Agilent ChemStation for UV-visible Spectroscopy

Understanding Your Dissolution Testing Software

Agilent Technologies

Notices

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Software Revision

This handbook is for B.02.xx revisions of the Agilent ChemStation software, where xx is a number from 00 through 99 and refers to minor revisions of the software that do not affect the technical accuracy of this handbook.

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In This Guide...

This handbook contains full details of the operation of the dissolution testing software for the Agilent ChemStation for UV-visible spectroscopy in both single and multibath modes. It describes the calculations used in the evaluation of the data and for making corrections for lost volume and sample dilution. the manual is designed to enable you to follow Good Laboratory Practice (GLP) guidelines. Using the information in the manual, you will be able to understand the data processing calculations from beginning to end and perform the data evaluation and corrections manually.

1 Dissolution Test Data

This chapter explains the configuration of sampling systems for single bath and multibath dissolution systems, describes the acquisition of dissolution test data, and describes the additional registers that are used in the dissolution testing modes.

2 Routine Data Analysis

This chapter explains the extraction and processing of wavelength data in routine analysis.

3 Advanced Data Analysis

This chapter explains the extraction and processing of wavelength data in advanced analysis, and includes the equations used in the calculations for single component and multicomponent analyses.

4 Evaluation

This chapter explains the calculations used in the evaluation of dissolution test data, including those used in corrections for dilution and lost volume.

5 Combined Reports

This chapter describes the requirements and procedures for the evaluation of dissolution test results according to USP 23.

Contents

1 Dissolution Test Data

Single Bath Dissolution Testing

Single bath dissolution testing allows either on-line or off-line sampling, depending on the configured sampling system.

In the on-line sampling setup a single dissolution bath per spectrophotometer can be controlled.

On-line Sampling

Automated on-line sampling can be configured using either a multicell transport system or a valve sampling system. In both cases, a maximum of eight vessels can be sampled.

A sampling (measurement) cycle consists of the sample transfer from the vessel to the spectrophotometer and the measurement of all configured vessels. The minimum cycle time is the minimum time difference between consecutive measurement cycles.

Multicell Transport System

The on-line multicell transport system allows up to eight vessels to be sampled in parallel (seven vessels using the 7-position multicell transport system). The sampling cycle is based on a user-defined timetable, with a minimum sampling cycle time of approximately 2.5 minutes; the cycle time is dependent on the pump time.

Valve Sampling System

The valve sampling system allows up to eight vessels to be sampled sequentially. The sampling cycle time can be configure to 5, 7.5 or 10 minutes. In order for the dissolution calculations to be accurate, the valve sampling system demands that the tablets be dropped at the same predefined intervals as the sampling; this is done manually, under instruction from the Agilent ChemStation.

Off-line Sampling

When sampling of the dissolution vessels is carried out off-line, and the samples are stored for later analysis (using a fraction collector for example), the samples can be analyzed using any of the available standard sampling systems:

- Sipper
- Off-line multicell transport
- Autosampler

Off-line sampling can accommodate up to 24 dissolution vessels.

Multibath Dissolution Testing

Multibath dissolution testing requires a multicell transport, and a valve sampling system for each bath. Up to four baths can be accommodated in a setup with a single spectrophotometer, each containing up to eight dissolution vessels. Each bath is sampled into an individual flow cell in the multicell transport system; each bath is equivalent to an on-line single bath system using the valve sampling system ("Valve Sampling System" on page 8).

Instrument Parameters

The instrument parameters that are available for user control depend on the sampling system that is configured. For all sampling systems except the valve-based sampling systems, the user has full control of all instrument parameters; for the valve system, all instrument parameters are fixed. Table 1 shows the instrument parameters and limits for each case.

Table 1 Instrument Parameters in Dissolution Testing

Parameter	Valve Sampling System	Other Sampling Systems
Wavelength Range	190–1100 nm fixed	190–1100 nm variable
Interval	1 nm fixed	1–100 nm variable
Integration Time	0.5 s fixed	0.1–25.5 s variable
Std. deviation	On	On or off
Stray light correction	On	On or off

Because multibath dissolution testing is a valve-based sampling system, the instrument parameters are fixed according to the valve sampling system column in Table 1.

2
Routine Data Analysis

Spectral Processing

Absorbance

Absorbance is the default data type of spectral storage in the Agilent ChemStation for UV-visible spectroscopy. The raw data format transmitted from the Agilent 8453 is absorbance.

Transmittance

Transmittance spectra are calculated using the Transmittance function; for a complete description of the mathematical processes involved in calculating transmittance spectra, “Transmittance” on page 18.

Derivative

First, second, third and fourth order derivative spectra can be calculated. The Derivative functions calculate the derivative of the data points (y-values) in the spectrum to the specified derivative order using a Savitsky-Golay algorithm with a filter length of 5 and a polynomial degree of 3. For a complete description of the Derivative function, “Derivative” on page 18.

Data Transformation

Use Wavelength

The Use Wavelength function selects a processed spectral intensity value at a single wavelength. The extracted value may then be subjected to further modification by background correction to produce the final result.

Background Correction

Background correction subtracts a calculated *background* value from the extracted spectral intensity value(s). There are five methods of calculating the background values:

- single reference wavelength
- subtract average over a range
- three-point drop line, used when two wavelengths are set
- absorbance value
- capsule background

Single Reference Wavelength

The single reference wavelength method subtracts the spectral intensity value at a specified wavelength, as in Figure 1 on page 14. The reference wavelength is usually selected at a point on the baseline beyond the sample absorbance.

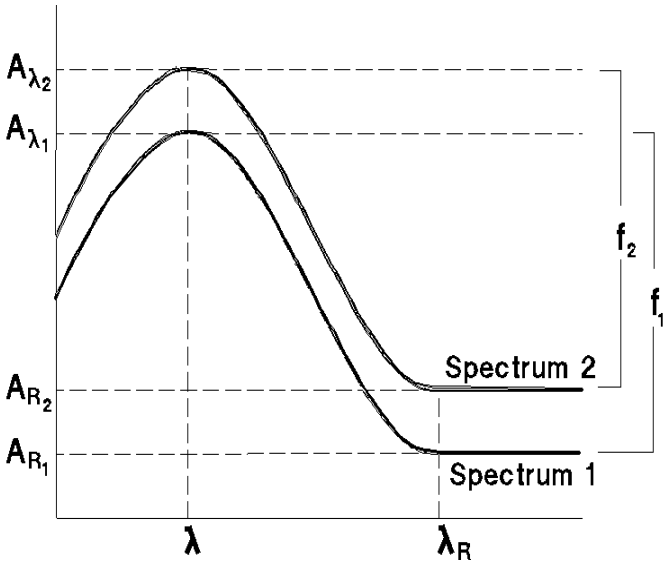


Figure 1 Background Correction at a Single Reference Wavelength

Subtraction is carried out using Equation 1:

$$f_\lambda = A_\lambda - A_{R_\lambda} \quad \textbf{(1)}$$

where

f_λ is the function result at wavelength λ

A_λ is the absorbance at wavelength λ

$A_{R\lambda}$ is the absorbance at reference wavelength λ

The variances (if available) are treated according to Equation 2:

$$\text{var}(f_\lambda) = \text{var}(A_\lambda) + \text{var}(A_{R_\lambda}) \quad \textbf{(2)}$$

where

$var(f_\lambda)$ is the variance of the function result at wavelength λ

$var(A_\lambda)$ is the variance of the absorbance at wavelength λ

$var(A_{R\lambda})$ is the variance of the absorbance at reference wavelength λ

Subtract Average Over Range

The subtract average over range method uses the same calculation as for a single reference wavelength (see "Single Reference Wavelength" on page 13), but replaces the spectral intensity value at the single wavelength with the average intensity value over a specified wavelength range.

$$f_\lambda = A_\lambda - \left(\frac{(A_{R_1} + A_{R_2} + A_{R_3} + \ldots + A_{R_n})}{n} \right)$$

where the terms are the same as for a single reference wavelength (see "Background Correction" on page 13).

