JWMVS-529

CD Multivariate Calibration Creation Program

Software Manual

Jasco

Preface

This instruction manual serves as a guide for using this software. It is intended to instruct first-time users on how to properly use the software, and to serve as a reference for experienced users.

Before using the software, read this instruction manual carefully, and make sure you fully understand its contents. This manual should be easily accessible to the operator at all times during software operation. When not using the software, keep this manual stored in a safe place. Should this instruction manual be 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.

Notices

(1)   JASCO shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.

(2)   Prohibitions on the use of JASCO software

- Copying software for purposes other than backup
- 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).

(7)   *Windows* is a registered trademark of *Microsoft Corporation* in the United States and other countries. In general, company names and product names are trademarks or registered trademarks of the respective companies. However, the ^{TM} and $^{®}$ marks are not used in all cases in this manual.

(8)   JASCO and the JASCO logo are registered trademarks of JASCO Corporation in Japan and other countries.

Limited Warranty

Products sold by JASCO, unless otherwise specified, are warranted for a period of one year from the date of shipment to be free of defects in materials and workmanship. If any defects in the product are found during this warranty period, JASCO will repair or replace the defective part(s) or product free of charge.

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
(7)   CONSUMABLES AND PARTS OF WHICH WARRANTY PERIOD IS SPECIFIED OTHERWISE.

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.

<div align="right">

JASCO Corporation
2967-5, Ishikawa-machi, Hachioji-shi
Tokyo 192-8537,
JAPAN

</div>

Notation Used

The following notational conventions are used throughout this manual:

General Notation

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 Calibration Creation] program that runs on *Microsoft Windows*. The [CD Multivariate Calibration Creation] 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 three 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 Calibration Creation] program, and describes the program's menus. Read this chapter first.

This program is for creating calibration models for analyzing the secondary structures of proteins. For information on the program for performing quantitation using the calibration models created by this program, refer to "CD Multivariate SSE Program Software Manual". Calibration models are created offline using data files. The [CD Multivariate SSE] program is capable of the simultaneous quantitation of multiple components (Helix, Sheet, Turn, Other, etc.) using the selected calibration model.

- Standards samples with known concentrations are measured to create a relational expression (calibration model) for the concentrations of its components. This model is then stored along with the data.
- The [CD Multivariate Calibration Creation] program creates calibration curve models offline from data files. Calibration curve models created in this program are loaded into the [CD Multivariate SSE] program to perform quantitative analysis.
- Quantitative analysis can also be performed by using the calibration curve models that come standard with the program.

2. Introduction to [CD Multivariate Calibration Creation] Program

This chapter describes in detail the procedures for starting the [CD Multivariate Calibration Creation] program and creating calibration models (calibration curve data files) to be used in the [CD Multivariate SSE] program. This will ensure that the user is familiar with how the program works.

3. [CD Multivariate Calibration Creation] Program Reference

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

1.2 Overview of the [CD Multivariate Calibration Creation] Program

The [CD Multivariate Calibration Creation] program is for creating calibration models (calibration curve data files) to use in the [CD Multivariate SSE] program. The program creates PCR and PLS calibration models by using multiple pieces of protein data with known protein secondary structures. At least as many spectra as there are secondary structure components are necessary to create the calibration model. For detailed information on the PLC and PCR methods, see Chapter 4 "Appendix" in "CD Multivariate SSE Program Software Manual".

Note: This manual is for the [CD Multivariate Calibration Creation] program. For information on estimating the secondary structures of proteins, see "CD Multivariate SSE Program Software Manual".

1.3 Features of the [CD Multivariate Calibration Creation] Program

- When creating calibration models, always start the [CD Multivariate Calibration Creation] program on the analysis side.
- Calibration models can be created from up to 100 spectra.
- The number of components of the protein secondary structure can be changed from the four components (Helix, Sheet, Turn, and Other) in the initial parameters.
- Data can be edited (validation and invalidation of spectrum and others) on the calibration curve.

1.4 Using Calibration Models That Come Standard in CFR Version Programs

When using the calibration models that come standard with this program in the CFR version program, copy them to a work folder according to the following procedure and then sign it with an electronic signature.

Note: With the CFR version, it is not possible to select [Save in]. Only a subfolder within the prespecified folder can be selected.

(1) Copy the folders in the following installation folder to the work folder.
    Copy the CalibrationModel folder located in C:\ProgramFiles\JASCO\SpectraManager\ Common\CDOption\CDMultiVariSSE to the workgroup folder. This will allow the calibration models to be opened from the [CD Multivariate Calibration Creation] program. When doing this, make sure to leave the CalibrationModel folder in its original folder (C:\ProgramFiles\JASCO\SpectraManager\Common\CDOption\CDMultiVariSSE), i.e., copy it rather than move it.
(2) Log in with Managers rights and then open the calibration model to use in the [CD Multivariate Calibration Creation] program. Save it using a different name. The calibration models that come standard cannot be overwritten.

(3) In Spectra Manager CFR, review and approve the calibration model that was saved using a different name.

Managers can review and approve, while Analysts can only review. Operators cannot perform any kind of electronic signing. Login with the Managers rights, and create, review, and sign. Note that in the CFR version program, only calibration models approved by Managers can be saved in binary format (.jcdqt). Quantitative analysis can be performed with unapproved calibration models, but the results can only be saved in text format. Managers, Analysts, and Operators can save results in text format.

To use the CD spectra that come standard with the program to create calibration models, copy the Samples folder located in C:\ProgramFiles\JASCO\SpectraManager\Common\ CDOption\CDMultiVariSSE to the workgroup folder. This will allow them to be opened from the [CD Multivariate Calibration Creation] program. Save them using a different name and sign them electronically, so that they can be used to create calibration models.

1.5 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.

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

JASCO Spectra Manager[TM] 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[TM] software provides functionality ranging from analytical instrument control and spectral data processing to sophisticated and specialized data analysis programs. JASCO Spectra Manager[TM] 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[TM] CFR consists of three core modules: Administrative Tools and Security Manager modules; intrinsic components of the Spectra Manager[TM] CFR software; and a database, which is an external component to the Spectra Manager[TM] 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.
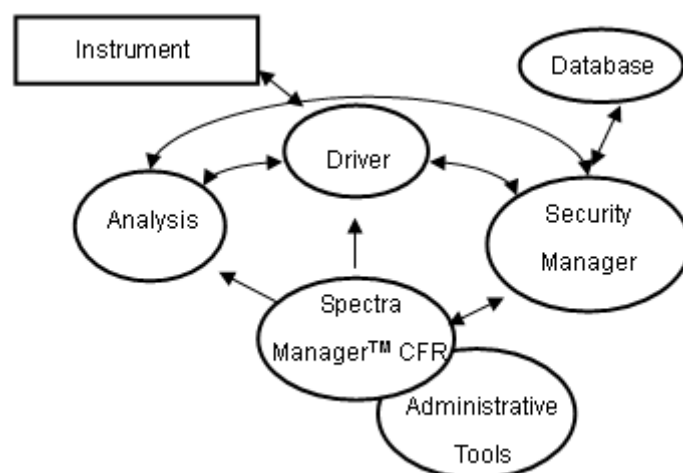

Figure 1.1 Structure of Spectra Manager^{TM} CFR

All of the security management functions of JASCO Spectra Manager^{TM} 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^{TM} CFR has two levels of security: system-level security and workgroup-level security.

When a user is registered in JASCO Spectra Manager^{TM} 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^{TM} 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^{TM} 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.
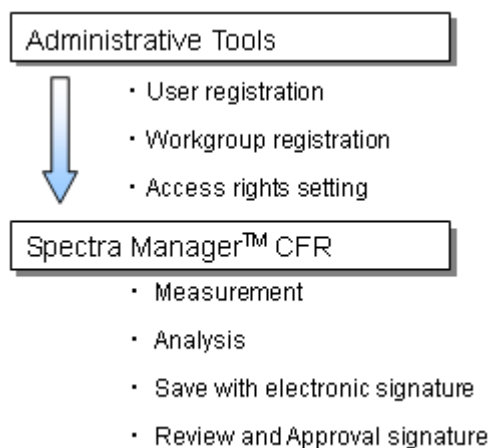

Figure 1.2 Operational flow

2. Introduction to the [CD Multivariate Calibration Creation] Program

This chapter describes in detail the procedures for starting the [CD Multivariate Calibration Creation] program, examining calibration parameters for spectra, creating calibration models for proteins, and saving/printing the results.

Note: See "CD Multivariate SSE Program Software Manual" for information on how to perform quantitative analysis on unknown samples using the calibration models created in the [CD Multivariate Calibration Creation] program.

2.1 Operation Overview

Starting the [CD Multivariate Calibration Creation] program.   See Section 2.2.

↓

Creating a new calibration model.   See Section 2.3.

↓

Examining the optimal parameters for protein secondary structure using the PCR and PLS methods.

See Section 2.4 and 2.5.

↓

Checking the validity of calibration models.   See Section 2.6.

↓

Saving calibration curves.   See Section 2.7.

↓

Saving calibration models.   See Section 2.8.

↓

Exiting the [CD Multivariate Calibration Creation] program.   See Section 2.9.

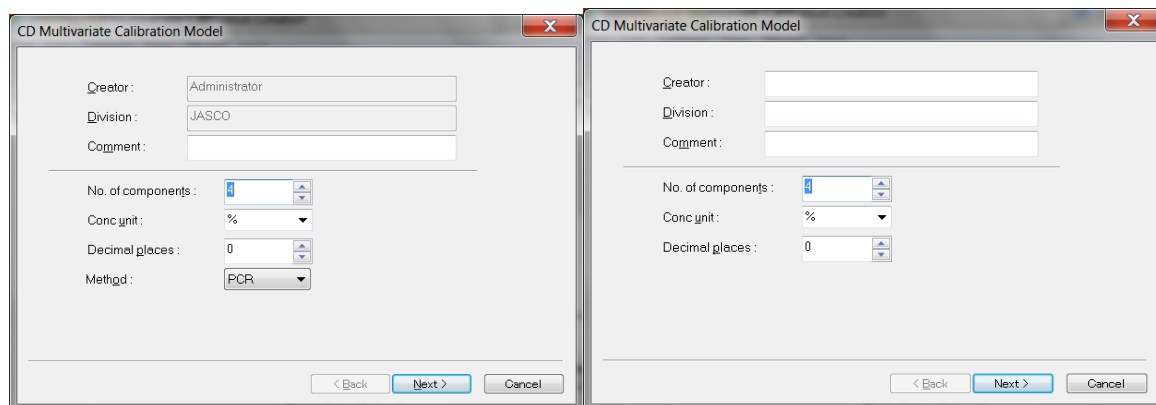## 2.2 Starting the [CD Multivariate Calibration Creation] Program

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

Figure 2.1 [CD Multivariate Calibration Creation] window

2.3 Creating New Calibration Models
2.3.1 Setting Data Files

(1) Select [New] from the [File] menu or click the ⬜ tool button. The dialog box in Fig. 2.2 is
displayed.

CFR version   Non-CFR version

Figure 2.2 [CD Multivariate Calibration Model] dialog box

(2) Enter the following parameters:

[Creator]	Enters any creator name desired. In the CFR version, the user name of the user that logged in will automatically be entered.
[Division]	Enters any name desired. In the CFR version, the division of the user that logged in will automatically be entered.
[Comment]	Enters any comment desired.
[No. of components]	Enters the number of protein secondary structures to be quantitated. The default setting is 4.
[Conc unit]	Selects a unit from the drop-down list.
[Decimal places]	Enters the number of decimal places for data.
[Method]	Selects the PLC or PCR method for the CFR version only.

Note 1: Increase the value in [No. of components] to increase the number of components in the protein secondary structure.

Note 2: In the non-CFR version, the quantitation method can be changed in the [CD Multivariate Calibration Creation] program, but in the CFR version, the method selected in advance is applied.

(3) Click the <Next> button. The dialog box shown in Fig. 2.3 is displayed. Enter component names corresponding to the number of components set in the dialog box in Fig. 2.2.

Figure 2.3 [Component Name] dialog box

Note: If the number of components in the protein secondary structure was increased beyond the default value in the dialog box in Fig. 2.2, click a [Component] text box starting from number five and enter a component name.

2.3.2 Loading Standard Spectra

 Click the <Next> button in the dialog box in Fig. 2.3 to display the dialog box in Fig. 2.4. Click the <Browse...> button, select spectra data with a known protein secondary structure to use in creating the calibration model, and then click <Open>. Up to 100 spectra data files can be opened.
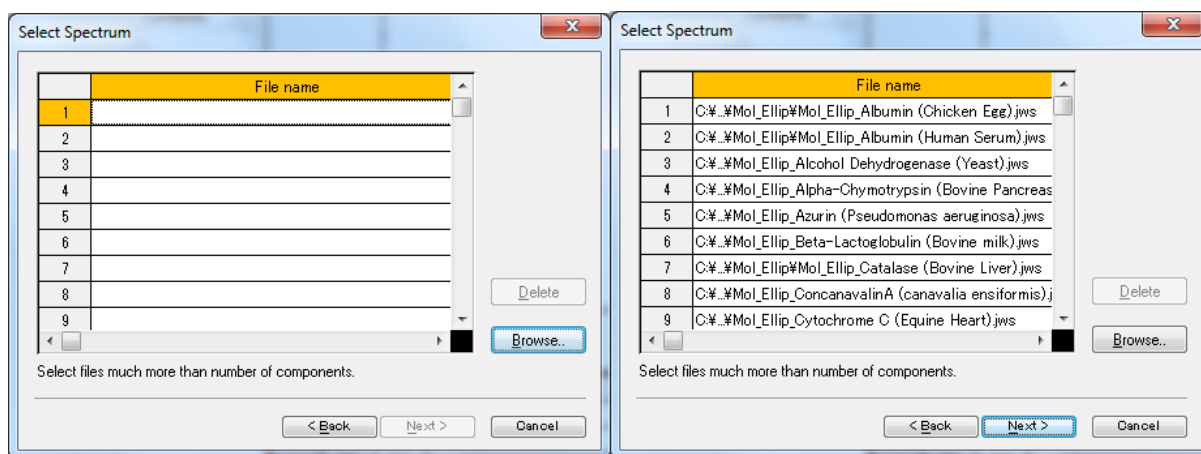
Before data is loaded   After data is loaded

Figure 2.4 [Select Spectrum] dialog box

Note 1: Multiple spectrum files can be loaded at once by holding down Shift or Ctrl while clicking the file names. Data for calibration models requires at least the number of secondary structure components that were set.

Note 2: In the CFR version, only electronically signed CD spectra can be opened.

2.3.3 Setting Calculation Parameters

(1) Click the <Next> button in the dialog box in Fig. 2.4 to display the dialog box in Fig. 2.5. Set the parameters here. The values for [PCR principal components] and [PLS factor] can be changed after creating a calibration model. In this example, enter "4" for [PCR principal components] and "3" for [PLS factor].

Note 1: In the CFR version, only [PCR principal components] or [PLS factor] will be active, depending on the quantitation method selected in the dialog box in Fig. 2.2.

Note 2: See Sections 2.4.1 and 2.5.1 for details about setting the calculation range.

CFR version   Non-CFR version
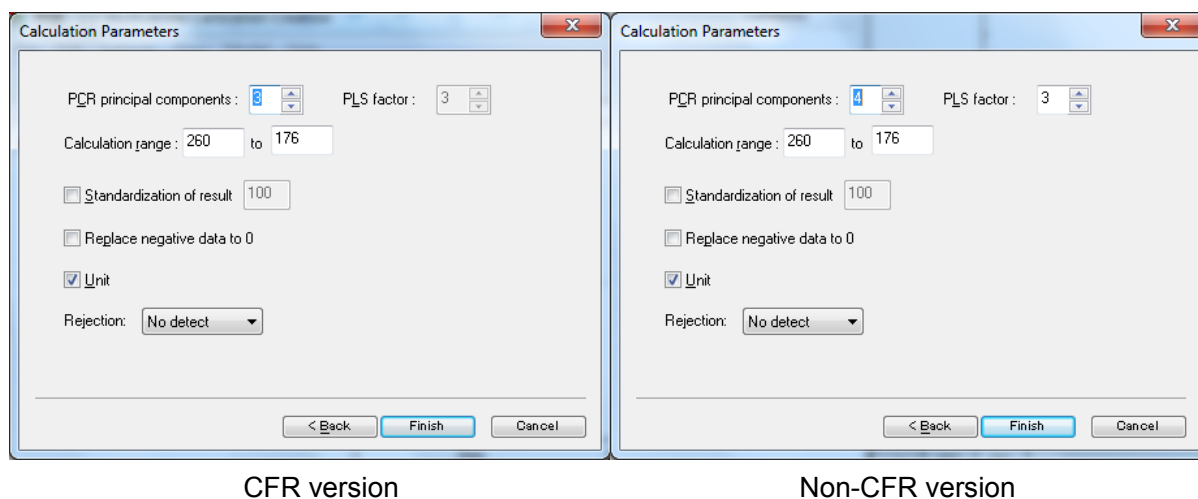
Figure 2.5 [Calculation Parameters] dialog box

(2) Click the <Finish> button. The spectrum names are displayed in the spectrum display field and the file names in the data sheet.

