system. When creating a PCR calibration model, it is possible to determine the optimal number of principal components (H) by plotting a graph of the number of principal component spectra as a function of the correlation coefficient.

A correlation curve is created from *n* standard spectra by removing a single sample (i.e., by using *n*-1 samples). A quantitative value is calculated using the above-mentioned correlation curve created using the excluded spectrum, and the difference between the predicted value (the calculated concentration) and the true value (the measured concentration) is obtained. These steps are repeated, with a different sample excluded each time. The root-mean-square error of prediction (RMSEP) is obtained by taking the sum of the squares of the concentration differences, dividing this by the number of standard samples used and then taking the square root of the result.

$$RMSEP = \sqrt{\frac{\sum \Delta^2}{N}}$$

Another method is to calculate the correlation coefficient between a known concentration value and a calculated value instead of the RMSEP value to determine the value H that maximizes the correlation coefficient. The PCR calibration model is made up of H factor vectors and H regression curves. PCR quantitative analysis determines the score of the first quantitative analysis factor with respect to a spectrum of unknown concentration to obtain the spectrum residual. This is repeated H times to obtain the scores of the H factor vectors. The concentration of the unknown spectrum is then calculated from these scores and the corresponding regression curves.

4.3 Notes on Measurement/Analysis

4.3.1 Notes on Measurement and Sample Preparation

(1) The CD spectra of 26 types of proteins that come standard with the program were measured by JASCO. Each protein was measured in an aqueous solution of a commercially available reagent. The concentration of proteins is calculated based on the molar absorbance coefficient.

The ratio of secondary structures of the 26 types of proteins was calculated based on the information at the PROTEIN DATA BANK (http://www.rcsb.org/pdb/home/home.do) (January 2010).

Helix: Total number of α-Helix and 3/10-Helix residues
Sheet: Number of strand residues
Turn: Number hydrogen bonded turn residues
Other: All other residues

The 26 types of proteins that come standard with the program and the IDs (PDB ID) in the PROTEIN DATA BANK are as follows.

Protein Name	PDB ID
Albumin_Chicken Egg	1ova DSSP
Albumin_Human Serum	1bm0 DSSP
Alcohol Dehydrogenase_Yeast	2hcy DSSP
Alpha-Chymotrypsin_Bovine	5cha DSSP
Azurin_Pseudomonas aeruginosa	1azc DSSP
Beta-Lactoglobulin_Bovine milk	1beb DSSP
Catalase_Bovine Liver	8cat DSSP
Concanavalin A_Canavalia ensiformis	2ctv DSSP
Cytochrome C_Horse Heart	1hrc DSSP
Elastase_Porcine Pancreas	3est DSSP
Glyceraldehyde-3-phosphate-dehydrogenase_Rabbit muscle	1j0x DSSP
Hemoglobin Bovine	2qss DSSP
IgG_Bovine	6fab DSSP
Lactate Dehydrogenase_Chicken Heart	6ldh DSSP
Lysozyme_Chicken Egg	1lys DSSP
Myoglobin_Horse Heart	2vlx DSSP
Papain_Carica papaya	9pap DSSP
Pepsinogen_Porcine stomach	3psg DSSP

Ribonuclease A_Bovine Pancreas	3rn3 DSSP
Ribonuclease S_Bovine Pancreas	1n3z DSSP
Subtilisin Carlsberg_Bacillus licheniformis	1c3l DSSP
Superoxide dismutase_Bovine Erythrocytes	2sod DSSP
Thermolysin_Bacillus thermoproteolyticus	8tln DSSP
Triose phosphate isomerase_Rabbit muscle	1r2t DSSP
Trypsin_Bovine Pancreas	1k1i DSSP
Trypsin inhibitor [Kunitz]_Soybean	1ba7 DSSP

(2) Sampling of aqueous solutions

When a protein aqueous solution is measured using a CD spectrometer, a concentration of 1 mg/mL in a 0.1 mm cell is generally used. Change the concentration and cell length as required. Since absorption by the salts included in the buffer solution affects CD measurement in the vacuum UV region, select a buffer solution with low absorption.

4.3.2 Notes on Analysis

(1) Selecting a quantitative analysis method (PCR or PLS)

Quantitation results will differ between PCR and PLS quantitative analysis. PLS quantitative analysis is recommended when the secondary structure component ratio in the sample is thought to be similar to that in any of the 26 proteins, and PCR quantitative analysis is recommended when the secondary structure component ratio in the sample is thought to be very different to that in the model protein. The reason for this is that, in general, quantitation precision readily falls even when the difference in the component ratio of proteins used in creating the calibration model and secondary structures is large. In contrast, even when the difference in the component ratio of proteins used in creating the calibration model and secondary structures is large in the case of PCR quantitation, precision does not readily fall. The PCR method is recommended for samples that give results containing an unusually large amount of components (for example, Helices). The PLS method is recommended for samples that give results containing a nearly uniform amount of components.

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