Three-Point Drop Line

For a three-point drop-line background correction, the spectral intensity and variance values from two reference wavelengths are taken, giving A_{R_1}, A_{R_2}, $\mathrm{var}(A_{R_1})$ and $\mathrm{var}(A_{R_2})$. In this case, the reference wavelengths define a straight line (as in Figure 2) which is used to calculate A_{R_λ} and $\mathrm{var}(A_{R_\lambda})$ using Equation 3 and Equation 4.

$$A_{R_\lambda} = \frac{1}{\lambda_{R_2} - \lambda_{R_1}} \{ (\lambda_{R_2} - \lambda) A_{R_1} + (\lambda - \lambda_{R_1}) A_{R_2} \} \quad \textbf{(3)}$$

$$\mathrm{r}(A_{R_\lambda}) = \frac{1}{(\lambda_{R_2} - \lambda_{R_1})^2} \left\{ (\lambda_{R_2} - \lambda)^2 \mathrm{var}(A_{R_1}) + (\lambda - \lambda_{R_1})^2 \mathrm{var}(A_{R_2}) \right\} \quad \textbf{(4)}$$

where the terms are the same as for a single reference wavelength (see "Background Correction" on page 13). In Figure 2, the upper trace shows the spectrum before background correction, with the three-point drop line superimposed. The lower trace shows the spectrum after background correction.

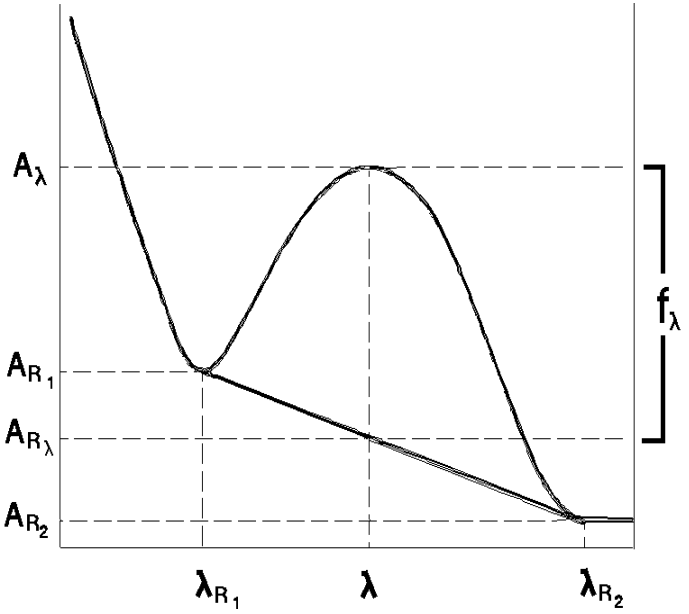


Figure 2 Three-Point Drop Line

Absorbance Value

The absorbance value background correction subtracts an absorbance value (in AU) from the absorbance at the specified wavelength.

Capsule Background

The capsule background method subtracts the spectral data value at the same wavelength in the capsule background spectrum from the data value at the specified wavelength.

Interactive Math Functions

The Dissolution Testing mode provides several math functions which can be used to manipulate spectra interactively. The results of interactive math processing are placed in the Math Result register and displayed in the Math Result window. There are two types of math function:

Unitary functions operate on individual spectra; they can be used to process several spectra in the same operation.

Binary functions operate on two spectra; any attempts to operate binary functions on a number of spectra other than two results in an error message.

Absorbance

Absorbance is a unitary function; it is the default data type of spectral storage in the Agilent ChemStation. The Absorbance function uses logarithm and scalar multiply functions to transform transmittance spectra into absorbance spectra using Equation 5. If the data are already absorbance, no processing is performed.

$$A = -\log_{10}\left(\frac{T}{100}\right) \quad \textbf{(5)}$$

where

A is absorbance

T is transmittance in percent

The variances of the spectrum (if available) are transformed by Equation 6:

$$\mathrm{var}(A) = \left(\frac{1}{T \cdot \ln 10}\right)^2 \mathrm{var}(T) \quad \textbf{(6)}$$

Variance data are displayed as standard deviation data in the Tabular Data of Spectrum window.

Transmittance

Transmittance is a unitary function; it uses exponential, reciprocal and scalar multiply functions to transform absorbance spectra into transmittance spectra using Equation 7. The transmittance spectra are placed in the math result register.

$$T = 100 \cdot 10^{-A} \quad \textbf{(7)}$$

The variances, if available, are transformed by Equation 8:

$$\mathrm{var}(T) = (100 \cdot \ln 10 \cdot 10^{-A})^2 \cdot \mathrm{var}(A) \quad \textbf{(8)}$$

All additional information, such as annotations, are preserved unchanged in the transmittance spectrum.

Derivative

Derivative is a unitary function; it calculates the derivative of the data points (y-values) in the spectrum using a Savitsky-Golay algorithm and places the resulting spectrum in the math result register.

Savitsky-Golay Algorithm The Savitsky-Golay algorithm uses the Derivative Order, Filter Length and Polynomial Degree from the Derivative Parameter dialog box. For each data point in the trace, the calculation takes a set of data points equal to the filter length around the current data point and fits a curve of the specified polynomial degree, using a least squares fit. The fitted curve is then used to calculate the new value for the current data point, and the derivative of that point.

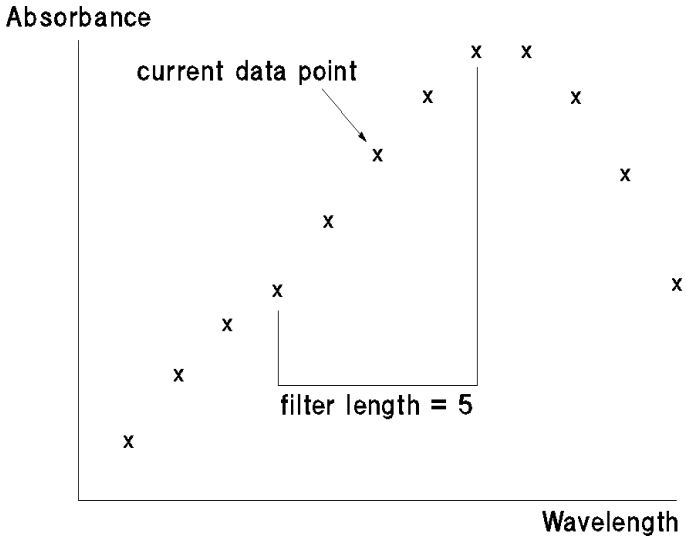


Figure 3 Filter Length in the Savitsky-Golay Algorithm

For each y-value, y_i,

$$\text{deriv}(y_i) = \sum C_{kj} \cdot y_{i+j-1} \quad \textbf{(9)}$$

where

i is *1 ... data points-(filter length-1)*

j is *1 ... filter length*

k is 2 for derivative

deriv(y1) corresponds to the $\left(\frac{(\text{filter length-1})}{2} + 1\right)$ th value. For example, if *filter length* is 5, then *deriv(y1)* becomes y_3. For this reason, the length of the spectrum is reduced by *(filter length - 1)* values; $\left(\frac{(\text{filter length-1})}{2}\right)$ values at the beginning of the spectrum and $\left(\frac{(\text{filter length-1})}{2}\right)$ values at the end of the spectrum are not processed.

In Equation 9, C is the coefficient matrix:

$$C = N^{-1} \cdot F^{T} \quad \textbf{(10)}$$

where

N^{-1} is the inverse of N, the product of F^{T} and F

F^{T} is the transpose of F, the matrix of the powers of the polynomials

The matrix of the powers of the polynomials is generated using Equation 11:

$$F_{ij} = k^{(j-1)} \quad \textbf{(11)}$$

where

i is $1 \ldots filter\ length$

j is $1 \ldots degree + 1$

k is $i - \frac{\text{filter length} - 1}{2} - 1$

At the end of the calculation, the y-values are multiplied by the reciprocal of the step:

$$y_i = \frac{y_i}{\text{step}} \quad \textbf{(12)}$$

where

step is the increment of the equidistant x-axis.

All additional information, such as annotations, are preserved unchanged in the derivative spectrum.