2.3.4 Entering Protein Secondary Structures

(1) Click the blank cells under the components in the data sheet and then enter a value.
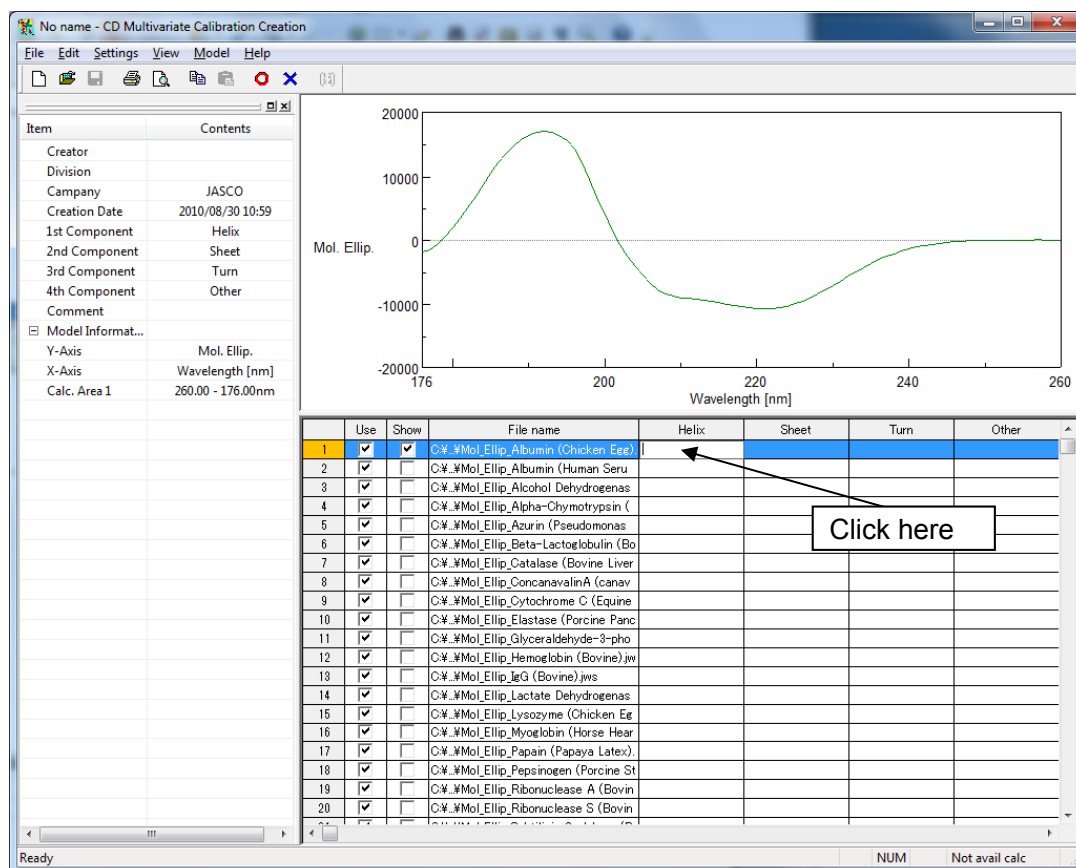
Figure 2.6 [CD Multivariate Calibration Creation] window (after loading data)

(2) Enter the same number of values for protein secondary structures as the number of components set.

Note: *The following operations cannot be performed until values for all the cells corresponding to the number of components set have been entered.*

13

2.3.5 Calculating

(1) Select [Calculate] from the [Model] menu or click the ⬚ tool button to calculate the calibration model.

To omit spectra, clear the [Use] check box of the spectra to be omitted (see Fig. 2.6) and then recalculate. This will create a calibration model without the omitted spectra.

Note: See Sections 2.4.1 and 2.5.1 for details about setting the calculation range.

2.4 Using PCR Method to Examine Optimal Number of Principal Components in Analysis Parameters for Protein Secondary Structures

2.4.1 Using [Correlation Coefficient Spectrum] to Determine X-Axis Calculation Range

(1) Select [PCR] - [Correlation Coefficient Spectrum...] from the [Model] menu. The dialog box in Fig. 2.7 is displayed.
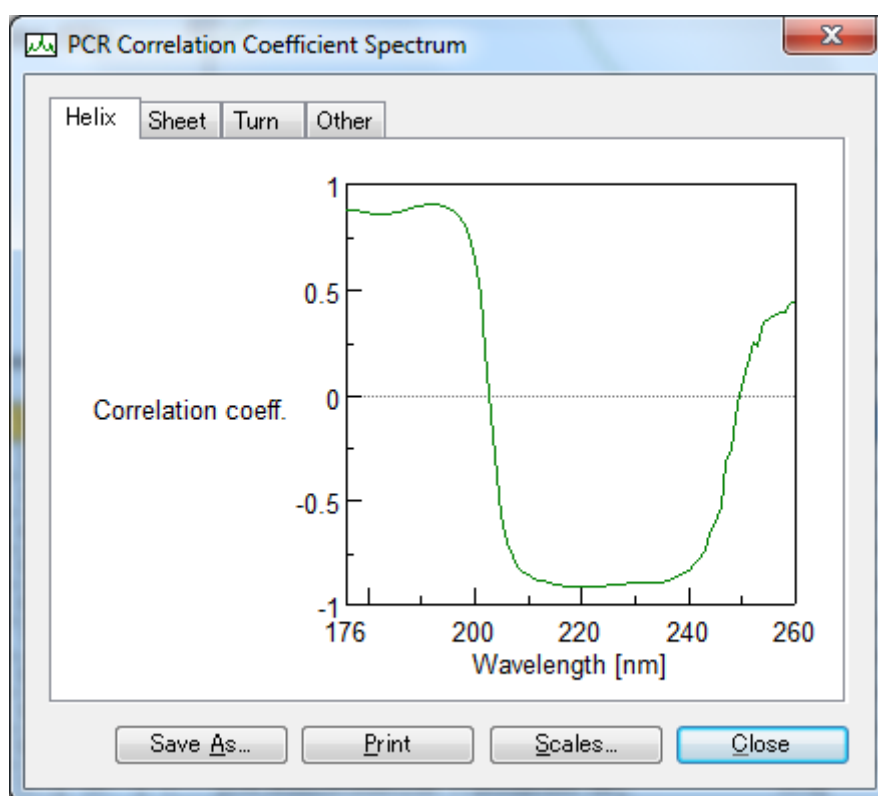
Figure 2.7 [PCR Correlation Coefficient Spectrum] dialog box

(2) Decide the calculation range of the x-axis based on the correlation coefficient spectrum. A correlation coefficient value near a range between +1 and –1 is decided for the calculation range. With the spectrum in this case, there is a region with a dramatic change from -1 to +1, but a range between 260 and 176 nm is appropriate. The calculation range determined here will be used in Section 2.4.4 "Resetting the Number of Principal Components", so write it down for future use.

Note 1: If the x-axis values are difficult to read, drag the mouse diagonally over the graph to enlarge it. Right-click the graph to restore the graph to its original scale.

Note 2: Save/print the graph as required.

2.4.2 Using [Correlation Coefficient vs. No. of Principal Components...] to Determine Optimal Number of Principal Components

Display the [Correlation Coefficient vs. No. of Principal Components] graph to determine the optimal number of principal components.

(1) Select [Model] - [PCR] - [Correlation Coefficient vs. No. of Principal Components...]. The true values (entered values) of each of the components corresponding to the number of principal components and the correlation coefficient with the evaluation value are calculated and the results are displayed.

Note: The maximum value of the correlation coefficient will be the minimum value of the root mean squared error of prediction (RMSEP).
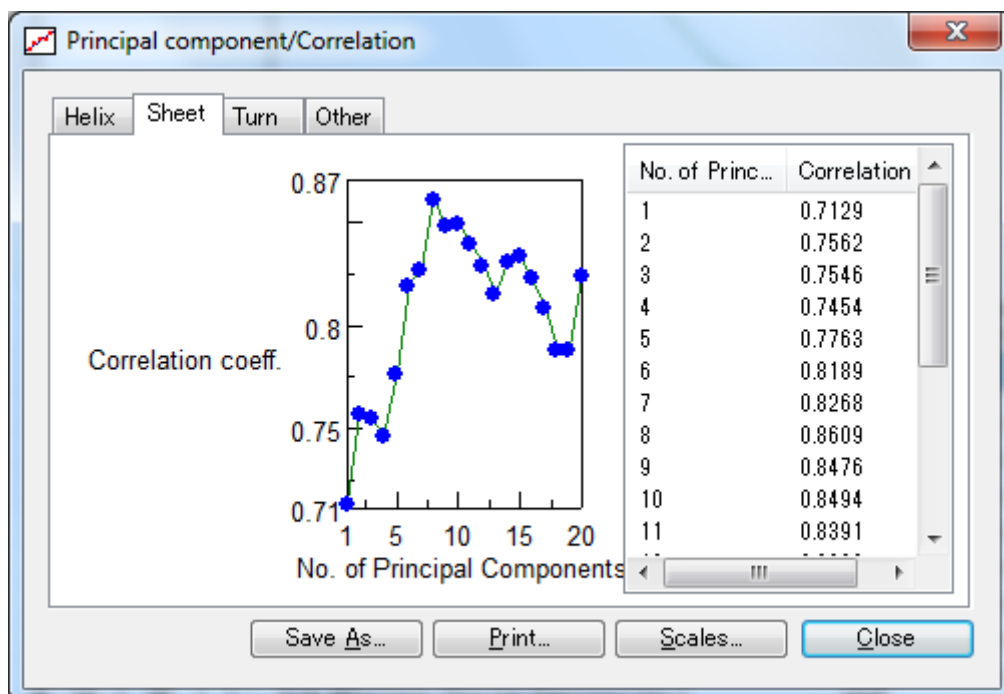
Figure 2.8 [Principal component/Correlation] dialog box

(2) The value when the correlation coefficient is the highest is the optimal number of principal components. Select that number of principal components from the graph. Calculation time increases as the number of principal components increases. When the correlation coefficients are identical, use the one with fewer principal components and write down the value of the determined principal components for future use.

2.4.3 Using [RMSEP vs. No. of Principal Components...] to Determine Optimal Number of Principal Components

(1) Select [Model] - [PCR] - [RMSEP vs. No. of Principal Components...]. The RMSEP values corresponding to the number of principal components are calculated and the results are displayed.
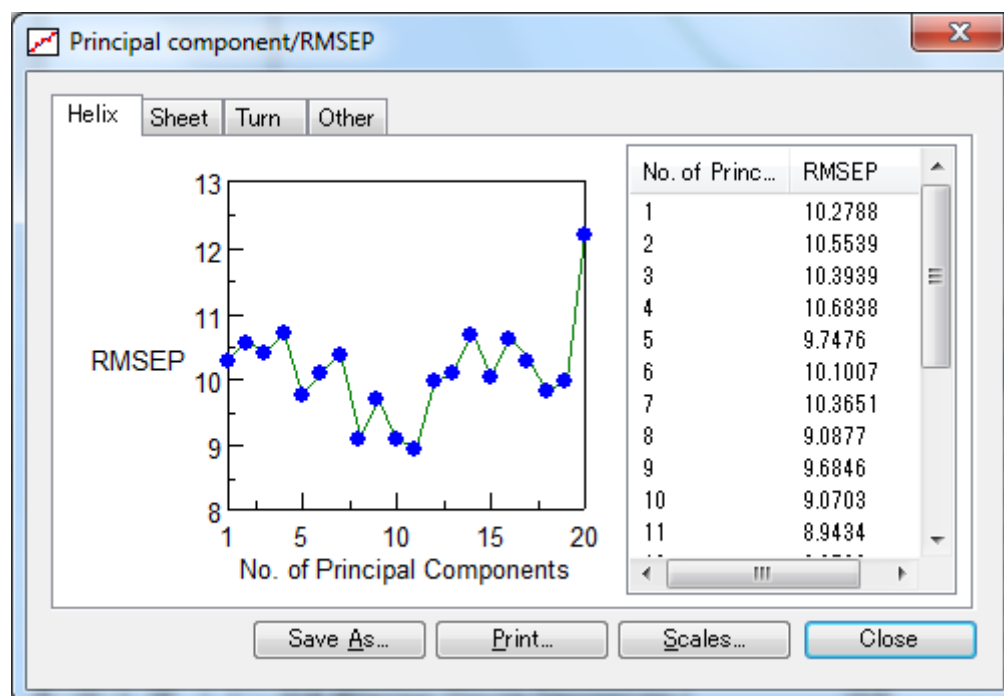

Figure 2.9 [Principal component/RMSEP] dialog box

(2) The value where RMSEP is the lowest is the optimal number of principal components. Since calculation time increases as the number of principal components increases, when the RMSEP values are identical, use the one with fewer components and write down the value of the selected principal components for future use.

2.4.4 Resetting the Number of Principal Components

(1) Select [Parameters...] from the [Setting] menu. The dialog box in Fig. 2.2 is displayed.

(2) Click the <Next> button until the [Calculation Parameters] dialog box in Fig. 2.5 is displayed. In [PCR principal components], enter the values written down as instructed in Sections 2.4.2 and 2.4.3 and then click the <Finish> button.

(3) In [Calculation range], enter the range written down, as instructed in Section 2.4.1.

(4) Select [Calculate] from the [Model] menu or click the ⸢12⸣⸤34⸥ tool button. The calibration model is recalculated.

Note 1: If the number of principal components found by the principal component/correlation coefficient graph differs from the number found by the principal component/RMSEP graph, vary the number of principal components while repeating the calculation in step 4) above. Ideally, the number of principal components will be identical on both graphs. Finally, display and check the calibration curve (see Section 2.6).

Note 2: Always recalculate after changing the calculation range or number of principal components. Calibration curves cannot be displayed and calibration models cannot be saved until you recalculate.

2.5 Using PLS Method to Examine Optimal Number of Factors in Analysis Parameters for Protein Secondary Structures

2.5.1 Using [Correlation Coefficient Spectrum] to Determine X-Axis Calculation Range

(1) Select [PLS] - [Correlation Coefficient Spectrum...] from the [Model] menu. The dialog box in Fig. 2.10 is displayed.
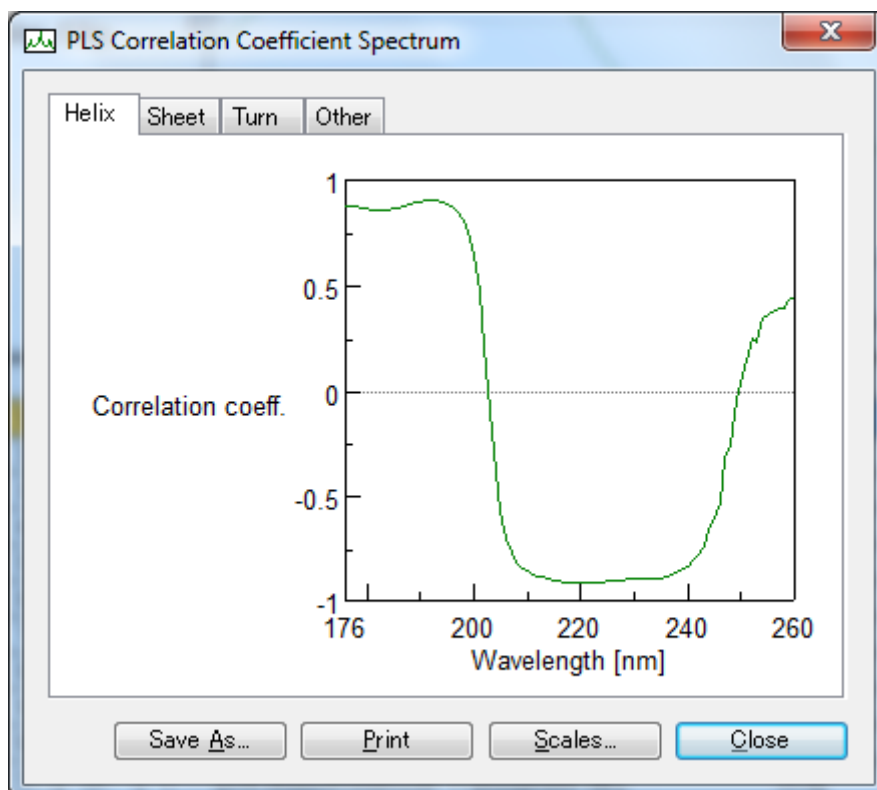

Figure 2.10 [PLS Correlation Coefficient Spectrum] dialog box

(2) Decide the calculation range of the x-axis based on the correlation coefficient spectrum. A correlation coefficient value near a range between +1 and –1 is decided for the calculation range. With the spectrum in this case, there is a region with a dramatic change from -1 to +1, but a range between 260-176 nm is appropriate. The calculation range determined here will be used in Section 2.5.4 "Resetting Number of Principal Components", so write it down for future use.

Note 1: If the x-axis values are difficult to read, drag the mouse diagonally over the graph to enlarge it. Right-click the graph to restore the graph to its original scale.

Note 2: Save/print the graph as required.

2.5.2 Using [Correlation Coefficient vs. Factor...] to Determine Optimal Number of Factors

Display the [Factor/Correlation] graph to determine the optimal number of factors.

(1) Select [PLS] - [Correlation Coefficient vs. Factor...] from the [Model] menu. The true values (entered values) of each of the components corresponding to the number of factors and the correlation coefficient with the evaluation value are calculated and the results are displayed.
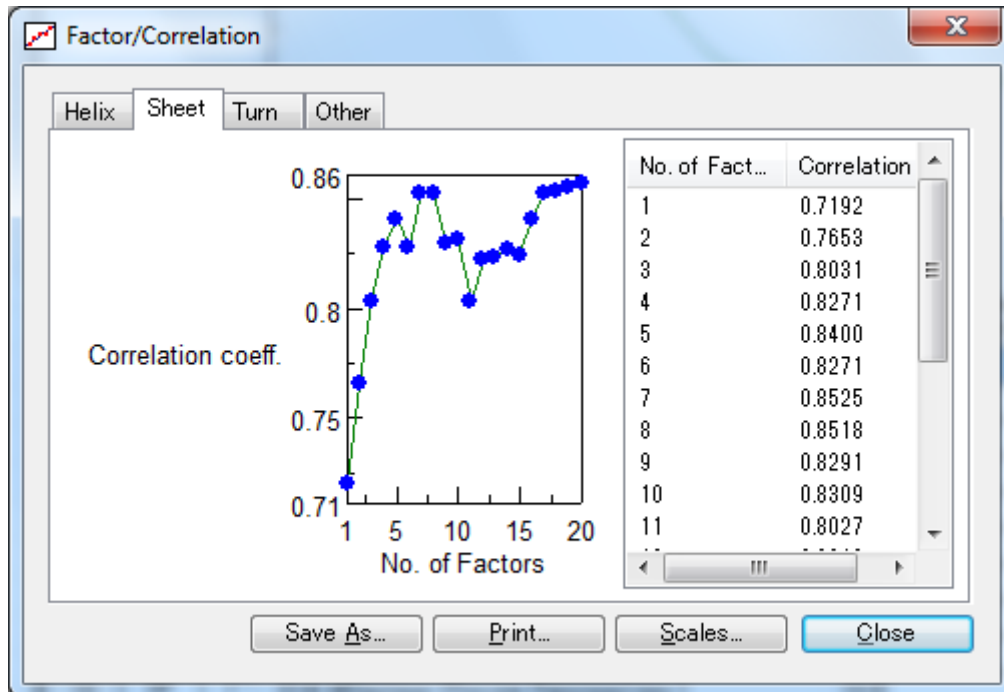
Figure 2.11 [Factor/Correlation] dialog box

(2) The value where the correlation coefficient is the highest is the optimal number of factors. Select that value from the graph. Calculation time increases as the number of factors increases. When the correlation coefficients are identical, use the one with fewer factors and write down the values of the determined factors for future use.

2.5.3 Using [RMSEP vs. Factor...] to Determine Optimal Number of Factors

(1) Select [PLS] - [RMSEP vs. Factors...] from the [Model] menu. The RMSEP values corresponding to the number of factors are calculated and the results are displayed.
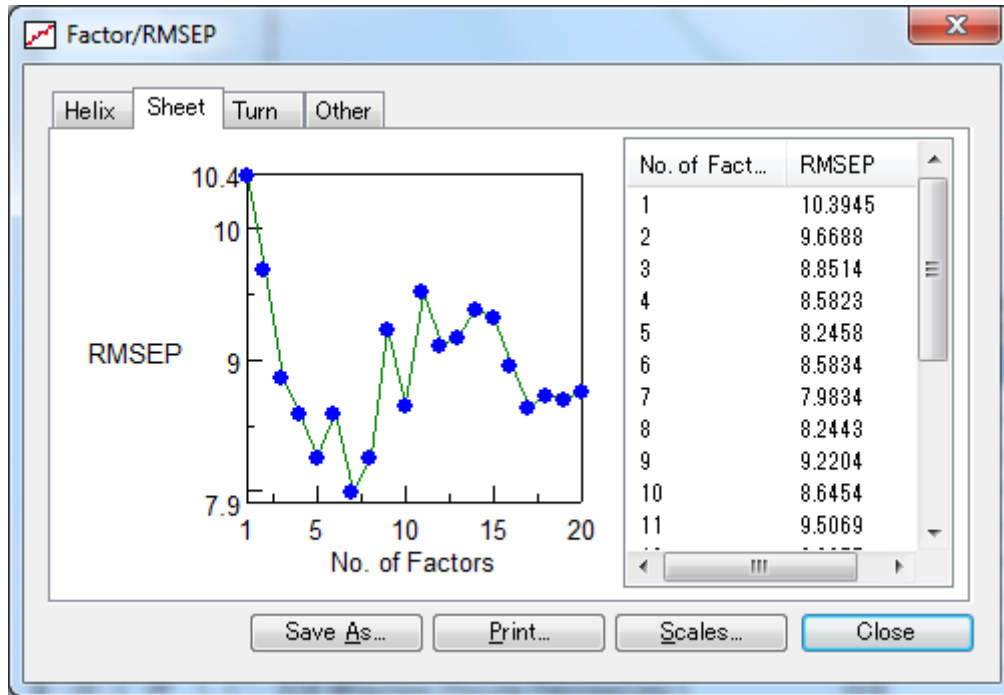
Figure 2.12 [Factor/RMSEP] dialog box

Note: The maximum value of the correlation coefficient is the minimum value of RMSEP.

(2) The value where RMSEP is the lowest is the optimal number of factors. Since calculation time increases as the number of factors increases, when the RMSEP values are identical, use the one with fewer factors and write down the values of the determined factors for future use.

2.5.4 Resetting the Number of Factors

(1) Select [Parameters...] from the [Setting] menu. The dialog box in Fig. 2.2 is displayed.

(2) Click the <Next> button until the [Calculation Parameters] dialog box in Figure 2.7 is displayed. In [PLS Factors], enter the values written down as instructed in Sections 2.5.2 and 2.5.3 and then click the <Finish> button.

(3) In [Calculation range], enter the range written down, as instructed in Section 2.5.1.

(4) Select [Calculate] from the [Model] menu or click the tool button. The calibration model is recalculated.

Note 1: If the number of factors found by the factors/correlation coefficient graph differs from the number found by the factors/RMSEP graph, vary the number of factors while repeating the calculation in step four above. Ideally, the number of factors will be identical on both graphs. Finally, display and check the calibration curvet (see Section 2.6).

Note 2: Always recalculate after changing the calculation range or number of factors. Calibration curves cannot be displayed and calibration models cannot be saved until you recalculate.

2.6 Validating Calibration Models
2.6.1 Using PCR Method to Validate Calibration Models

Check the correlation coefficient of each component based on a PCR method calibration curve.

(1) Select [PCR] - [Leave-one-out Cross Validation...] from the [Model] menu. The dialog box in Fig. 2.13 is displayed.
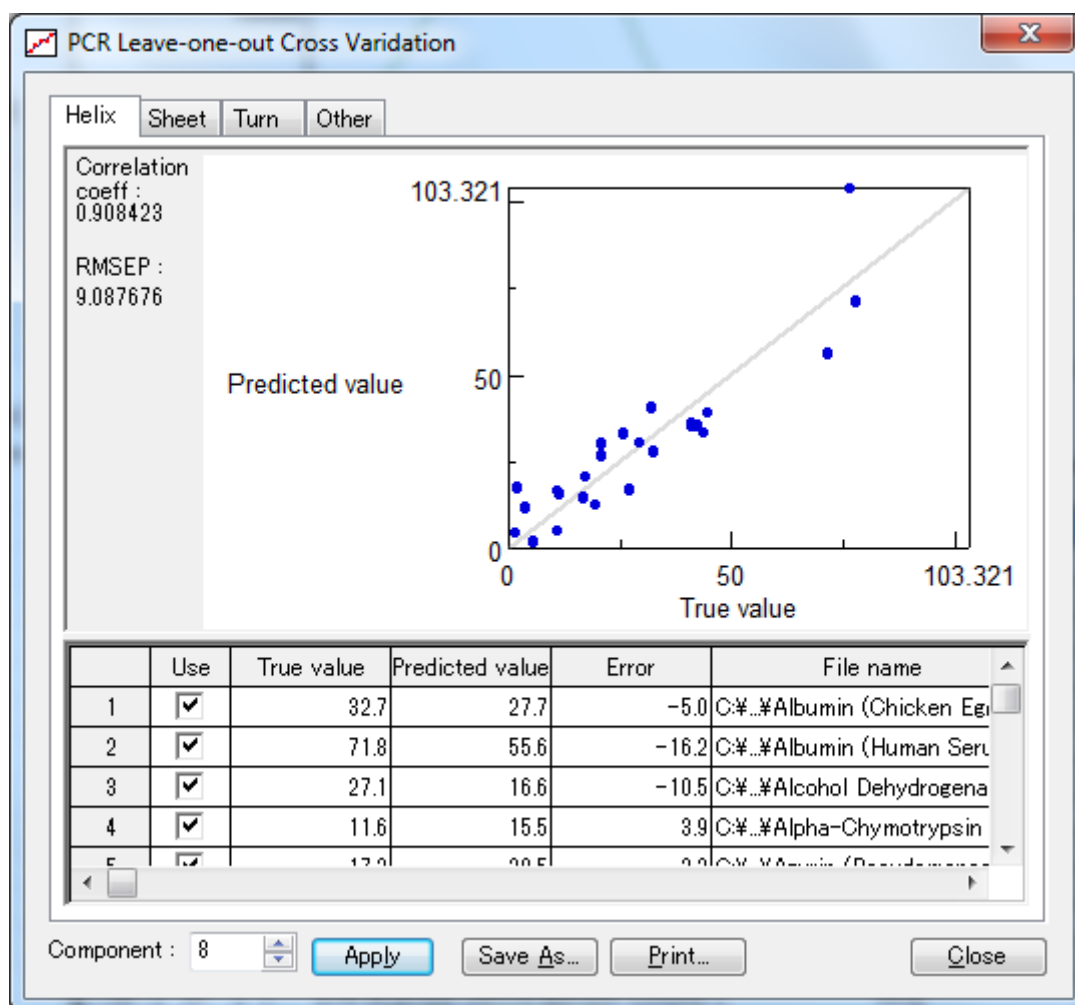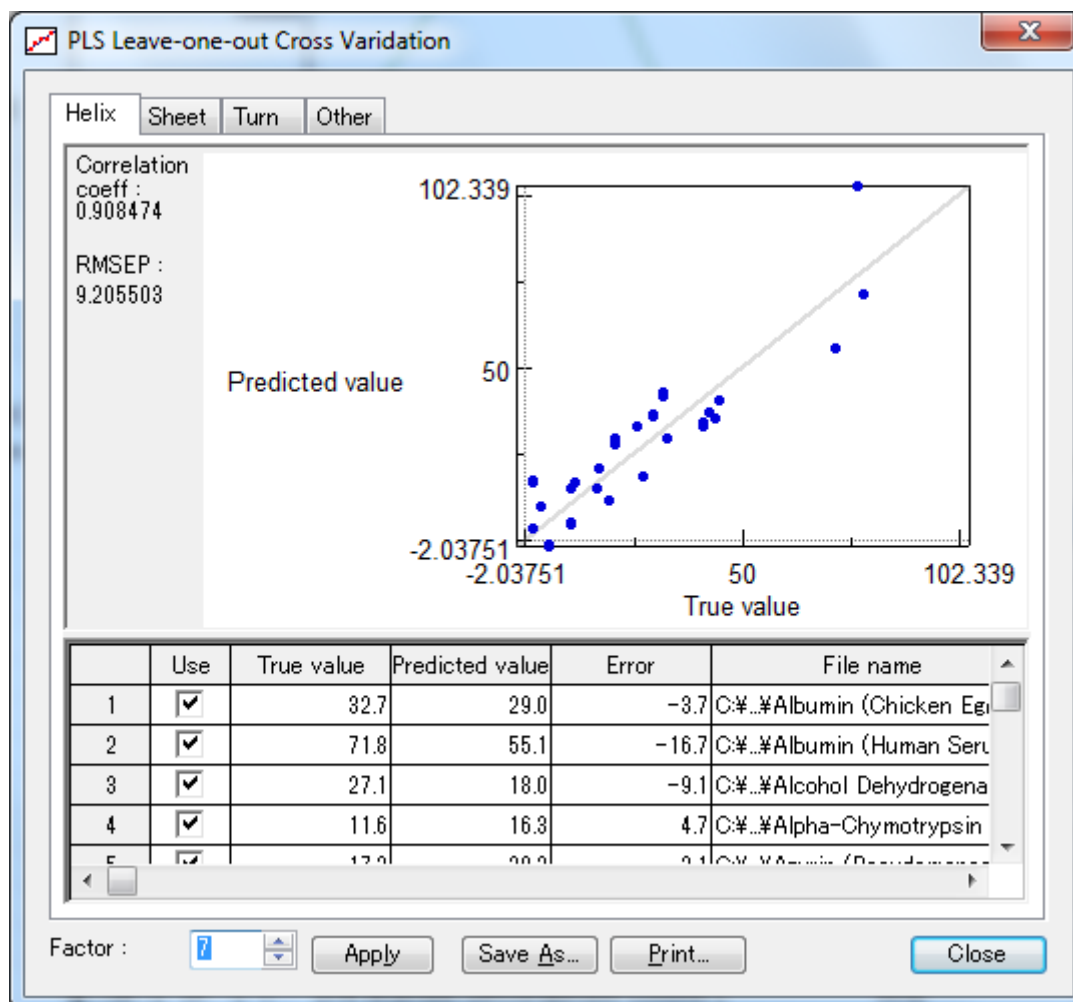

Figure 2.13 [PCR Leave-one-out Cross Validation] dialog box

(2) Optimally, each point of the evaluation values for the standard spectrum should be along the calibration curve. Change the number of principal components or the calculation range, or omit points that diverge dramatically from the calibration curve and then recalculate as required.

2.6.2   Using PLS Method to Check Validity of SSE Calibration Models

Check the correlation coefficient of each component based on a PLS method calibration curve.

(1) Select [PLS] - [Leave-one-out Cross Validation...] from the [Model] menu. The dialog box in Fig. 2.14 is displayed.

Figure 2.14 [PCR Leave-one-out Cross Validation] dialog box

(2) Optimally, each point of the evaluation values for the standard spectrum should be along the calibration curve. Change the number of factors or the calculation range, or omit points that diverge dramatically from the calibration curve and then recalculate as required.

2.7 Saving Calibration Curves

Save the calibration curve values in text format by using the following procedures.

(1) Click the <Save As> button in the dialog box in Fig. 2.13 or 2.14 to display the dialog box in Fig. 2.15. Enter a file name and then click the <Save> button to save the calibration curve in text (.txt) format.
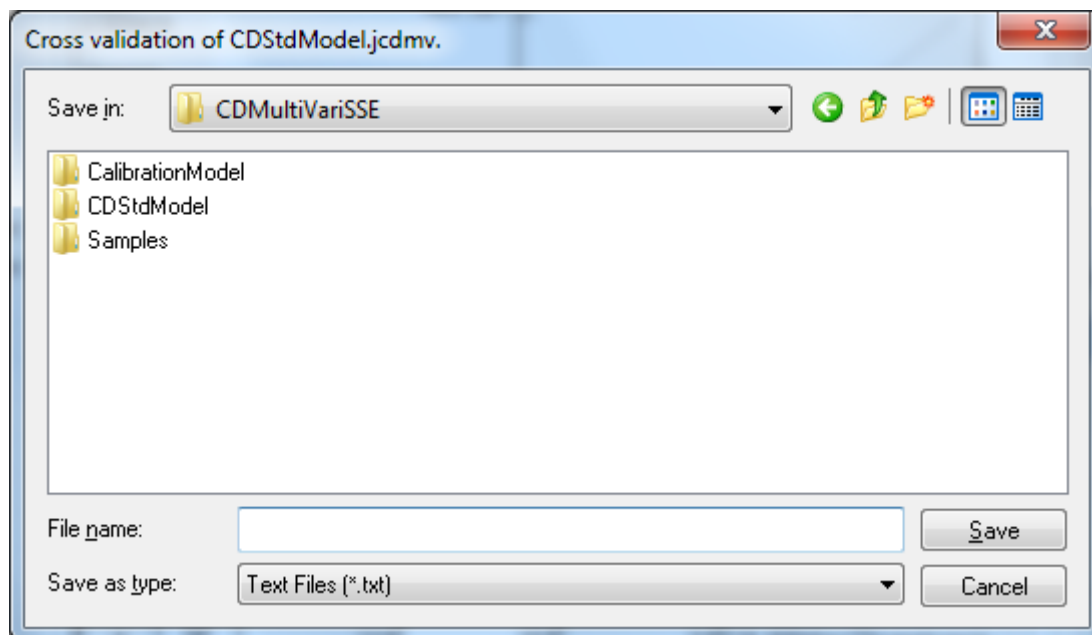
Figure 2.15 [Cross Validation of CDStdModel.jcdmv] dialog box

2.8 Saving Calibration Models

Save SSE calibration models used in the [CD Multivariate Calibration Creation] by using the following procedures.