Spline

The spline function constructs a cubic splined curve through the data points (y-values) in the spectrum and places the resulting spectrum in the math result register. Unlike the smooth function, the spline function does not smooth the spectrum; the splined curve passes through all the original data points, and the spline process inserts additional points between the original ones to produce a continuous curve. The additional data points are calculated by a two-stage process:

Stage 1: Calculating the Second Derivative The algorithm for calculating the second derivative originates in the following tri-diagonal system of linear equations:

$$(x_i - x_{i-1})y''_{i-1} + 2(x_{i+1} - x_{i-1})y''_i + (x_{i+1} - x_i)y''_{i+1}$$

$$= 6\left(\frac{y''_{i+1} - y''_i}{x_{i+1} - x_i} - \frac{y''_i - y''_{i-1}}{x_i - x_{i-1}}\right) \quad \textbf{(13)}$$

or, in simplified form,

$$\text{diff1} \cdot y''_{i-1} + 2(\text{diff1} + \text{diff2} \cdot y''_i) + \text{diff2} \cdot y''_{i+1} = 6(\text{quot2} - \text{quot1}) \quad \textbf{(14)}$$

where

diff1 is $x_i - x_{i-1}$

diff2 is $x_{i+1} - x_i$

quot1 is $\frac{y''_i - y''_{i-1}}{\text{diff1}}$

quot2 is $\frac{y''_{i+1} - y''_i}{\text{diff2}}$

The solution is achieved in a two-step process:

1 decomposition and forward substitution

2 back substitution

Stage 2: Calculating the Splined Values The new y-values to be inserted between the original y-values are calculated according to Equation 15:

$$y_x = ((A \cdot \text{diffX} + B)\text{diffX} + C)\text{diffX} + D \quad \textbf{(15)}$$

where

diffX is the distance between the current x-value and the original x-value, x - x_i

In Equation 16, the coefficients A, B, C, and D are given by:

$$A = \frac{y''_{i+1} - y''_i}{6 \cdot \text{interval}} \quad \textbf{(16)}$$

where

interval is x_{i+1} - x_i

i is the current index, starting at 1 and ending at one less than the number of original values

$$B = \frac{y''_i}{2} \quad \textbf{(17)}$$

$$C = \frac{y_{i+1} - D}{\text{interval}} - \frac{1}{6} \cdot \text{interval} \cdot (y''_{i+1} + 4B) \quad \textbf{(18)}$$

where

interval is x_{i+1} - x_i

i is the current index, starting at 1 and ending at one less than the number of original values

$$D = y_i \quad \textbf{(19)}$$

The variances of the original spectrum, if available, are deleted.

All additional information, such as annotations, are preserved unchanged in the splined spectrum.

Scalar Multiply

Scalar Multiply is a unitary function; it multiplies each of the data points (y-values) in the spectrum or trace by a constant value and places the result in the math result register.

$$y_{\text{new}} = y \times C \quad \textbf{(20)}$$

The scalar multiply function can be used to divide by a constant value by using the reciprocal of the desired divisor, $1/C$.

All other additional information, such as annotations, are preserved unchanged in the resulting spectrum or trace.

Scalar Add

Scalar Add is a unitary function; it adds a constant value to each of the data points (y-values) in the spectrum or trace and places the result in the math result register.

$$y_{\text{new}} = y + C \quad \textbf{(21)}$$

The scalar add function can be used to subtract a constant value by using a negative constant.

All other additional information, such as annotations, are preserved unchanged in the resulting spectrum or trace.

Add

Add is a binary function; it adds two spectra or traces together and places the result in the math result register. The spectrum or trace selected first (item A) is taken as the model and provides the x-value range (or list) and resolution for the resulting spectrum or trace. Item A is first copied into the math result register, then the y-values (for example absorbance) from the second selected spectrum or trace (item B) are added to the y-values of the item in the math result register as follows:

Items of different x-value resolutions If the x-values of item A do not completely agree with those of item B (for example spectra with different wavelength resolutions):

- The y-values of item B are interpolated before adding them to the y-values of the result when the interval of the x-value in item A is less than that of item B.
- The y-values at intermediate x-values in item B are ignored when the interval of the x-value of item A is greater than that of item B, and only the y-values from item B at the x-values corresponding to item A are added to the y-values of the result.

Items of different x-value ranges If the x-range of item A is greater than the x-range of item B (for example spectra with different wavelength ranges), the extra y-values in item A remain unchanged in the result.

If the x-range of item A is less than the x-range of item B, the extra y-values in item B are ignored.

If there is no overlap between the x-ranges of the items, item A remains unchanged.

Additional information, such as annotations, are taken solely from item A; all such items from item B are ignored.

Subtract

Subtract is a binary function; it subtracts one spectrum or trace from another and places the result in the math result register. The spectrum or trace selected first (item A) is taken as the model and provides the x-value range (or list) and resolution for the resulting spectrum or trace. Item A is first copied into the math result register, then the y-values (for example absorbance) from the second selected spectrum or trace (item B) are subtracted from the y-values of the item in the math result register as follows:

Items of different x-value resolutions If the x-values of item A do not completely agree with those of item B:

- The y-values of item B are interpolated before subtracting them from the y-values of the result when the interval of the x-value in item A is less than that of item B.
- The y-values at intermediate x-values in item B are ignored when the interval of the x-value of item A is greater than that of item B, and only the y-values from item B at the x-values corresponding to item A are subtracted from the y-values of the result.

Spectra of different x-value ranges If the x-range of item A is greater than the x-range of item B, the extra y-values in item A remain unchanged in the result.

If the x-range of item A is less than the x-range of item B, the extra y-values in item B are ignored.

Additional information, such as annotations, are taken solely from item A; all such items from item B are ignored.

Calibration

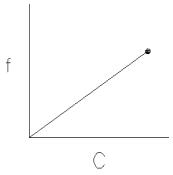
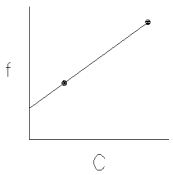
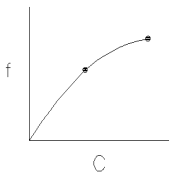
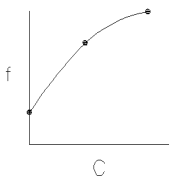
Calibration is the correlation of the **function result** with known concentrations of sample. The basic assumption is that the variance in the measured data is less than the variance in the standard concentrations. As a result of this correlation a **calibration curve** can be fitted to the data points and **calibration coefficients** can be calculated. The concentrations of unknown samples can then be calculated from the calibration coefficients and the function result of the samples.

Calibration Curve Types

Real data may deviate slightly from the ideal linear relationship described in "Beer-Lambert Law" on page 38. The relationship between the function result and concentration may be described more accurately by adding an offset (a non-zero intercept) or a quadratic term (or both) to the equation.

The ChemStation provides four different calibration curve types, shown in Table 2. These calibration curve graphs are shown in the more traditional way with the concentration on the x-axes and the function results on the y-axes.

Table 2 Calibration Curve Types for Single Component Analysis

	Curve Type	Curve	Equation
I	Linear without zero offset		$c = k_1 f$
II	Linear with zero offset		$c = k_0 + k_1 f$
III	Quadratic without zero offset		$c = k_1 f + k_2 f^2$
IV	Quadratic with zero offset		$c = k_0 + k_1 f + k_2 f^2$

c is the concentration

f is the function result

k_0, k_1, k_2 are calibration coefficients

Number of Standards

There is no fixed limit to the number of calibration standards that can be incorporated into a calibration; the more points that can be placed on the curve, the more accurately the curve can be characterized. However, each of the calibration curve types requires a minimum number of standards of different concentrations (see Table 3).

Table 3 Minimum Number of Standards for the Calibration Curve Types

Calibration Curve Type	Minimum Number of Standards
Linear, no zero offset (Beer's Law)	1
Linear with zero offset	2
Quadratic, no zero offset	2
Quadratic with zero offset	3

Calibration Standards

If the absorbance of the analyte at the wavelength(s) you select strictly obeys Beer's law, then one standard is sufficient to characterize the calibration curve (linear without zero offset). However, the use of two standards at different concentrations will confirm adherence to Beer's law, or show up any irregularities. Two standards are necessary to characterize a linear calibration curve with zero offset. Addition of a third standard at a different concentration is sufficient to identify and characterize a non-linear calibration curve.

Spectral Match

The spectral match function compares two spectra by linear regression. The match factor is calculated over the common wavelength range of the two spectra.