(1) Select [Save As...] from the [File] menu in the dialog box in Fig. 2.9 to display the dialog in Fig. 2.16.
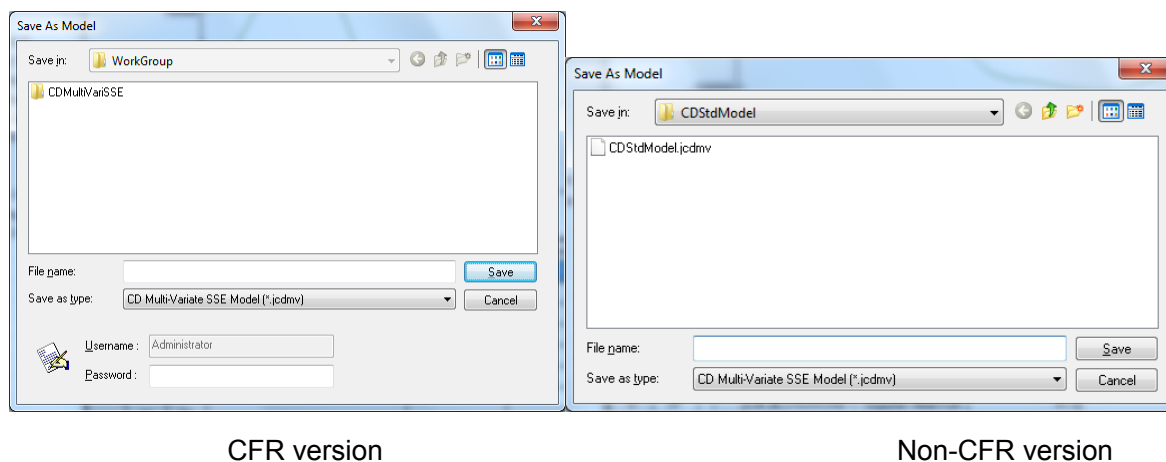
CFR version   Non-CFR version

Figure 2.16 [Save As Model] dialog box

(2) Enter a file name and then click the <Save> button.

> *Note 1: Make sure to save calibration models that you create. You can only use them in the [CD Multivariate SSE] program once they have been saved.*
>
> *Note 2: In the CFR version of the [CD Multivariate SSE] program, calibration models must be electronically signed.*

2.9 Exiting the Program

Exiting the [CD Multivariate Calibration Creation] program.

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

> *Note: When there are unsaved data, a warning message is displayed. Perform the action recommended by the message.*

Exiting the [Spectra Manager] program.

    (2) Select [Exit] from the [Program] menu.

3. [CD Multivariate Calibration Creation] Program Reference

The window in Fig. 3.1 is displayed when the [CD Multivariate Calibration Creation] program is started from the [Spectra Manager] window and a calibration model is opened.

Note: Descriptions of features identical to those in the standard [Spectra Analysis] program are omitted here. For details about spectral analysis, see the "Spectra Analysis Program Software Manual".
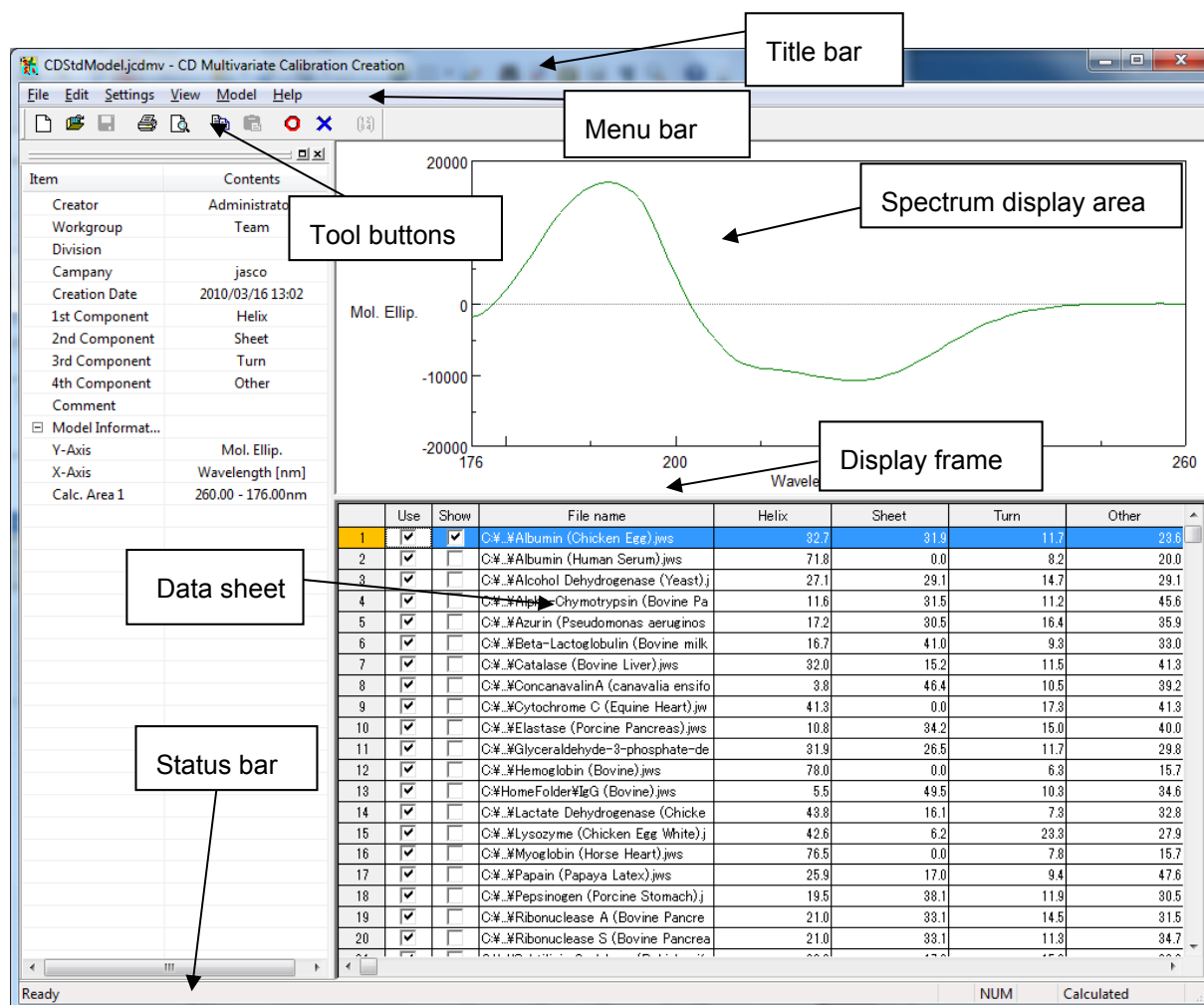
Figure 3.1 [CD Multivariate Calibration Creation] window

(1)   Menu

Menus

[File] menu   Creates, loads, and saves SSE calibration models and prints data
                                     sheets, etc.

[Edit] menu   Enables, disables, adds, changes, and deletes data on data sheets
                                     and copies data to the Clipboard.

[Settings] menu   Sets calculation parameters for calibration models (component

26

|	names, concentration units, calculation ranges, and calculation parameters, etc.).
[View] menu	Changes the scale, font, gridlines, pattern, and style of spectra, and changes the font of data sheets and the color/style of input cells.
[Model] menu	Displays calculations, quantifications, correlation coefficients, calibration curves and so on for PCR and PLS calibration models.
[Help] menu	Displays the version information for the program.

(2)　　　Toolbar

 : Creates a new SSE calibration model ([File]-[New]).

 : Loads an SSE calibration model ([File]-[Open]).

 : Saves an SSE calibration model ([File]-[Save]).

 : Prints a data sheet ([File]-[Print]).

 : Displays a print preview of a data sheet ([File]-[Print Preview]).

 : Copies the currently selected data to the Clipboard ([Edit]-[Copy]).

 : Pastes copies of a table from a spreadsheet program to the data sheet ([Edit]-[Paste]).

 : Enables disabled data ([Edit]-[Spectra]-[Enable]).

 : Disables the currently selected data ([Edit]-[Spectra]-[Disable]).

 : Calculates an SSE calibration model ([Model]-[Calculate]).

 : Displays the version information for the program ([Help]-[About]).

3.1 [File] menu
3.1.1 [New...]

Creates a new SSE calibration model.
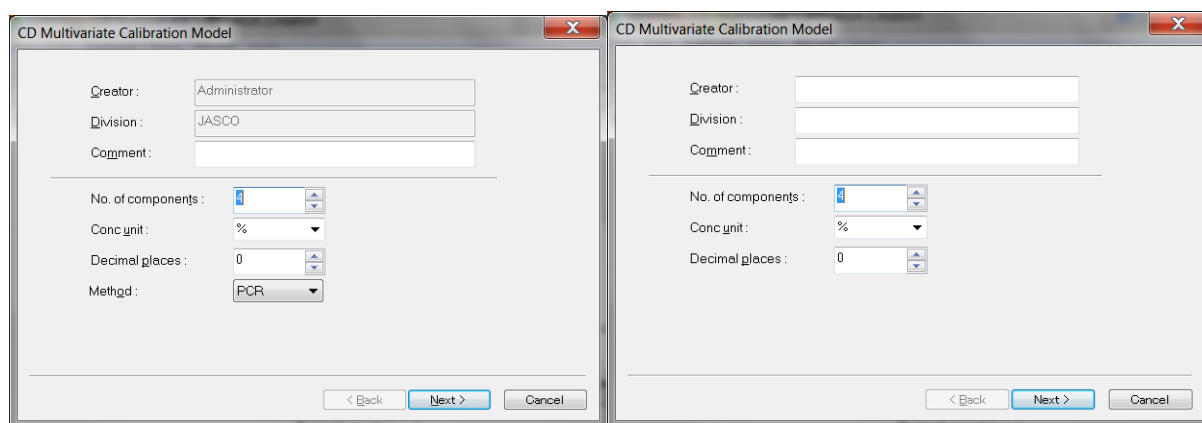
CFR version   Non-CFR version

Figure 3.2 [CD Multivariate Calibration Model] dialog box

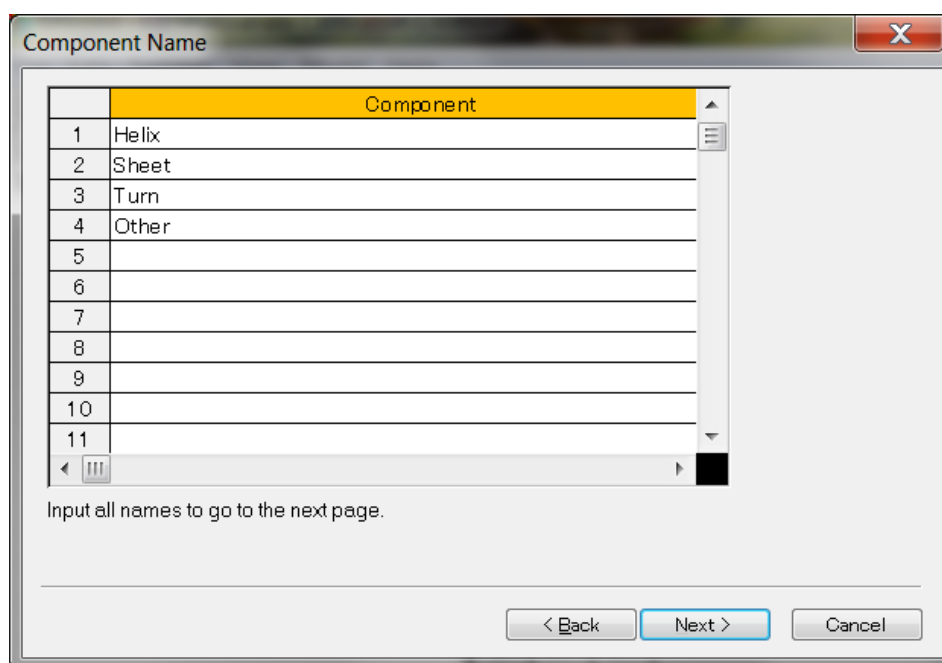|	
[Creator]	Records the creator name entered here. Enter this information as required. In the CFR version, the user name of the user that logged on will automatically be entered.
[Division]	Records the division name entered here. Enter this information as required. In the CFR version, the division of the user that logged on will automatically be entered.
[Comment]	Records the comment entered here. Enter a comment as required.
[No. of components]	Enters the number of protein secondary structures to be quantitated. The default setting is 4.
[Conc unit]	Enters the concentration unit. Available units: %, M, mM, nM, g/l, ppm, and ppb
[Decimal places]	Enters the number of decimal places for data.
[Method]	Selects the PLC or PCR method for the CFR version only.
<Next>	Displays the dialog box in Fig. 3.3.

Figure 3.3 [Component Name] dialog box

[Component]	Enters the component names of protein secondary structures.
<Back>	Returns to the dialog box in Fig. 3.2.
<Next>	Displays the dialog box in Fig. 3.4.

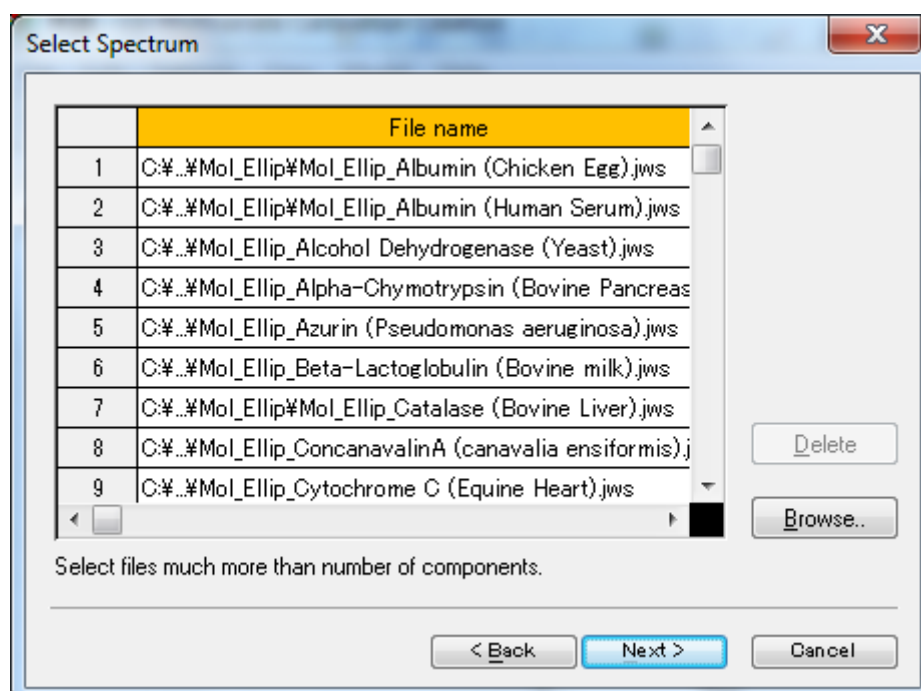Figure 3.4 [Select Spectrum] dialog box

[File name]	Displays fields for selecting spectral data when creating a calibration model.

<Browse...>	Loads a standard spectrum in the dialog box. Clicking this button opens the [Select Spectrum] dialog box. Select a file and click the <Open> button. Multiple spectrum files can be loaded at once by holding down $\boxed{\text{Shift}}$ or $\boxed{\text{Ctrl}}$ while clicking the file name. In the CFR version, only electronically signed CD spectra can be loaded.
<Delete>	Deletes the currently selected file from the file list. Click this button after clicking a number.
<Back>	Returns to the dialog box in Fig. 3.3.
<Next>	Displays the dialog box in Fig. 3.5.