The match factor is calculated by Equation 22:

$$\text{Match Factor} = \frac{10^3\left(\sum y_A \cdot y_B - \left(\frac{\sum y_A \sum y_B}{n}\right)\right)^2}{\left(\sum y_A^2 - \frac{(\sum y_A)^2}{n}\right)\left(\sum y_B^2 - \frac{(\sum y_B)^2}{n}\right)} \quad \textbf{(22)}$$

where

y_A, y_B are the weighted intensity values of spectrum A and spectrum B respectively. The weighted intensity values, w, are calculated by multiplying them by the reciprocal of the square roots of the variances: $w = \frac{y}{\sqrt{\text{var}}}$

n is the number of data points

At the extremes, a match factor of 0 indicates totally dissimilar spectra and a match factor of 1000 indicates identical spectra. Generally, values above 990 indicate an acceptable degree of similarity; values between 900 and 990 indicate some similarity, but with a degree of doubt; values below 900 show that the spectra are different.

3
Advanced Data Analysis

Spectral Processing

Instead of using the data at a single wavelength, you can define several wavelengths or wavelength ranges for use in the calculation of an **analytical function**. The result of the analytical function calculation is the **function result**; if you select a single wavelength, the function result is equal to the data value (e.g. absorbance) at that wavelength.

Analytical Wavelength

The analytical wavelength is the primary wavelength that you select for analyzing your sample. The analytical wavelength is typically the wavelength at the absorbance maximum, although the full-spectrum acquisition and virtually absolute wavelength reproducibility of the diode-array spectrophotometer allow you to select any wavelength in the absorbance band. If the maximum absorbance is high (e.g. greater than 2 AU), selecting wavelengths at the side of an absorbance band, where the absorbance is lower, can avoid non-linearity due to stray light. Measurements on the side of absorbance bands are usually also included when measuring over a wavelength range, and in multicomponent analysis, when measurements over a wide spectral range are used.

Range of Wavelengths

Selecting a wavelength range instead of a single wavelength can improve sensitivity and reproducibility. As more data points are averaged together either side of the absorbance maximum, the average absorbance decreases slowly at first, then more quickly as low absorbances on the side of the band are included (see Figure 4). At the same time as data points are averaged, noise decreases as the square root of the number of data points. Thus, the signal-to-noise ratio improves as more points are averaged, but eventually declines as more low absorbance values are included in the average. There is an optimum range which is generally equivalent to the bandwidth at the half-height of the absorbance band, that is the natural bandwidth (NBW).

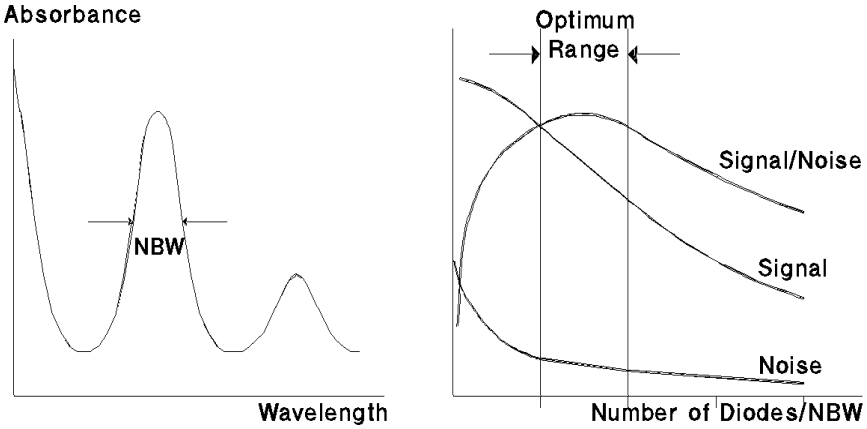


Figure 4 Optimum Signal-to-Noise Ratio

When more than one wavelength range is specified, the function result is the average of the average absorbance values of all specified ranges:

$$f = \frac{1}{N_A + \ldots + N_N}\left(\sum_{i\varepsilon\lambda_{A_1}:\lambda_{A_n}} A_i + \ldots + \sum_{i\varepsilon\lambda_{N_1}:\lambda_{N_n}} A_i\right) \quad \textbf{(23)}$$

where

$i\varepsilon\lambda_{A_1}:\lambda_{A_n}$ through $i\varepsilon\lambda_{A_1}:\lambda_{A_n}$ specify the analytical wavelength ranges

$N_A \ldots N_N$ specify the number of data points within each range

For each wavelength range, the **Step** specifies the interval between wavelength values for the calculation of the average absorbance. Where the step is different from the spectral resolution of the data, a linear interpolation is made where necessary; for example, if a step value of 3 nm is specified for data with a spectral resolution of 2 nm, a linear interpolation is made for every other point. Similarly, a linear interpolation is made for every point when the step value is the same as the spectral resolution of the data, but the starting wavelength of the range does not correspond exactly with an actual wavelength value. For multiple ranges, the specification of a smaller step value adds weight to the influence of the range in the function result.

Analytical Function

The Analytical Function option allows you to include internal referencing in the calculation of the function result. For details of internal referencing, see "Background Correction" on page 13. The analytical function wavelength parameters include a Factor, which is an alternative method for increasing or decreasing the influence of specific wavelength range in the function result. When the factor is not equal to 1, the average absorbance value of the wavelength range is multiplied by the factor before the function result is calculated:

$$f = \frac{1}{N_A + \ldots + N_N}\left(\sum_{i\varepsilon\lambda_{A_1}:\lambda_{A_n}} F_A \cdot A_i + \ldots + \sum_{i\varepsilon\lambda_{N_1}:\lambda_{N_n}} F_N \cdot A_i\right) \quad \textbf{(24)}$$

where

$i\varepsilon\lambda_{A_1}:\lambda_{A_n}$ through $i\varepsilon\lambda_{A_1}:\lambda_{A_n}$ specify the analytical wavelength ranges

$N_A \ldots N_N$ specify the number of data points within each range

$F_A \ldots F_N$ are the specified factors for the ranges

Evaluation

Advanced mode offers a choice of four evaluation procedures:

- None (no evaluation)
- Equation
- SCA (see "Single Component Analysis" on page 37)
- MCA (see "Multicomponent Analysis" on page 51)

When no evaluation is selected, the analysis results are the contents of the Wavelength Results register: the function result and the results from the individual wavelenth(s). In this case no further calculation of dissolution results is possible.

Equation Evaluation

When an equation evaluation is specified, the analysis results are the results of up to four equations specified in the Equation Parameter dialog box. In the definition of the equations, you can use any result generated by the Use Wavelength(s) and stored in the Wavelength Results register (function result, results from individual wavelengths, average absorbance of a wavelength range) and any other variables that you have entered into the Sample Spectra table. For example, if you wish to use the weight of an analyte in an equation, you can add *weight* as an Analyte in the Sample Spectra table, and enter the appropriate value and units for the weight of the analyte. You can use the *weight* of the sample in the equation by defining *weight* as one of the four variables A1 to A4 in the Equation Parameter dialog box. When path length correction is switched on, it normalizes the results to a 1-cm path length by dividing the function result by the value of the Path Length parameter in the Sample Spectra table:

$$f_{\text{corr}} = \frac{f}{l} \quad \textbf{(25)}$$

where

f_{corr} is the function result after path length correction

f is the uncorrected function result

l is the path length

When dilution factor correction is switched on, the result is multiplied by the entry in the Sample Information dialog box:

$$f_{corr} = f \times d$$

where

f_{corr} is the function result after path length correction

f is the uncorrected function result

d is the dilution factor

Results from individual wavelengths or wavelength ranges are corrected for the path length and dilution factor in the same way as the function result.

Single Component Analysis

Two statistical methods are available for establishing the relationship between the concentration of a set of standards and their measured variables (for example their absorptions):

Least Squares Method

The Least Squares Method is the default method. The calibration curve parameters are calculated to minimize the squares of the differences between the measured data points and the calculated points on the calibration curve. Under these conditions, the overall deviation of the calculated calibration curve from the measured data points is minimized.

Method of Maximum Likelihood

The Method of Maximum Likelihood is a two-stage curve fit that makes use of the statistical variances that the diode-array spectrophotometer provides to determine the precision of each data point. Each point is then weighted according to both its precision, and the precision of the analyte concentration (using the standard deviation of the analyte concentration from the Standard Spectra table). The calibration curve is then fitted to the statistically-weighted points by a least squares method.

Beer-Lambert Law

The Beer-Lambert law states that the absorbance of a solute is directly proportional to its concentration:

$$A = Ecd \tag{26}$$

In Equation 26:

A is absorbance

E is molar absorptivity or molar extinction coefficient (l mol^{-1} cm^{-1})

c is analyte concentration (mol l^{-1})

d is cell path length (cm)

The concept of **analytical functions**, created by combining absorbance or derivative data from different parts of the spectrum to give a **function result**, f, means that these function results can be used instead of absorbance data in the following equations.

To calculate the concentration of an unknown compound, the above equation can be solved for a given function result, f:

$$c = \frac{f}{Ed} \tag{27}$$

The reciprocal of the product of molar extinction coefficient and cell path length is often called the calibration coefficient, k.

$$c = kf \tag{28}$$

SCA Calibration

Calibration Curve Types

Real data may deviate slightly from the ideal linear relationship described in "Beer-Lambert Law" on page 38. The relationship between the function result and concentration may be described more accurately by adding an offset (a non-zero intercept) or a quadratic term (or both) to the equation.

The ChemStation provides four different calibration curve types, shown in Table 2 on page 27. These calibration curve graphs are shown in the more traditional way with the concentration on the x-axes and the function results on the y-axes.

Number of Standards There is no fixed limit to the number of calibration standards that can be incorporated into a calibration; the more points that can be placed on the curve, the more accurately the curve can be characterized. However, each of the calibration curve types requires a minimum number of standards of different concentrations (see Table 4).

Table 4 Minimum Number of Standards for the Calibration Curve Types

Calibration Curve Type	Minimum Number of Standards
Linear, no zero offset (Beer's law)	1
Linear with zero offset	2
Quadratic, no zero offset	2
Quadratic with zero offset	3

Calibration Standards

If the absorbance of the analyte at the wavelength(s) you select strictly obeys Beer's Law, then one standard is sufficient to characterize the calibration curve (linear without zero offset). However, the use of two standards at different concentrations will confirm adherence to Beer's Law, or show up any irregularities. Two standards are necessary to characterize a linear calibration curve with zero offset. Addition of a third standard at a different concentration is sufficient to identify and characterize a non-linear calibration curve.

Calibration Curve Fits

The mathematical problem is to find the calibration coefficients in a given curve type which will allow the best determination of a future unknown sample.

Least Squares Method (LSQ)

The least squares method uses the analytical function data from Use Wavelengths of all standards to determine the calibration coefficients of the chosen calibration curve type by a least squares calculation.

If each i^{th} data set (f_i,c_i) of the n standard data sets is expected to obey a function in the p coefficients, k_j, although the real (measured) values may cluster around the function because of statistical errors, ε_i, then the general equation is:

$$c_i = k_0 + k_1 f_i + k_2 f_i^2 + \varepsilon_i \quad \textbf{(29)}$$

where

i is *1, 2, 3,...n* (the total number of standards)

The calibration coefficients, k_j, can be estimated using the least squares method, that is minimizing the sum of the squares of the errors, e_i (the differences between the measured value and the calibration curve).

$$\sum_{i=1}^{n} (c_{actual_i} - c_{calculated_i})^2 = \sum_{i=1}^{n} \varepsilon_i^2 = minimum \quad \textbf{(30)}$$

In matrix notation:

$$C = Fk + \varepsilon \quad \textbf{(31)}$$

where

C is n-concentration column vector

F is n × p function result data matrix

k is p-calibration coefficient column vector

e is n-error column vector

The elements used in the calibration matrix F and the coefficient vector k are given in Table 5 with dimension p of the coefficient vector.

Table 5 Elements Used in Calibration Matrix *F*

Curve Type	p	$F_{(i)th\ row}$			k		
I	1	f_i	—	—	k_1	—	—
II	2	1	f_i	—	k_0	k_1	—
III	2	f_i	f_i^2	—	k_1	k_2	—
IV	3	1	f_i	f_i^2	k_0	k_1	k_2

Example

Using curve type III, where $p = 2$, for 5 standards, $n = 5$:

$$c_1 = k_1 f_1 + k_2 f_1^{\,2} + \varepsilon_1$$

through to

$$c_1 = k_1 f_5 + k_2 f_5^{\,2} + \varepsilon_5$$

That is:

$$\begin{bmatrix} c_i \\ \dots \\ c_5 \end{bmatrix} = \begin{bmatrix} f_1 & f_1^2 \\ \dots & \dots \\ f_5 & f_5^2 \end{bmatrix} \begin{bmatrix} k_1 \\ k_2 \end{bmatrix} + \begin{bmatrix} \varepsilon_i \\ \dots \\ \varepsilon_5 \end{bmatrix} \quad \text{or, in matrix notation: } C = Fk + \varepsilon$$

By defining F and k for each of the calibration curve types, the coefficients are given by the general equation:

$$k = (F^T F)^{-1} F^T C \qquad \textbf{(32)}$$

where

F^T denotes the transpose of F

Example Continued:

$$\begin{bmatrix} k_1 \\ k_2 \end{bmatrix} = \left(\begin{bmatrix} f_1 & \cdots & f_5 \\ f_1^2 & \cdots & f_5^2 \end{bmatrix} \begin{bmatrix} f_1 & f_1^2 \\ \cdots & \cdots \\ f_5 & f_5^2 \end{bmatrix} \right)^{-1} \left(\begin{bmatrix} f_1 & \cdots & f_5 \\ f_1^2 & \cdots & f_5^2 \end{bmatrix} \begin{bmatrix} c_1 \\ \cdots \\ c_5 \end{bmatrix} \right)$$

Method of Maximum Likelihood

In the maximum likelihood calculation, the standard deviations of the function results of the standards (calculated from the variances provided by the spectrophotometer) and the standard deviations of the concentrations (from the Analytes of Standards table) are used in a weighted calibration.

Now, instead of Equation 30 on page 40, the weighed sum of the squares of the residuals is minimized:

$$S_{MLH} = \sum_{i=1}^{n} \omega_i (C_{actual_i} - C_{calculated_i})^2 \quad \textbf{(33)}$$

The weights ω_i are the reciprocals of the squares of the estimated standard deviations of the residuals:

$$\omega_i = \frac{1}{s_{\varepsilon_i}^2} \quad \textbf{(34)}$$

From Equation 29 on page 40, it follows that ε_i depends on C_i and f_i, both of which are subject to error. Using the technique of error propagation, the standard deviations of ε_i can be estimated from the standard deviation of concentration, s_{C_i}, and the standard deviation of the function result, s_{f_i}, of the i^{th} standard.

$$s_{e_i}^2 = s_{C_i}^2 + k_1^2 s_{f_i}^2 + 4k_1 k_2 f_i s_{f_i}^2 + 4k_2^2 f_i^2 s_{f_i}^2 \quad \textbf{(35)}$$

If maximum likelihood is specified, the least squares coefficients, k_1 and k_2 are used to evaluate $s_{e_i}^2$ in Equation 35.

When S_{MLH} is minimized, the estimates, k, of the calibration coefficients can be obtained using Equation 36:

$$k = (F^T W F)^{-1} F^T W C \quad \textbf{(36)}$$

where the weighting matrix W is $diag(w_1, \ldots, w_n)$ with w_i of Equation 34.

SCA Calibration Results

This section contains the equations used in the calculations of the results given in the SCA Summary and the SCA Calibration Results table. For an explanation of the usage of the SCA Calibration Results, see "SCA Evaluation" on page 48.

Residual

$$e_i = C_{actual_i} - C_{calculated_i} \quad \textbf{(37)}$$

%Error

$$d_i = \frac{e_i}{C_{calculated_i}} \times 100 \quad \textbf{(38)}$$

where

e_i is the residual of the i^{th} standard ("Residual")

Std.Dev of Calibration

- Least Squares Method

$$s = \sqrt{\frac{1}{(n-p)} \sum_{i=1}^{n} e_i^2} \quad \textbf{(39)}$$

where

e_i is the residual of the i^{th} standard

n is the number of standards

p is the number of coefficients in the equation of the calibration curve

- Maximum Likelihood

$$s_{sd} = \sqrt{\frac{n \cdot S_{MLH}}{\sum_{i=1} w_i}} \quad \textbf{(40)}$$

The calculation for S_{MLH} is given in Equation 33 on page 42.