CFR version   Non-CFR version
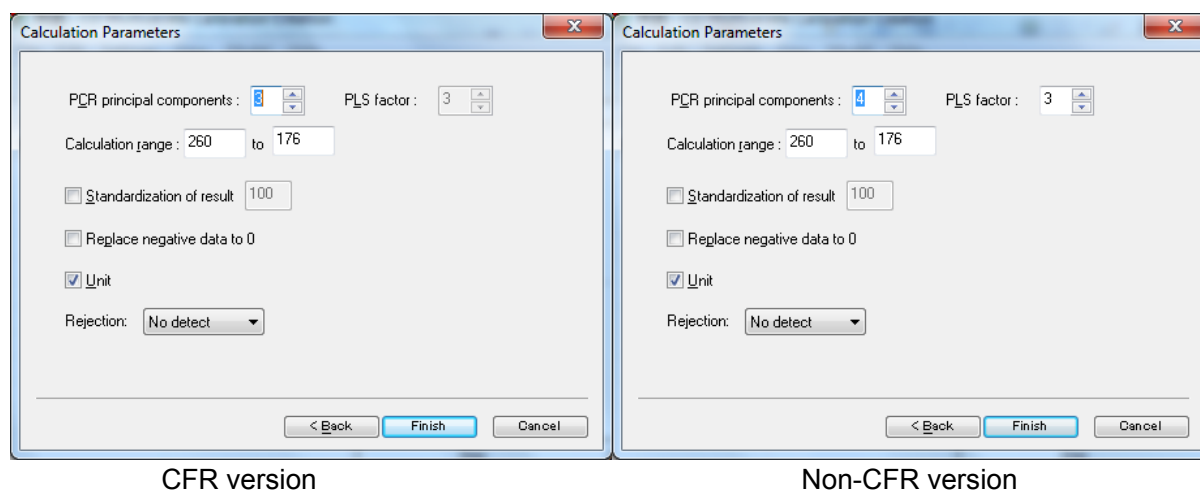
Figure 3.5 [Calculation Parameters] dialog box

[PCR principal components]
Enters the number of PCR principal components. See Section 2.4 for details about setting the parameters.

[PLS factor]
Enters the number of PLS factors. See Section 2.5 for details about setting the parameters.

[Calculation range]
Creates a calibration model from the data in the specified wavelength range. Refer to the correlation coefficient spectrum among other information when deciding the range.

[Standardization of result]
Adjusts the composition ratio of quantitated secondary structures to the specified percentage. For example, when a value of 100% is specified and the total of the analysis result is 50%, the value of each component will be doubled and the sum adjusted to 100%.

[Replace negative data to 0]
When analysis results are negative, the composition ratio will be replaced with 0%.

[Unit]

Selects whether to display % unit in the results of CD multivariate SSE analysis.

[Rejection]

Selects the rejection rate of F detection for analysis results. See "Setting Analysis Parameters" in the "CD Multivariate SSE Program Software Manual" for details about the rejection rate.

3.1.2 [Open]

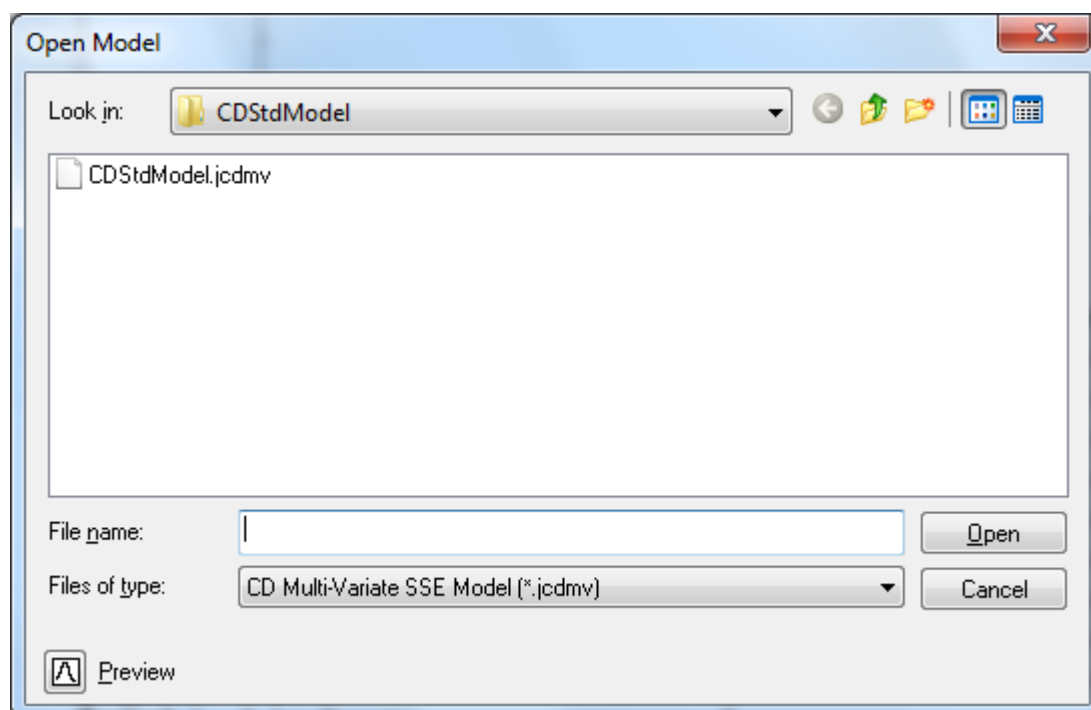Opens an existing calibration model. Specify a calibration model and then click the <Open> button.

Figure 3.6 [Open Model] dialog box

Note 1: Opening an existing calibration model is also possible using the 🖼 tool button.
Note 2: In the CFR version, the [Look in] drop-down list cannot be used to select a folder. However, a subfolder within the prespecified folder can be selected.

3.1.3 [Close]

Closes the currently opened calibration model. If the calibration model has been updated, the dialog box in Fig. 3.7 is displayed. Perform the action recommended by the message.
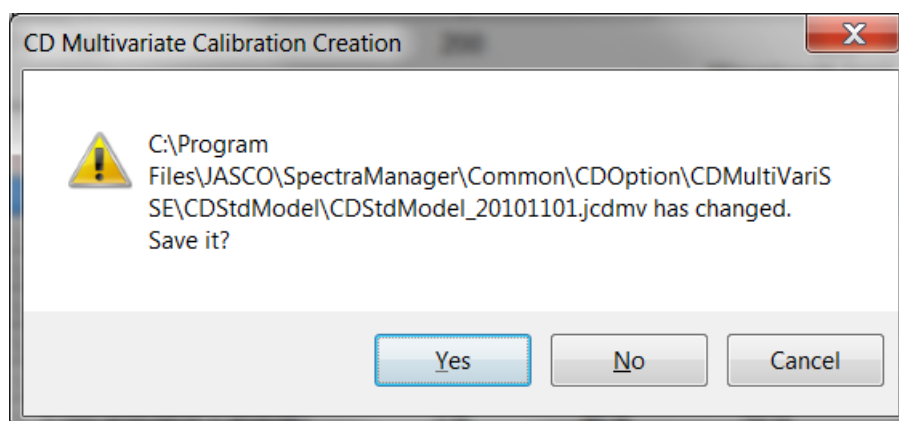

Figure 3.7 Message if calibration mode has not been saved

3.1.4 [Save]

Saves a calibration model that has been opened and changed using the identical file name. The old file will be overwritten when using this command. The [Save As] command described in Section 3.1.5 is automatically used when the [Save] command is used to save newly created data. In the CFR version, the files that can be saved by overwriting differ by workgroup level and file signature. Using this command erases the previous data.

Table 3.1 Privileges assigned to Save function

	Managers	Analysts	Operators
Creation signature only	√	√	√*
Creation and review signatures	√		
Creation, review, and approval signatures			

Note: The calibration models that come standard with this program cannot be overwritten. When changing them, save the file using a different name.

3.1.5 [Save As]

Saves a created calibration model using a new name. Specify where to save the file, enter a file name, and then click the <Save> button. Clicking the <Cancel> button cancels the save operation.
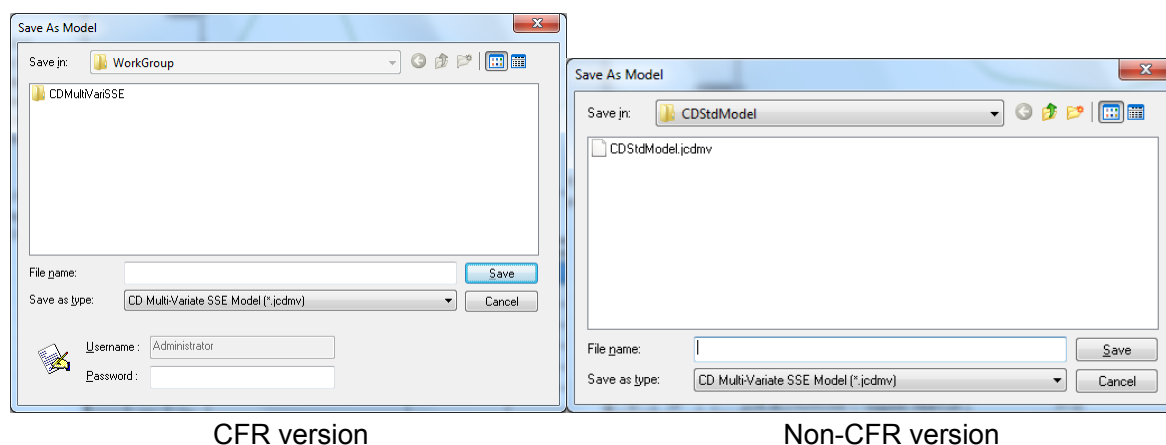

CFR version   Non-CFR version

Figure 3.8 [Save As Model] dialog box

> *Note:*   *In the CFR version, the [Look in] drop-down list cannot be used to select a folder. However, a subfolder within the prespecified folder can be selected.*

3.1.6 [Print]

Prints a list of file names and concentrations in the data sheet for created calibration models, the calculation area from the PCR calculation, and calculation parameters. The spectrum, however, is not be printed by using this command.

3.1.7 [Print Preview]

Displays a print preview for the selected calibration model.

3.1.8 [Print Items]

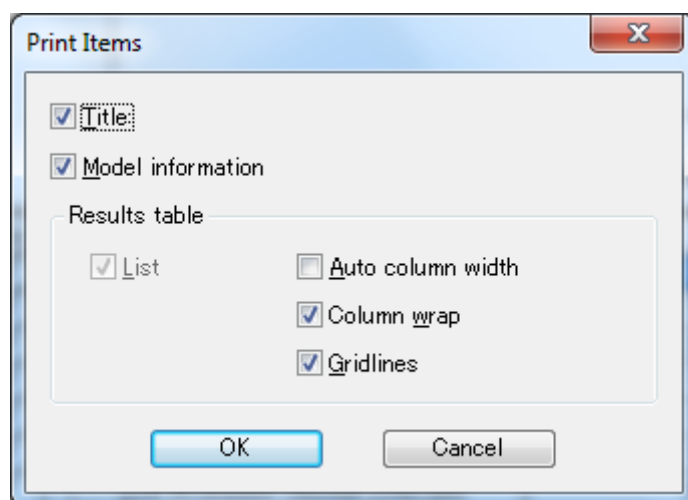Selects the items to print and their display format.

Figure 3.9 [Print Items] dialog box

3.1.9 [Print Setup]

Specifies the target printer and print parameters. The content of this dialog box varies depending on the connected printer.
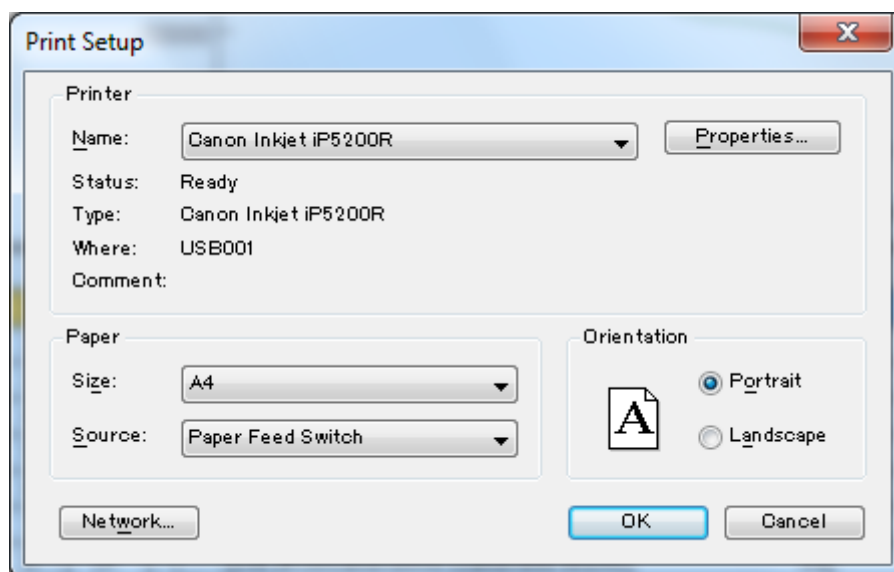

Figure 3.10 [Print Setup] dialog box

3.1.10 [Exit]

Exits the [CD Multivariate Calibration Creation] program. The dialog box in Fig. 3.11 is displayed if there are any calibration models that have not been saved.
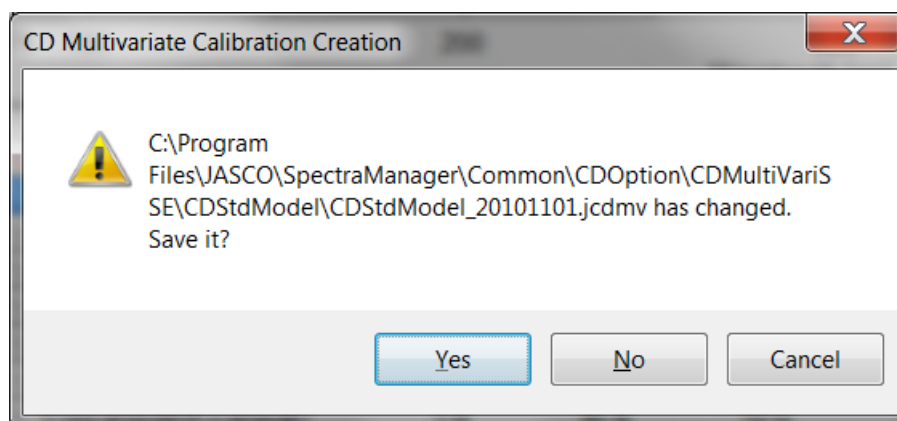

Figure 3.11 Message if there are any unsaved calibration models

3.2 [Edit] Menu
3.2.1 [Spectra]

[Enable]	Selects the data to use in creating a calibration model. Select this command after clicking data numbers in the data sheet. A checkmark will be displayed in the checkboxes of selected data in the data sheet. Only the data selected in the [Use] column will be used in creating a calibration model.
[Disable]	Selects the data not to use in creating a calibration model. Select this command after clicking data numbers in the data sheet. The checkmarks in the checkboxes in the [Use] column will be removed, and the data text will turn red. You can also select whether or not to use data in calibration models by clicking the [Use] checkboxes in the main window.
[Add...]	Adds new data to the data sheet. Clicking this command opens the dialog box in Fig. 3.4. Click the <Open> button after selecting the files to add.
[Delete]	Deletes the currently selected data. Click this command after clicking the data number(s) to delete. When the confirmation message for deleting data is displayed, click the <Yes> button to delete the data. After data is deleted, subsequent rows will be shifted up by one.
[Change...]	Changes the data that is currently selected. Click this command after clicking the data number to change. The dialog box in Fig. 3.4 is displayed. Select the file to change and then click the <Open> button.

3.2.2 [Component]

[Add...]	Adds components for the selected protein secondary structure.
[Delete]	Deletes components of the selected protein secondary structure.
[Name...]	Changes the component names of the protein secondary structure.

3.2.3 [Copy]

Copies the currently displayed data sheet (file name and ratio of a secondary structure for a protein) to the Clipboard. Use it to, for example, copy data sheets to a spreadsheet program.

3.2.4 [Paste]

Pastes the content of the Clipboard (a secondary structure for a protein copied from, for example, a spreadsheet program) into the data sheet column of the main window in Fig. 3.1.

3.3 [Settings] Menu
3.3.1 [Parameters...]