Relative Fit Error (Maximum Likelihood only)

$$s_{r_f} = \sqrt{\frac{S_{MLH}}{n-p}} \quad \textbf{(41)}$$

Std.Dev of Coefficient k

- Least Squares Method

$$s_{k_j} = s\sqrt{(F^T F)^{-1}_{jj}} \quad \textbf{(42)}$$

where

$(F^T F)^{-1}_{jj}$ is the j^{th} diagonal element of $(F^T F)^{-1}$

s is the standard deviation of calibration (see "Std.Dev of Calibration" on page 44)

- Maximum Likelihood

$$s_{k_j} = s_{r_f}\sqrt{(F^T W F)^{-1}_{jj}} \quad \textbf{(43)}$$

where

j is $1, \ldots, p$

Correl. Coeff. (R^2)

- Least Squares Method

$$R^2 = \frac{\sum_{i=1}^{n} (C_{calculated_i} - \bar{C})^2}{\sum_{i=1}^{n} (C_{actual_i} - \bar{C})^2} \quad \textbf{(44)}$$

For curve types II and IV (see "Calibration Curve Types" on page 26),

$$\bar{C} = \frac{1}{n}\sum_{i=1}^{n} C_i \quad \textbf{(45)}$$

For curve types I and III (see "Calibration Curve Types" on page 26), $\overline{C}$ is set to zero.

- Maximum Likelihood

$$R^2 = \frac{\sum_{i=1}^{n} w_i (C_{calculated} - \overline{C})^2}{\sum_{i=1}^{n} w_i (C_{actual} - \overline{C})^2} \quad (46)$$

For curve types II and IV (see "Calibration Curve Types" on page 26),

$$\overline{C} = \frac{\sum_{i=1}^{n} w_i C_i}{\sum_{i=1}^{n} w_i} \quad (47)$$

The calculation of w_i is given in Equation 34 on page 42.

For curve types I and III (see "Calibration Curve Types" on page 26), $\overline{C}$ is set to zero.

Leverage

- Least Squares Method

$$h_i = (F(F^T F)^{-1} F^T)_{ii} \quad (48)$$

- Maximum Likelihood

$$h_i = (W^{1/2} F (F^T W F)^{-1} F W^{1/2})_{ii} \quad (49)$$

95% CI

$$CI_i = t_{n-p,\,97,5} \cdot s\sqrt{h_i} \quad (50)$$

where

$t_{n-p,\,97,5}$ is the percentage point of the t distribution

s is the standard deviation of calibration (see "Std.Dev of Calibration" on page 44)

h_i is the leverage of the i^{th} standard (see "Leverage" on page 46)

Uncertainty

$$Uncertainty = \frac{PI_{i_{max}}}{C_{\text{calculated}_{i_{max}}}} \times 100 \quad (51)$$

where

$PI_{i_{max}}$ is the 95% Prediction Interval ("95% PI" on page 50) of the standard with the highest absolute value of function result.

Studentized Residual (Stud.Res.)

- Least Squares Method

$$t_i = \frac{e_i}{s\sqrt{1-h_i}} \quad (52)$$

where

e_i is the residual of the i^{th} standard ("Residual" on page 43)

s is the standard deviation of calibration ("Std.Dev of Calibration" on page 44)

h_i is the leverage of the i^{th} standard ("Leverage" on page 46)

- Maximum Likelihood

$$t_i = \frac{w_i e_i}{s_{rf}\sqrt{1-h_i}} \quad (53)$$

where

e_i is the residual of the i^{th} standard (see "Residual" on page 43)

s_{rf} is the square root of the relative fit error (see "Relative Fit Error (Maximum Likelihood only)" on page 44)

h_i is the leverage of the i^{th} standard (see "Leverage" on page 46)

w_i is the weight of the i^{th} standard (see "Leverage" on page 46)

Cook's Dist. (Cook's Distance)

$$CD_i = \frac{t_i^2}{p} \cdot \frac{h_i}{1 - h_i} \quad \textbf{(54)}$$

where

t_i is the studentized residual of the i^{th} standard (see "Studentized Residual (Stud.Res.)" on page 47)

h_i is the leverage of the i^{th} standard (see "Leverage" on page 46)

p is the number of coefficients

SCA Evaluation

When the standards have been measured, and the calibration coefficients have been determined, the unknown concentration of a measured sample can be calculated simply by calculating C_{unk} in the equation of the calibration curve using the function result data, f_{unk} of the unknown sample:

$$C_{unk} = k_0 + k_1 f_{unk} + k_2 f_{unk}^2 \quad \textbf{(55)}$$

where k_0, k_1 or k_2 may be zero depending on the calibration curve type (see Table 2 on page 27).

SCA Quantification Results

Std.Dev.

- Least Squares Method

$$s_{C_{unk}} = s\sqrt{f_{unk}(F^T F)^{-1} f_{unk}^T} \quad (56)$$

where

s is the standard deviation of calibration (see "Std.Dev of Calibration" on page 44)

f_{unk} is (f_{unk}) for calibration curve type I

or $(1, f_{unk})$ for calibration curve type II

or (f_{unk}, f_{unk}^2) for calibration curve type III

or $(1, f_{unk}, f_{unk}^2)$ for calibration curve type IV (see "Calibration Curve Types" on page 26)

F is the calibration matrix

- Maximum Likelihood

$$s_{C_{unk}} = s_{rf}\sqrt{f_{unk}(F^T W F)^{-1} f_{unk}^T} \quad (57)$$

95% PI

- Least Squares Method

$$PI = t_{n-p,\,97,5} \cdot s\sqrt{f_{unk}(F^T F)^{-1} f_{unk}^T + 1} \quad \textbf{(58)}$$

where

$t_{n-p,\,97,5}$ is the percentage point of the t distribution

s is the standard deviation of calibration (see “Std.Dev of Calibration” on page 44)

f_{unk} and F are the same as for the standard deviation (see “Std.Dev.” on page 49)

- Maximum Likelihood

$$PI = t_{n-p,\,97,5} \cdot s_{rf}\sqrt{f_{unk}(F^T W F)^{-1} f_{unk}^T + s_{unk}^2} \quad \textbf{(59)}$$

s_{unk} is calculated from Equation 35 on page 42, setting $s_{C_i}^2$ to zero and f_i equal to f_{unk}, $s_{f_i}^2$ equal to $s_{f_{unk}}^2$.

Multicomponent Analysis

Multicomponent analysis is based on an extension of Beer's law to m components:

$$A = \sum_{i=1}^{m} E_i c_i d \quad \textbf{(60)}$$

where

A is the absorbance of a mixture of m components

E_i is molar absorptivity or molar extinction coefficient of the i^{th} component (l mol^{-1} cm^{-1})

c_i is the concentration of the i^{th} component (mol l^{-1})

d is cell path length (cm)

Equation 60 is applied at each wavelength. As with single component analysis (see "Single Component Analysis" on page 37), the multicomponent calibration uses the analytical function concept of combining absorbance or derivative data to give function results. However, instead of calculating the analytical function as an average, as in single component analysis, multicomponent analysis calculates individual function results for each wavelength.

The basic assumption in multicomponent analysis is that Equation 60—Beer's law—applies at each wavelength except for a statistical error brought about by the use of real (measured) values.

$$f_j = \sum_{i=1}^{m} E_{ji} c_i d + \varepsilon_j \qquad j = 1, \ldots, n \tag{61}$$

where

m is the number of components

n is the number of wavelengths

f_j is the function result at the j^{th} wavelength

E_{ij} is molar absorptivity or molar extinction coefficient of the i^{th} component at the j^{th} wavelength

ε_j is the statistical error at wavelength j

Calculation Methods

In the multicomponent analysis calibration, the n times m individual molar adsorptivity constants are calculated from standards, which may be either pure components or mixtures of the m components at known concentrations.

The calibration of component behavior by the standards can be formulated as follows:

$$f_{jk} = \sum_{i=1}^{m} E_{ji} c_{ik} d + \varepsilon_{jk} \qquad j = 1, \ldots, n \qquad k = 1, \ldots, p \tag{62}$$

where

p is the number of standards

c_{ik} is the concentration of the i^{th} component of the k^{th} standard; $c_{ik} = 0$ if the k^{th} standard has no i^{th} component

ε_{jk} is the statistical error of the k^{th} standard at wavelength j

A more compact form of Equation 62 is given by the matrix notation:

$$F = HC + \varepsilon \tag{63}$$

where

H is called the calibration coefficient matrix; it contains the E_{ji}

C is the standard concentration matrix; the elements are the products of d and C_{ik}

As with single component analysis (see “Single Component Analysis” on page 37), two calculation methods (least squares and maximum likelihood) are offered for the determination of the coefficient matrix H.