Selecting [Parameters...] from the [Settings] menu allows the setting of calibration model parameters. The dialog box in Fig. 3.2 is displayed. From this point, the same dialog boxes as those when creating a new model are displayed up to the one in Fig. 3.5. Change the settings displayed as required. Note that the number of components in Fig. 3.2 cannot be changed in this case. See Section 3.1.1 for details about creating a new calibration model.

3.4 [View] Menu
3.4.1 [Graph]

Changes the scale, font, gridlines, pattern, and style of a spectrum. A detailed explanation of this command has been omitted here because it is identical to the command in the [View] menu of the [Spectra Analysis] program. Refer to "Spectra Analysis Program Software Manual" as required.

[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.
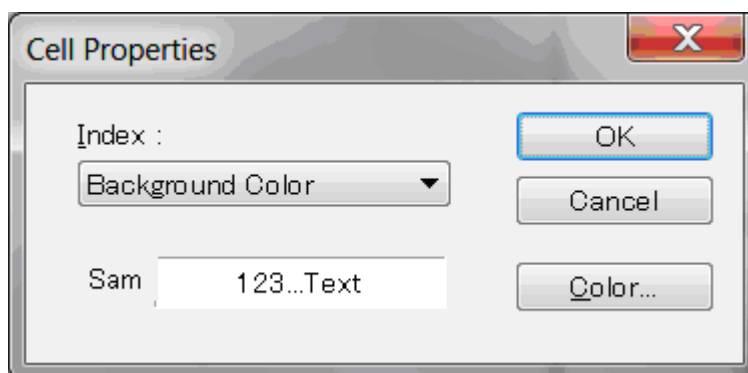
3.4.2 [Work Sheet]

Changes the data sheet font and the color of input cells/text.

3.4.2.1 [Fonts...]

Changes the font of the currently selected sheet.

3.4.2.2 [Cell Property...]

Changes the color of the currently selected cell and entered text.

s

[Index]   Displays the current setting in the [Sam] field. To change it, select an item
                      from the drop-down list and then click the <Color...> button.

<Color...>   Sets the display color of the data sheet in Fig. 3.1. Clicking this button
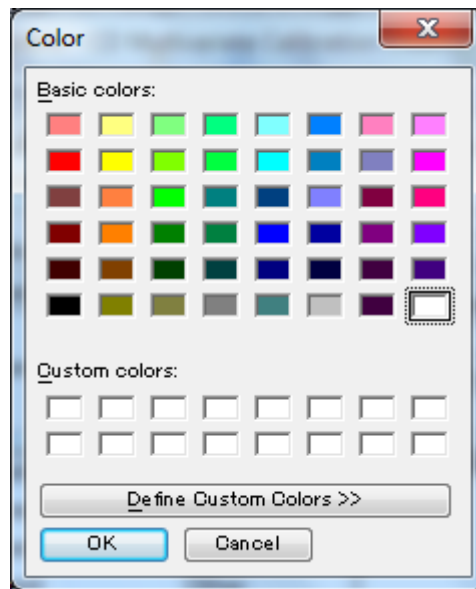   displays the dialog box in Fig. 3.13.

Figure 3.13 [Color] dialog box

[Basic colors]   Selects a color from the list. Click the <OK> button to apply the selected color.

3.5 [Model] menu
3.5.1 [Calculate...]

Calculates an SSE calibration model. If calculation parameters or the calculation range have been changed using the [Settings] menu, select this command and recalculate. Calculation is possible once values for all component names have been entered.

3.5.2 [Quantitative Analysis...]

Verifies the created calibration model. This cannot be selected until an SSE calibration model is calculated. Open a file and analyze the protein secondary structure. Selecting this command displays the [Select Spectrum] dialog box. Select one file on which to perform quantitative analysis and then click the <Open> button. A results dialog box is displayed. Quantitative analysis is performed on a single file. It is not possible to perform it simultaneously on multiple files here.
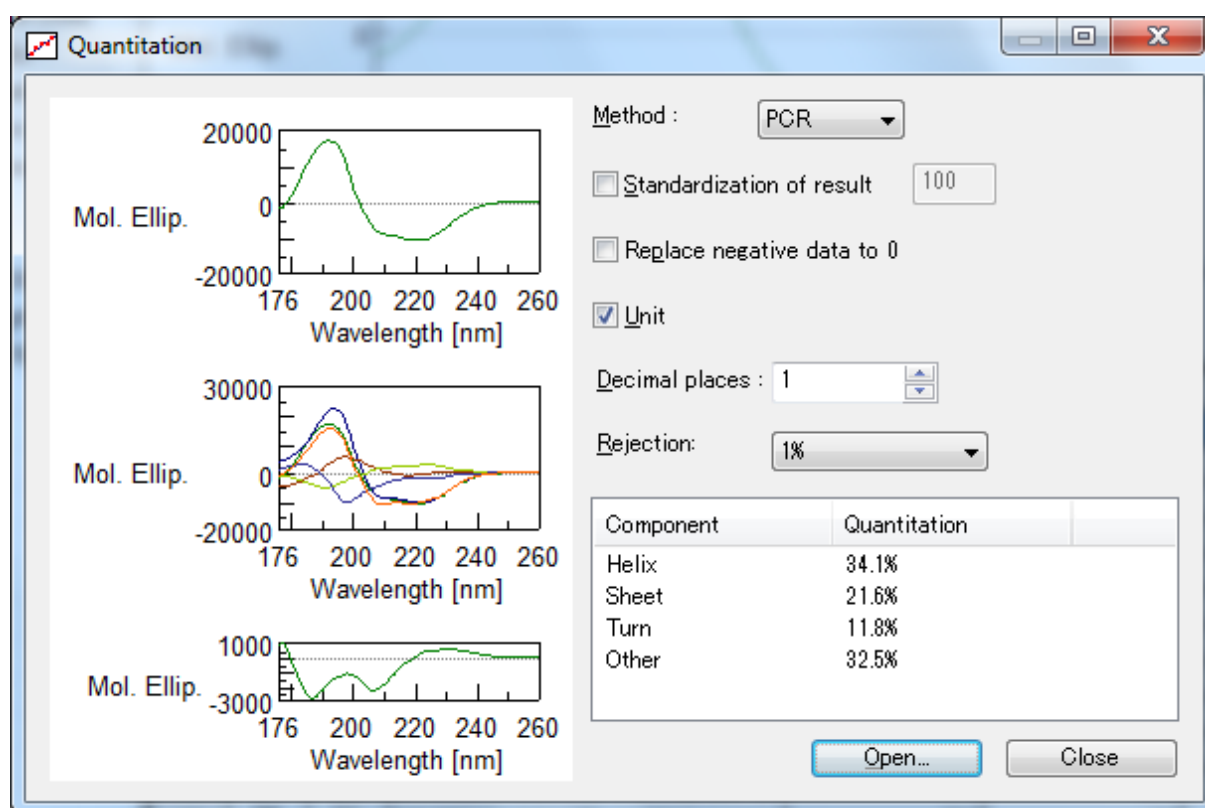
Figure 3.14 [Quantitation] dialog box

3.5.3 [PCR]
3.5.3.1 [Score Vector...]

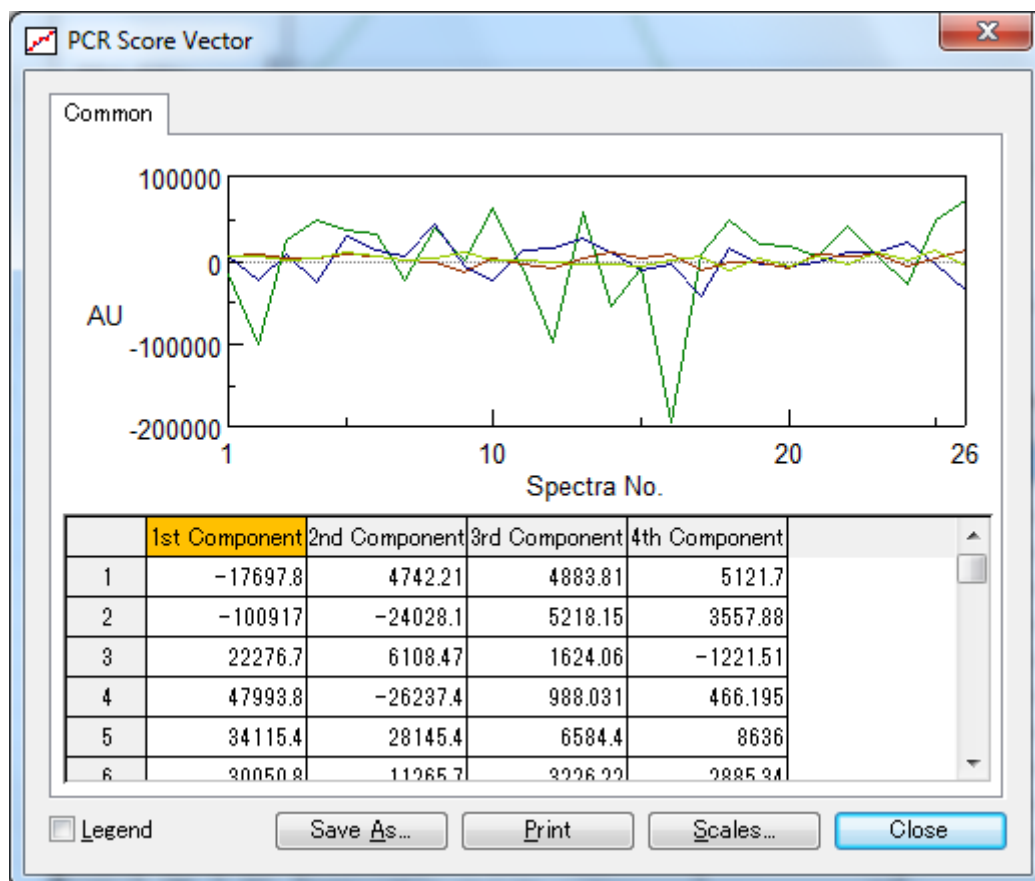Displays a score vector. This cannot be selected until a PCR calibration model has been calculated.

Figure 3.15 [PCR Score Vector] dialog box

<Save As...>	Saves the score vector in text format.
<Print>	Prints the score vector.
<Scales...>	Changes the scale of the score vector.

Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.

| [Legend] | Displays a legend for the score vector. |
| <Close> | Closes the dialog box. |

3.5.3.2 [Principle Component]

Displays the spectrum of the principal component. This cannot be selected until a PCR calibration model has been calculated.
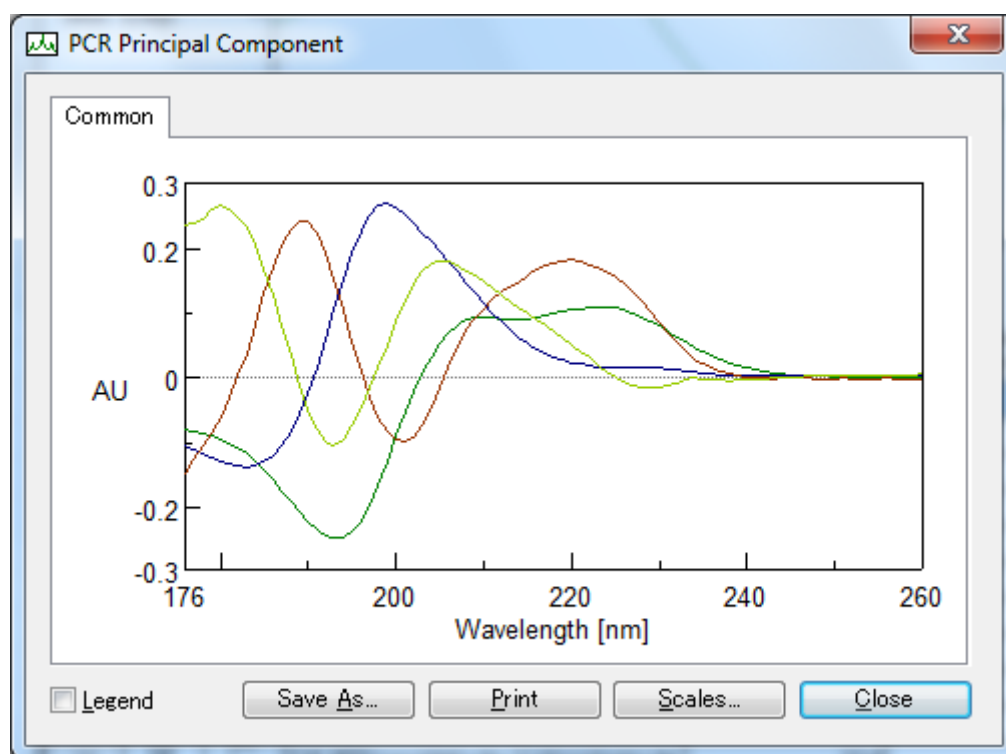
Figure 3.16 [PCR Principle Component] dialog box

<Save As...>	Saves the spectrum of the principal component.
<Print>	Prints the spectrum of the principal component.
<Scales...>	Changes the scale of the spectrum of the principal component.

Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.

[Legend]	Displays a legend for the spectrum of the principal component.
<Close>	Closes the dialog box.

3.5.3.3 [Regression Coefficient...]

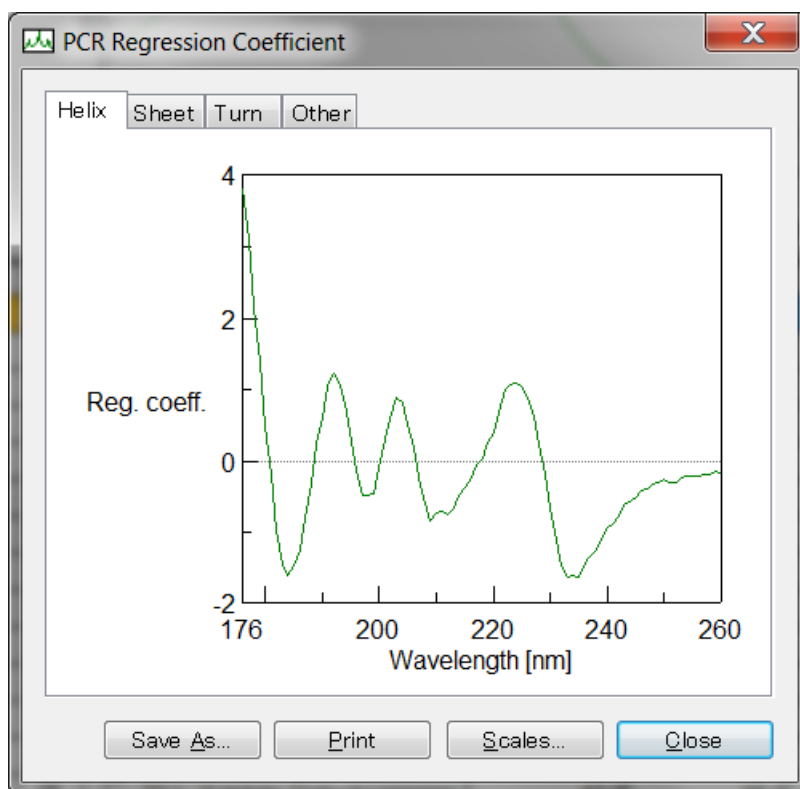Displays a graph of the regression coefficient. This cannot be selected until a PCR calibration model is calculated.

Figure 3.17 [PCR Regression Coefficient] dialog box

<Save As...>	Saves the graph of the regression coefficient in *.jws format.
<Print>	Prints the graph of the regression coefficient.
<Scales...>	Changes the scale of the graph of the regression coefficient.

Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.

<Close>	Closes the dialog box.

3.5.3.4 [Correlation Coefficient Spectrum...]