Least Squares

The least squares solution of Equation 63 on page 53 is given by Equation 64:

$$H = FC^{T}(CC^{T})^{-1} \tag{64}$$

Maximum Likelihood

In the case of maximum likelihood, the standard deviations, s_{jk} of the statistical errors ε_{jk} are estimated by error propagation from the standard deviations of the function results, $s_{F_{jk}}$, and the standard deviations of the concentrations entered in the Analytes of Standard table, $s_{C_{ik}}$.

$$s_{jk}^2 = s_{F_{jk}}^2 + \sum_{i=1}^{m} h_{ji} s_{C_{ik}}^2 \quad \textbf{(65)}$$

For each wavelength, j, we define a p by p weighting matrix,

$$\phi_j = diag(s_{jk}^2) \quad \textbf{(66)}$$

and use it to calculate the j^{th} row of the calibration coefficient matrix, H, from the j^{th} row of the standard matrix, F, by a weighted least squares method.

$$H_j = F_j \phi_j^{-1} C^T (C \phi_j^{-1} C^T)^{-1} \quad \textbf{(67)}$$

where

H_j is the j^{th} row of H

F_j is the j^{th} row of F

The maximum likelihood method works in two steps:

First, the matrix H is estimated by an ordinary least squares method.

The weighting matrixes, ϕ_j, are calculated for each wavelength, and the final matrix, H, is calculated row by row by a weighted least squares method.

Finally, the standard deviations of the calibration coefficients H_{ji} are calculated and stored for use in the analysis of the unknown samples.

$$s_{H_{ji}}^2 = \sum_{k=1}^{p} s_{F_{jk}}^2 (\phi_{j_{kk}}^{-1} C_{ki}^{+}) \quad \textbf{(68)}$$

where

C_{ki}^{+} is the individual element of the matrix product $C^T (C \phi^{-1} C^T)^{-1}$

MCA Calibration Results

Ind. of Stds, Std.Dev.Residual and Rel.Fit Error

At the end of the calibration process, the calibration standards are evaluated as unknowns (see "MCA Evaluation" on page 56), and the standard deviation of the residual is calculated using Equation 76 on page 59 (least squares) or Equation 77 on page 59 (maximum likelihood). The independence of standards is also calculated using Equation 71 on page 57. In the case of the maximum likelihood method, the relative fit error is also calculated, using Equation 78 on page 59.

The Statistical Approach

The parameters estimated by the ordinary least squares in Equation 64 on page 53 can be shown to be *efficient* or *best* (that is, with minimum variance) if the following assumptions are true:

1. The regression function, Equation 63 on page 53, is correct. In multicomponent analysis, this means that Beer's law is obeyed without offset and without chemical or instrumental (detector) non-linearity. For calibrations using pure standards, it also means that no interactions between the standards occur when they are in admixture that alter the absorbance. Equation 63 on page 53 also implies that the mixture contains no absorbing compounds other than those in the standards.
2. Columns in matrix F are linearly independent, that is, the standard spectra are sufficiently different that $F^{T}F$ is non-singular.
3. F is known, that is, F is error-free, or has considerably smaller errors than those of C.
4. The errors of random variables C are stochastically independent.
5. Errors have normal distribution, with zero mean errors. This means that any systematic error must be negligible; ideally, there should be no systematic error. Systematic errors can arise from distortion of standard or sample spectra due to light scattering, stray light and other effects.
6. Errors have constant variance, that is, there is no difference in expected noise of data at different wavelengths.

Integrity of the Analysis

When mathematical mechanisms are applied to real-life data, there are always some assumptions that are not rigorously true. The computed results are never mathematically exact. In the Quantification software, the assumptions 1, 5 and 6 in "The Statistical Approach" on page 55 are the most important causes of error; the maximum likelihood method addresses assumption 6. The best way to verify the integrity of the multicomponent analysis is to test it with known samples to ensure that the errors due to the violation of various assumptions are negligible relative to the required accuracy.

MCA Evaluation

The concentrations of an unknown mixture of the calibrated components can be predicted by solving Equation 63 on page 53, but now with known coefficients and unknown concentrations.

$$F_{unk} = HC_{unk} + \varepsilon_{unk} \quad \textbf{(69)}$$

where

F_{unk} is an n by 1 matrix of the function results of the sample

C_{unk} is an m by 1 matrix of the component concentrations of the sample

ε_{unk} is an n by 1 matrix of the statistical errors

Least Squares

The least squares solution of Equation 69 is given by Equation 70:

$$C_{unk} = (H^T H)^{-1} H^T F_{unk} \quad \textbf{(70)}$$

Maximum Likelihood

In the case of the maximum likelihood calculation, a weighting matrix, Ω, is calculated from the standard deviations of the calibration coefficients, $s_{H_{ji}}$, and the standard deviations of the function results of the sample, $s_{F_{unk_j}}$.

$$\Omega_{jj} = s^2_{F_{unk_j}} + \sum_{i=1}^{m} s^2_{H_{ji}} C^2_{unk_i} \quad \textbf{(71)}$$

where

C_{unk_i} is the least squares estimate of the unknown sample concentrations

The final maximum likelihood estimates of the unknown concentrations are given by the weighted least squares method, with Ω as the weighting matrix:

$$C_{unk} = (H^T \Omega^{-1} H)^{-1} H^T \Omega^{-1} F_{unk} \quad \textbf{(72)}$$

MCA Quantification Results

Ind.of Stds

To characterize the calibration and evaluation, the independence of standards (ios) is calculated. For the least squares method, the independence of standards is calculated using Equation 73:

$$ios = \frac{trace(\dot{H^T}H) \cdot trace((H^TH)^{-1})}{m^2} \quad \textbf{(73)}$$

where

trace() is the sum of the diagonal elements of a square matrix

In the case of maximum likelihood, the independence of standards is calculated using Equation 74:

$$ios = \frac{trace(H^T\dot{\Omega^{-1}}H) \cdot trace((H^T\Omega^{-1}H)^{-1})}{m^2} \quad \textbf{(74)}$$

Function Result Residual

To verify the quality of the analysis, the function result residual is calculated from the concentration results, C_{unk}, the calibration coefficient matrix, H, and the sample function results, F_{unk}:

$$e_{unk} = F_{unk} - HC_{unk} \quad \textbf{(75)}$$

Std.Dev.Residual

In the case of the least squares method, the standard deviation of residual, s_{res}, is given by Equation 76:

$$s_{res} = \sqrt{\frac{1}{n-m} e_{unk}^{T} e_{unk}} = \sqrt{\frac{1}{n-m} \sum_{j=1}^{n} e_{unk_j}^{2}} \quad \textbf{(76)}$$

In the case of maximum likelihood, the standard deviation of residual is given by Equation 77:

$$s_{res} = \sqrt{\frac{n}{n-m} \cdot \frac{\sum_{j=1}^{n} \frac{1}{\Omega_{jj}} e_{unk_j}}{\sum_{j=1}^{n} \frac{1}{\Omega_{jj}}}} \quad \textbf{(77)}$$

Rel.Fit Error

The relative fit error, s_{rf}, is calculated for the method of maximum likelihood by Equation 78:

$$s_{rf} = \sqrt{\frac{1}{n-m} e_{unk}^{T} \Omega^{-1} e_{unk}} = \sqrt{\frac{1}{n-m} \sum_{j=1}^{n} \frac{1}{\Omega_{jj}} e_{unk_j}^{2}} \quad \textbf{(78)}$$

Standard Deviations of Concentration

The standard deviations of the resulting concentrations are calculated using Equation 79 in the case of the least squares calculation and Equation 80 for the method of maximum likelihood.

$$s_{C_{unk_i}} = \sqrt{s_{res}^2 (H^T H)_{ii}^{-1}} \qquad i = 1, \ldots, m \tag{79}$$

$$s_{C_{unk_i}} = \sqrt{s_{rf}^2 (H^T \Omega^{-1} H)_{ii}^{-1} + (C\phi^{-1} C^T)_{ii}^{-1} C_{unk_i}^2} \tag{80}$$

In Equation 80,

C is the calibration concentration matrix

f is the average of all weighting matrixes ϕ_j of Equation 66 on page 54.