Calculates and displays the correlation coefficient spectrum. Use this command to estimate the calculation range of the x-axis used in quantitation. A correlation coefficient value near a range between +1 and –1 is decided for the calculation range. In the case of this spectrum, the range between 260 and 176 nm is appropriate.
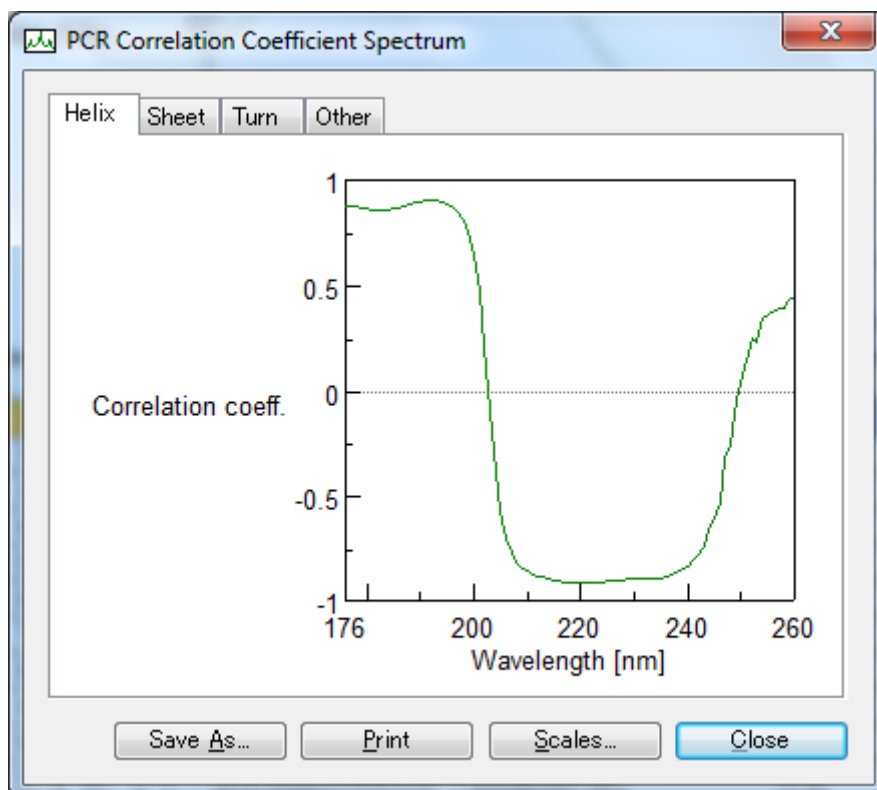

Figure 3.18 [PCR Correlation Coefficient Spectrum] dialog box

<Save As...>	Saves the correlation coefficient spectrum.
<Print>	Prints the correlation coefficient spectrum.
<Scales...>	Changes the scale of the correlation coefficient spectrum.

> *Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.*

<Close>	Closes the dialog box.

3.5.3.5 [Correlation Coefficient vs. No. of Principal Components...]

Calculates and displays the correlation coefficients between the true value and evaluated value versus the number of principal components. On the principal components/correlation coefficient graph, the principal components with the maximum number of correlation coefficients will be found. That value is the optimal number of principal components. However, calculation time increases when there are a large number of principal components. If correlation coefficients are identical, use the one with fewer principal components.
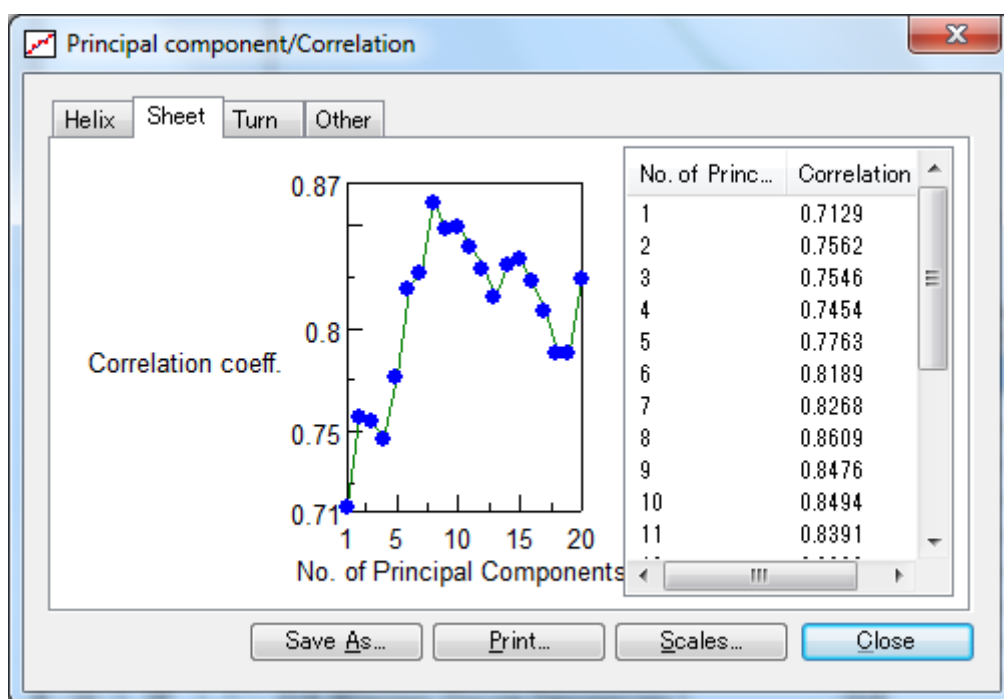

Figure 3.19 [Principal component/Correlation] dialog box

<Save As...>	Saves the principal component/correlation coefficient graph in text format.
<Print>	Prints the principal component/correlation coefficient graph.
<Scales...>	Changes the scale of the principal component/correlation coefficient graph.
<Close>	Closes the dialog box.

3.5.3.6 [RMSEP vs. No. Principal Components...]

Calculates and displays the RMSEP value corresponding to the number of principal components. On the principal components/RMSEP graph, the number of principal components where the RMSEP value is the lowest will be found. That value is the optimal number of principal components. However, calculation time increases when there are a large number of principal components. If RMSEP values are identical, use the one with fewer principal components.

> *Note: If the number of principal components found on the principal components/correlation coefficient graph and the number of principal components found on the principal components/RMSEP graph differ, repeatedly perform calculations while varying the number of principal components. It is ideal for the number of components on both graphs to be identical. After the calculations, display and check the calibration curve.*
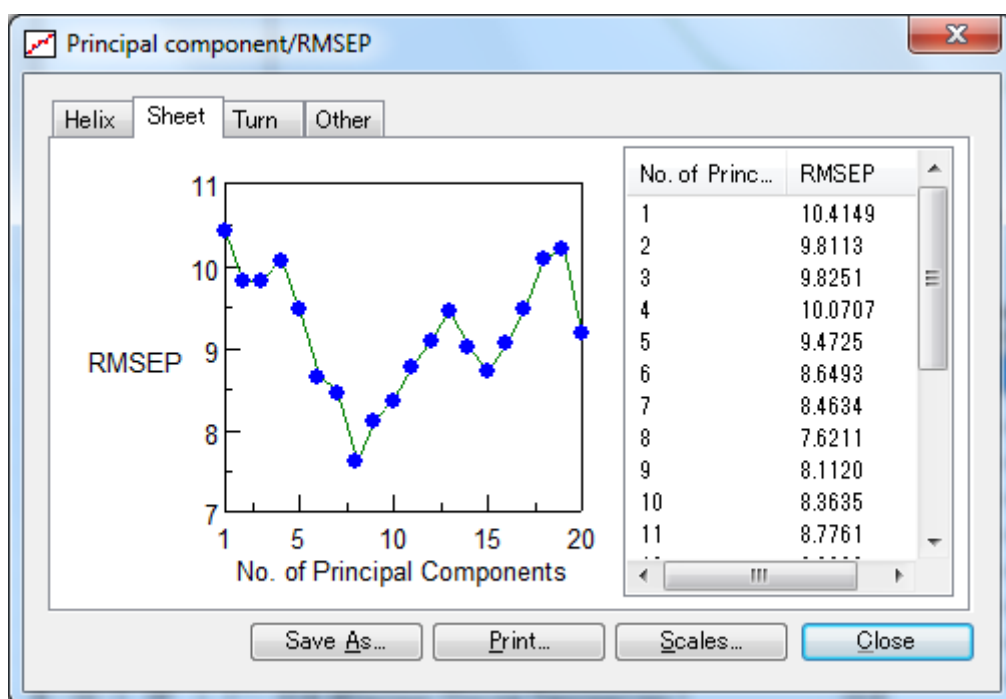
Figure 3.20 [Principal Component/RMSEP] dialog box

<Save As...>	Saves the principal component/RMSEP graph in text format.
<Print>	Prints the principal component/RMSEP graph.
<Scales...>	Changes the scale of the principal component/RMSEP graph.
<Close>	Closes the dialog box.

3.5.3.7 [Leave–one-out Cross Validation...]

Calculates and displays a calibration curve (a graph of true values and evaluated values). Use this command to validate PCR calibration models that are created. The model is satisfactory if points of concentration of the standard spectrum are on the calibration curve. Change the number of principal components and recalculate if necessary.
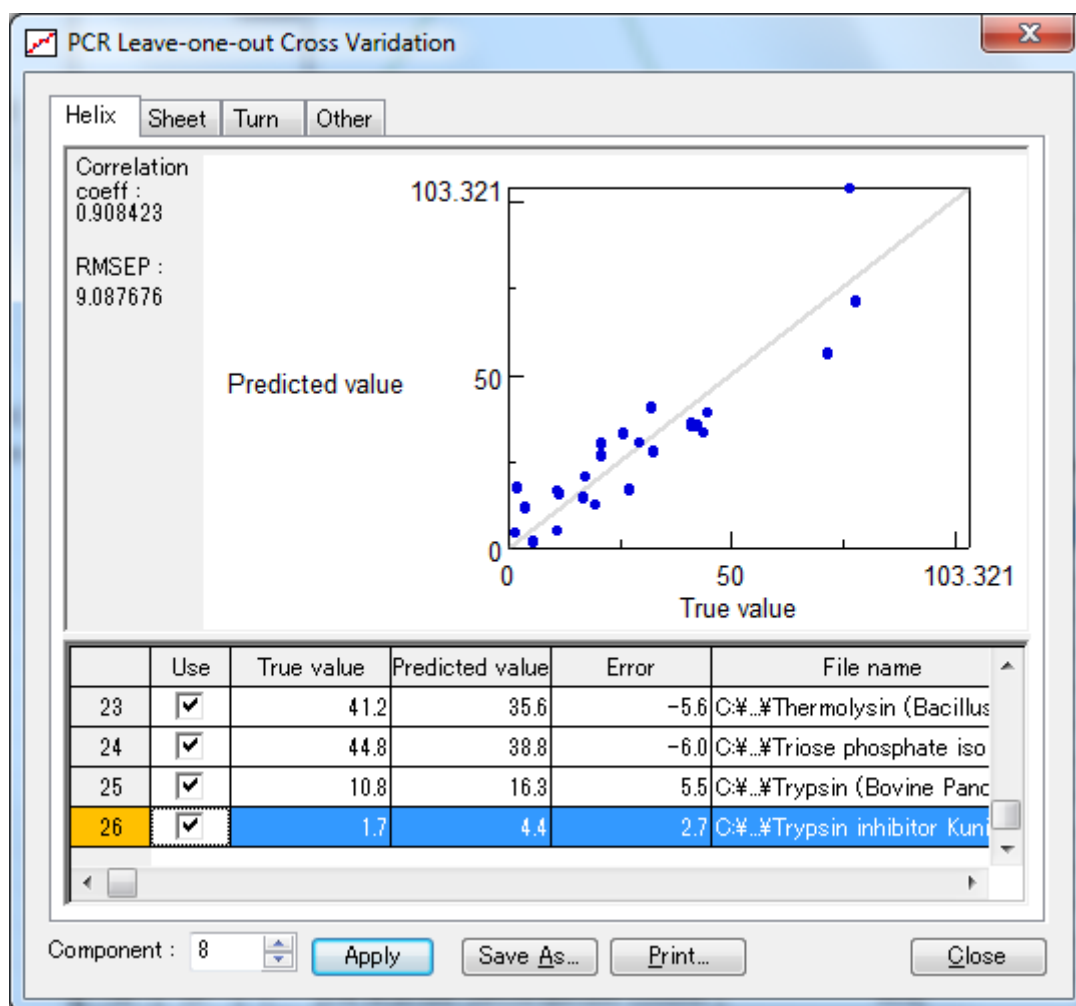

Figure 3.21 [PCR Leave–one-out Cross Validation] dialog box

[Component(s)]   Sets the number of principal components before recalculating.

<Apply>   Starts the recalculation process.

<Save As...>   Saves the calibration curve in text format.

<Print>   Prints the calibration curve.

<Close>   Closes the dialog box.

3.5.4 [PLS]
3.5.4.1 [Score Vector...]

Displays a score vector. This cannot be selected until a PLS calibration model is calculated.
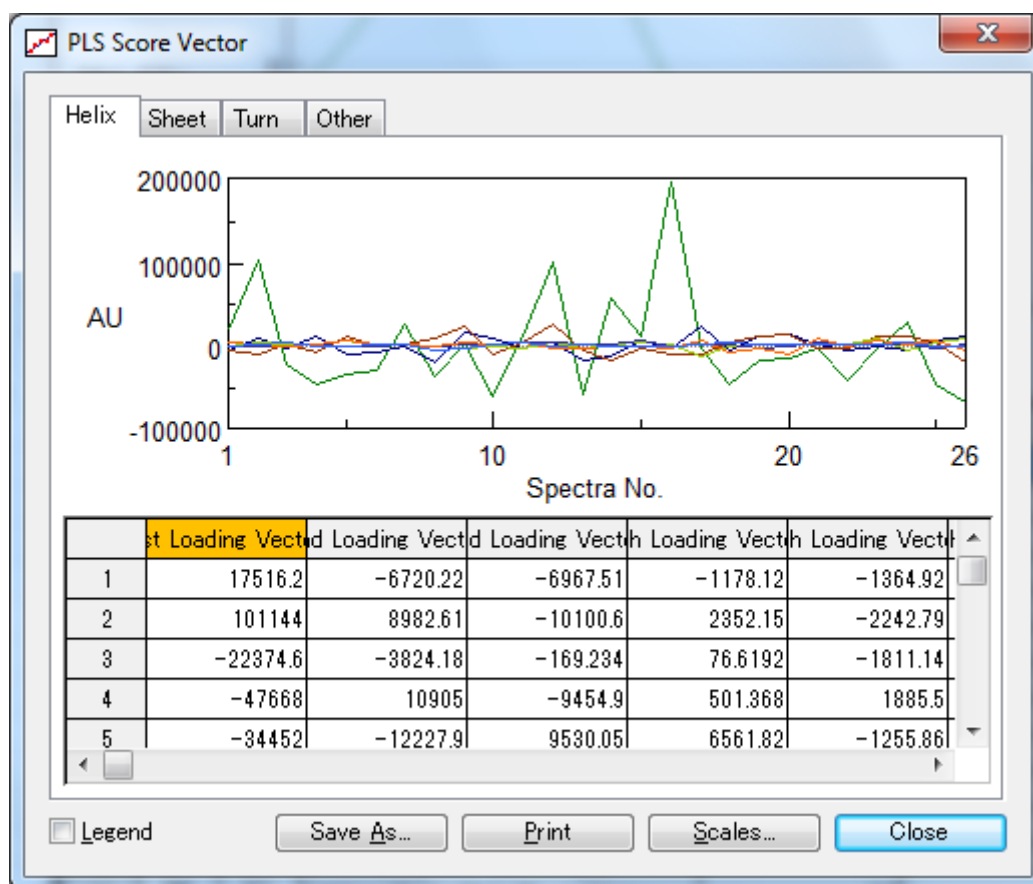

Figure 3.22 [PLS Score Vector] dialog box

\<Save <u>A</u>s...\>	Saves the score vector in text format.
\<<u>P</u>rint\>	Prints the score vector.
\<<u>S</u>cales...\>	Changes the scale of the score vector.

> *Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.*

[<u>L</u>egend]	Displays a legend for the score vector.
\<<u>C</u>lose\>	Closes the dialog box.

3.5.4.2 [Weight Loading Vector]

Displays a weight loading vector. This cannot be selected until a PLS calibration model has been calculated.
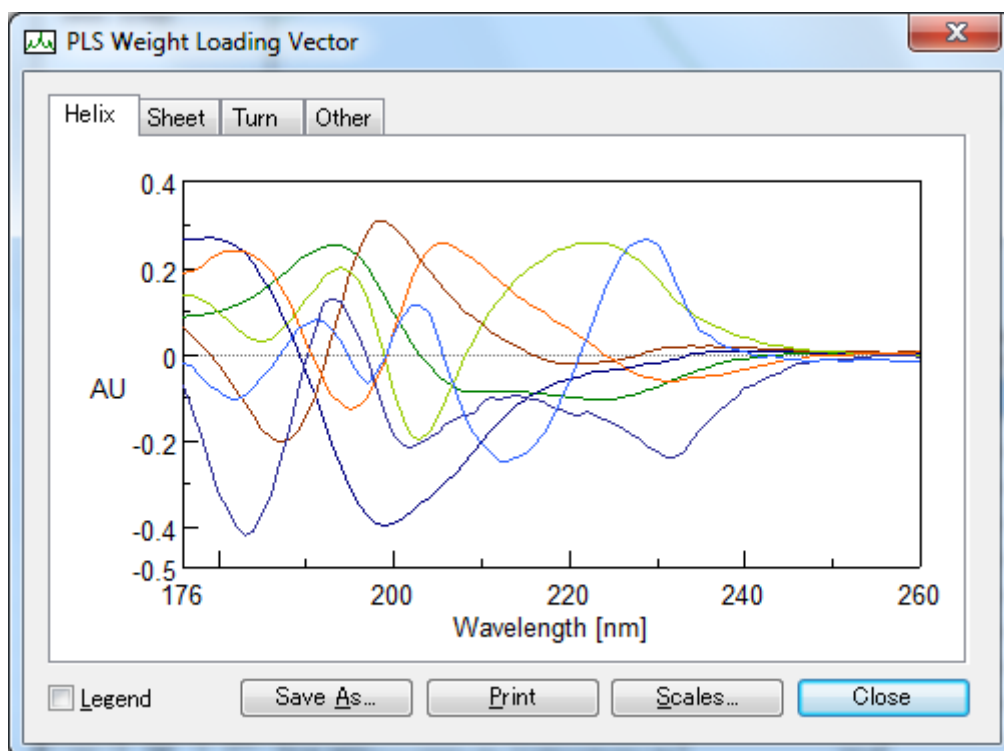

Figure 3.23 [PLS Weight Loading Vector] dialog box

<Save As...>   Saves the PLS weight loading vector.

<Print>   Prints the PLS weight loading vector.

<Scales...>   Changes the scale of the PLS weight loading vector.

> *Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.*

[Legend]   Displays a legend for the PLS weight loading vector.

<Close>   Closes the dialog box.

3.5.4.3 [Regression Coefficient...]

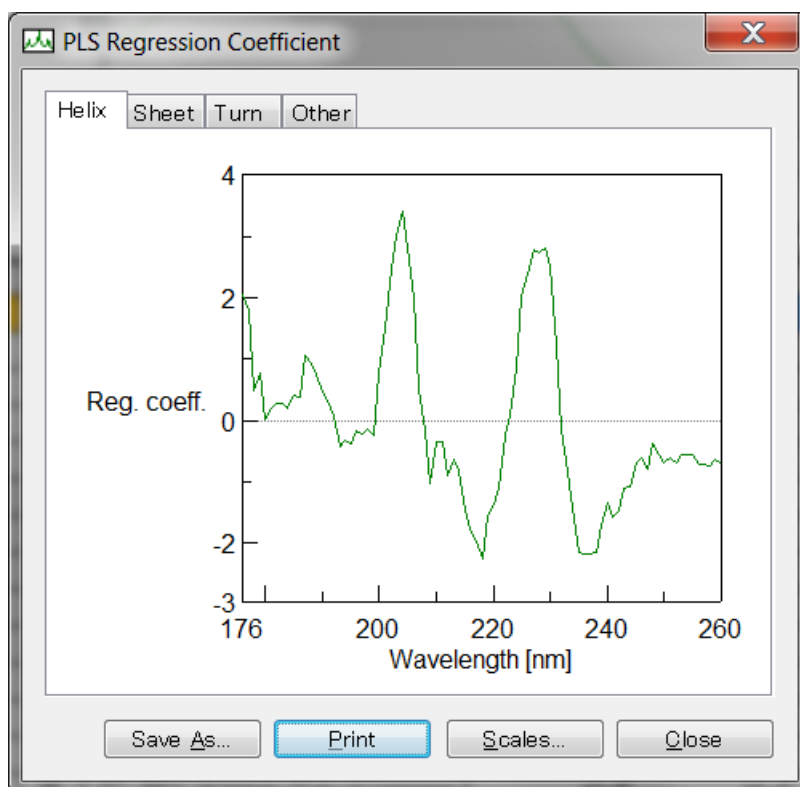Displays a regression coefficient graph. This cannot be selected until a PLS calibration model is calculated.

Figure 3.24 [PLS Regression Coefficient] dialog box

<Save As...>   Saves the graph of the regression coefficient in *.jws format.

<Print>   Prints the graph of the regression coefficient.

<Scales...>   Changes the scale of the graph of the regression coefficient.

Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.

<Close>   Closes the dialog box.

3.5.4.4 [Correlation Coefficient Spectrum...]

Calculates and displays a correlation coefficient spectrum. Use this command to estimate the calculation range of the x-axis used in quantitation. A correlation coefficient value near a range between +1 and −1 is decided for the calculation range. In the case of this spectrum, the range between 260 and 176 nm is appropriate.
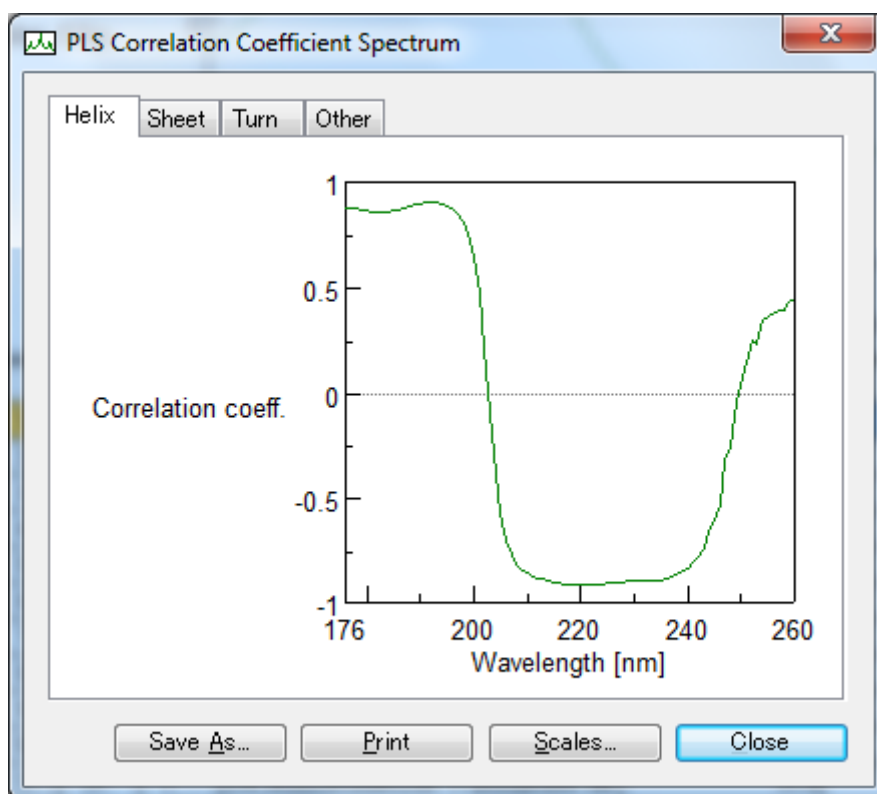

Figure 3.25 [PLS Correlation Coefficient Spectrum] dialog box

<Save As...>	Saves the correlation coefficient spectrum.
<Print>	Prints the correlation coefficient spectrum.
<Scales...>	Changes the scale of the correlation coefficient spectrum.

Note: Dragging the mouse diagonally over the graph enlarges it. Right-click the graph to restore the graph to its original scale.

<Close>   Closes the dialog box.

3.5.4.5 [Correlation Coefficient vs. Factor...]

Calculates and displays the correlation coefficients between the true value and evaluated value versus the number of factors. On the factors/correlation coefficient graph, the factors with the maximum number of correlation coefficients will be found. The value is the optimal number of factors. However, calculation time increases when there are a large number of factors. If correlation coefficients are identical, use the one with fewer factors.
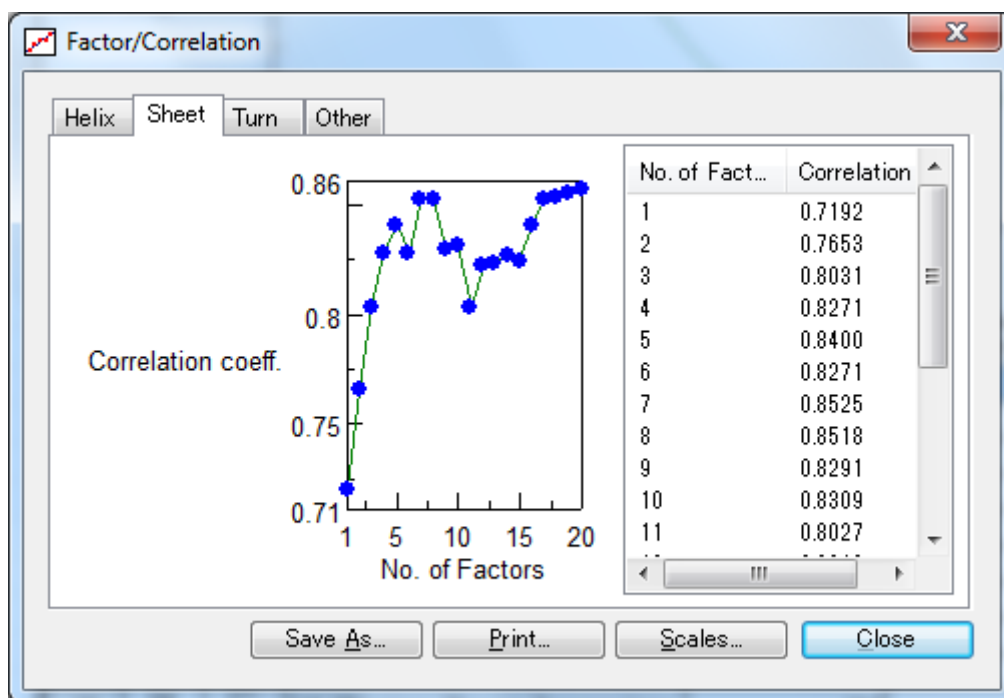

Figure 3.26 [Factor/Correlation] dialog box

<Save As...>   Saves the factor/correlation coefficient graph in text format.

<Print>   Prints the factor/correlation coefficient graph.

<Scales...>   Changes the scale of the factor/correlation coefficient graph.

<Close>   Closes the dialog box.

3.5.4.6 [RMSEP vs. Factor...]

Calculates and displays the RMSEP value for the number of principal components. On the factor/RMSEP graph, the number of factors where the RMSEP value is the lowest will be found. That value is the optimal number of factors. However, calculation time increases when there are a large number of factors. If RMSEP values are identical, use the one with fewer factors.

> *Note: If the number of factors found on the factor/correlation coefficient graph and the number of factors found on the factor/RMSEP graph differ, repeatedly perform calculations while varying the number of factors. It is ideal for the number of factors on both graphs to be identical. After the calculations, display and check the calibration curve.*
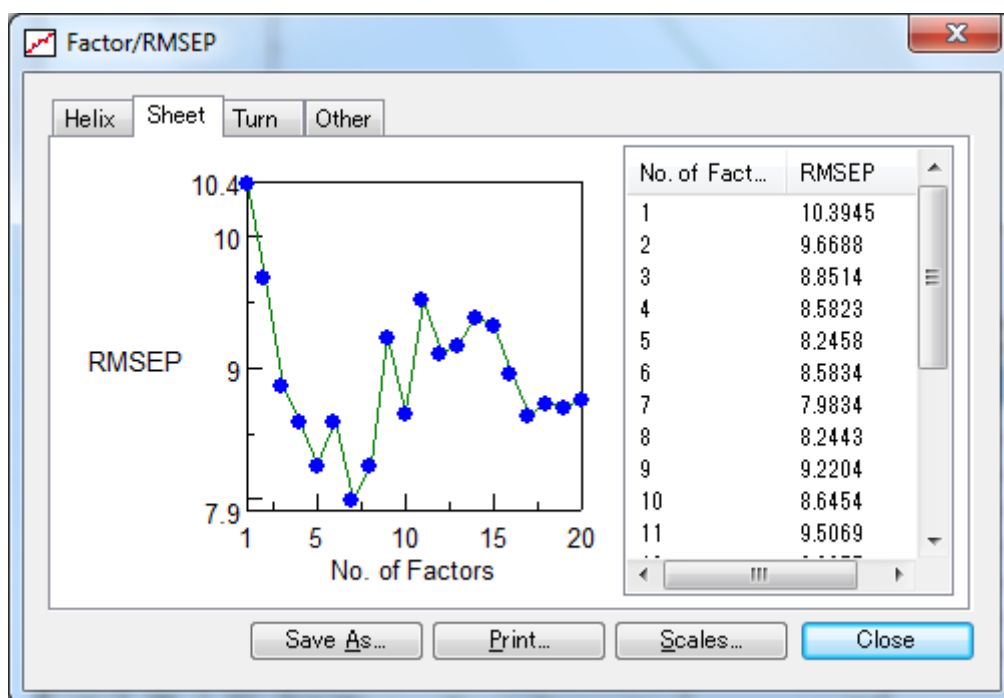

Figure 3.27 [Factor/RMSEP] dialog box

<Save As...>   Saves the factor/RMSEP graph in text format.

<Print>   Prints the factor/RMSEP graph.

<Scales...>   Changes the scale of the factor/RMSEP graph.

<Close>   Closes the dialog box.

3.5.4.7 [Leave –one-out Cross Validation...]

Calculates and displays a calibration curve (a graph of true values and evaluated values). Use it to validate PLS calibration models that are created. The model is satisfactory if points of concentration of the standard spectrum are on the calibration curve. Change the number of factors and recalculate if necessary.
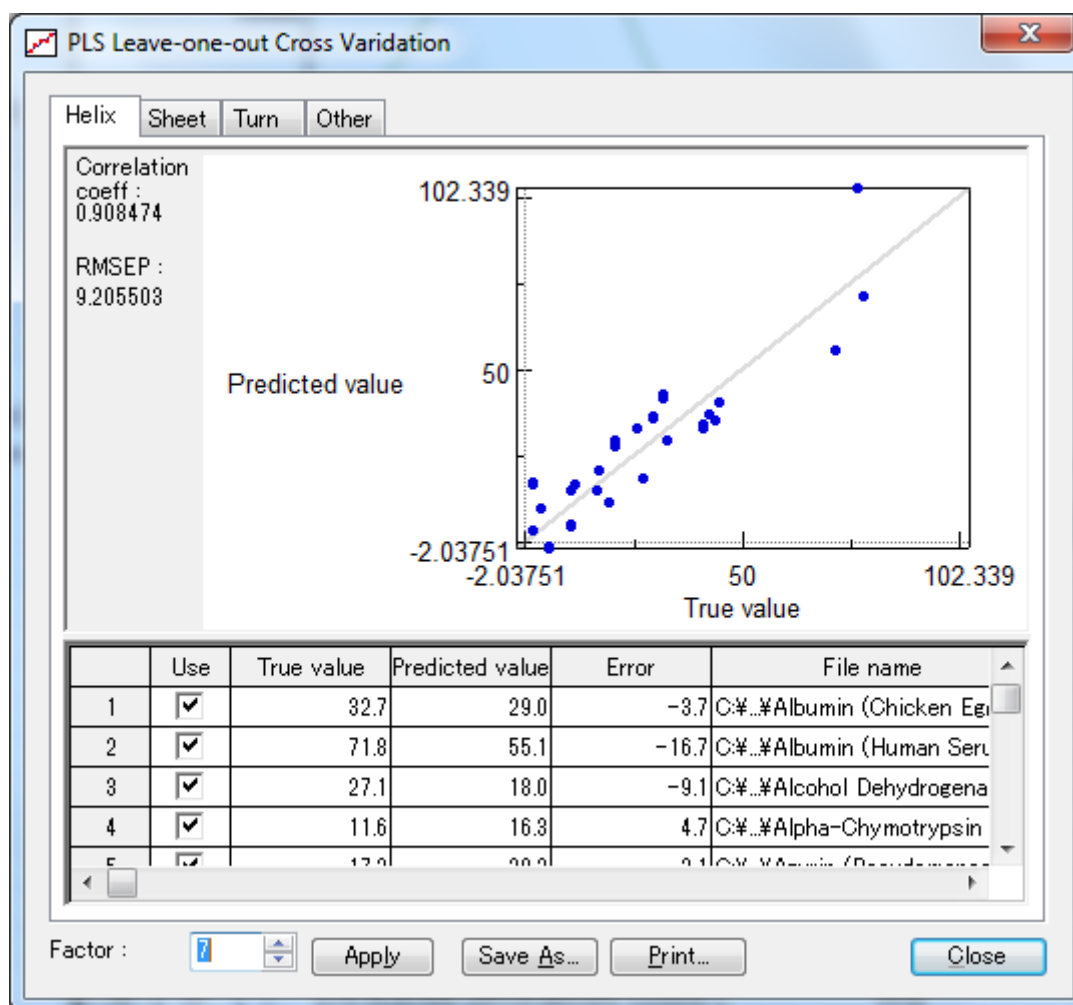

Figure 3.28 [PLS Leave–one-out Cross Validation] dialog box

[Factor(s)]	Sets the number of factors before recalculating.
<Apply>	Starts the recalculation process.
<Save As...>	Saves the calibration curve in text format.
<Print>	Prints the calibration curve.
<Close>	Closes the dialog box.

3.6 [Help] menu

| [About...] | Displays the version information for the program. |