4 Evaluation

Dissolution Calculations

The dissolution is calculated from the results of the evaluation according to the following equations:

Based on Label Weight

% dissolved or released = *D*

$$D = \frac{Cm_{(x)} \times Vm}{Wf} \times 100 \times F \quad \textbf{(81)}$$

where

$Cm_{(x)}$	is measured concentration of active ingredient in vessel
x	is vessel number
Vm	is volume of medium in dissolution vessel
Wf	is target weight of active ingredient in tablet
F	is factor (a user-defined factor, for example, potency or dilution)

Weight dissolved or released per tablet = *Wdt*

$$Wdt = Cm_{(x)} \times Vm \times F \quad \textbf{(82)}$$

where

$Cm_{(x)}$	is measured concentration of active ingredient in vessel
x	is vessel number
Vm	is volume of medium in dissolution vessel
F	is factor (a user-defined factor, for example, potency or dilution)

Weight dissolved or released per label weight of tablet = *Wdw*

$$Wdw = \frac{Cm_{(x)} \times Vm}{Wl} \times F \quad \textbf{(83)}$$

where

$Cm_{(x)}$	is measured concentration of active ingredient in vessel
x	is vessel number
Vm	is volume of medium in dissolution vessel
Wl	is label weight of tablet
F	is factor (a user-defined factor, for example, potency or dilution)

Based on Tablet Weight

% dissolved or released = *D*

$$D = \frac{Cm_{(x)} \times Vm}{Wf} \times 100 \times F \times \frac{Wl}{Wt_{(x)}} \quad \textbf{(84)}$$

where

$Cm_{(x)}$	is measured concentration of active ingredient in vessel
x	is vessel number
Vm	is volume of medium in dissolution vessel
Wf	is target weight of active ingredient in tablet
F	is factor (a user-defined factor, e.g. potency or dilution)
Wl	is label weight of tablet
$Wt_{(x)}$	is actual weight of tablet

Weight dissolved or released per tablet = *Wdt*

$$Wdt = Cm_{(x)} \times Vm \times F \times \frac{Wl}{Wt_{(x)}} \quad \textbf{(85)}$$

where

$Cm_{(x)}$	is measured concentration of active ingredient in vessel
x	is vessel number
Vm	is volume of medium in dissolution vessel
F	is factor (a user-defined factor, e.g. potency or dilution)
Wl	is label weight of tablet
$Wt_{(x)}$	is actual weight of tablet

Weight dissolved or released per label weight of tablet = *Wdw*

$$Wdw = \frac{Cm_{(x)} \times Vm}{Wl} \times F \times \frac{Wl}{Wt_{(x)}} \quad \textbf{(86)}$$

where

$Cm_{(x)}$	is measured concentration of active ingredient in vessel
x	is vessel number (in multibath dissolution testing)
Vm	is volume of medium in dissolution vessel
Wl	is label weight of tablet
F	is factor (a user-defined factor, for example, calibration or dilution)
$Wt_{(x)}$	is actual weight of tablet

Volume Correction

Some sampling configurations do not return the sample to the dissolution vessel; the volume remaining in the vessel is reduced after each sample is taken. Sometimes the volume in the dissolution vessel is kept constant by adding more dissolution medium. Normally, the volume change of each sample is small (for example, less than 5 ml) and when only a few samples are taken, the volume change is not important. However, when many samples are taken, the volume change can produce errors of several percent. In addition, the volume can be changed during a long dissolution run due to evaporation of the medium. The volume correction is a calculation that corrects for the changes in the volume.

Sample volume is removed from the vessel, but the volume of dissolution medium in the vessel is kept constant, resulting in dilution.

The weight calculation for each component is:

$$m_i = c_i V_i + \sum_{j=1}^{i-1} c_j V_s \quad \textbf{(87)}$$

$$V_i = V - V_s(i-1) - V_e + V_a(i-1) \quad \textbf{(88)}$$

where

m_i is corrected component weight for the i^{th} measurement

i is total number of measurements, including the current one

c_i is concentration result (weight/volume) for the i^{th} measurement

V is initial volume of dissolution medium

V_s is volume of dissolution medium removed for each measurement

V_i is actual volume of dissolution medium for the i^{th} measurement

V_e is volume of dissolution medium lost by evaporation

V_a is volume of dissolution medium added for each measurement

5 Combined Reports

The Combined Reports mode contains facilities for the evaluation and reporting of the three-stage dissolution test defined in USP 23. Three types of dosage forms are accommodated: Immediate Release, Extended Release and Delayed Release.

Usage of Dissolution Test Results

The standard dissolution tests specified in USP 23 stipulate a three-stage test with six units in each of the first two stages and twelve units in the final stage, a total of 24 units. The acceptance criteria are based on the cumulative results at each stage:

Stage 1 uses the results of the six units from Stage 1

Stage 2 uses 12 results: six from Stage 1 and six from Stage 2

Stage 3 uses 24 results: six each from Stages 1 and 2, and 12 from Stage 3

The Combined Report is designed to use dissolution test results for each stage as specified in USP 23, and has locations for results from 24 vessels, six locations in each of stages 1 and 2 and 12 locations in stage 3. As the dissolution results from the first test stage are loaded, they occupy the locations in stage 1. If there are less than six vessels in the test, the remaining locations are left empty to be filled up with results from stage 2. If there are more than six vessels in the test, the additional results occupy the first locations in stage 2. The remaining locations are filled with results from the next test stage until all locations are filled.

NOTE Acceptance results from a test stage can be calculated and evaluated only when all locations in that stage are filled.

Evaluation of Results

The evaluation of the test results is based on the standard acceptance criteria according to USP 23. The acceptance criteria are dependent on the dosage form of the product (see "Acceptance Tables According to USP 23" on page 71). For the evaluation of results, the QC Limits table in the Evaluation Parameters of the dissolution method must be completed with the requisite number of values. Depending on the dosage form of the product, one or more of the following values may be required:

Q-Value A required percentage of product or component dissolved at the specified time.

Minimum The minimum acceptable result for the product or component at the specified time.

Maximum The maximum acceptable result for the product or component at the specified time.

The required number of entries in the QC Limits table necessary for the evaluation of dissolution results according to USP 23 for each dosage form are given in Table 6. Empty cells denote that the value is not used in the evaluation.

Table 6 Values Necessary for Combined Report According to USP 23

Dosage Form		Q-Value	Minimum	Maximum
Immediate Release		At least one		
Extended Release			At least one	At least one
Delayed Release				
	Acid Stage			One only
	Buffer Stage	At least one		

If the number of values in the QC limits table is greater or less than required for the dosage form, the evaluation result is treated as in Table 7.

Table 7 Evaluation Rules for Combined Report

	Evaluation
Immediate Release	
No Q-value	No evaluation; the reason is shown in all reports.
One or more Q-values	Evaluate and report for each Q-value.
Extended Release	
No limits	No evaluation; the reason is shown in all reports.
One or more minimum, maximum or pair of limits	Evaluate and report for each limit.
Delayed Release (acid stage)	
No maximum	No evaluation; the reason is shown in all reports.
One maximum	Evaluate and report.
More than one maximum	Request that the user selects a value; evaluate and report using the selected value
Delayed Release (buffer stage)	
No Q-value	No evaluation; the reason is shown in all reports.
One or more Q-values	Evaluate and report for each Q-value.

Acceptance Tables According to USP 23

The following tables give the details of the acceptance criteria used in USP 23 for each dosage form and at each stage of the test.

Table 8 Immediate Release Acceptance Criteria

Stage	Number Tested	Acceptance Criteria
S_1	6	Each unit is not less than Q + 5%
S_2	6	Average of 12 units (S_1 + S_2) is equal to or greater than Q, and no unit is less than Q - 15%
S_3	12	Average of 24 units (S_1 + S_2 + S_3) is equal to or greater than Q, not more than 2 units are less than Q - 15% and no unit is less than Q - 25%

Table 9 Extended Release Acceptance Criteria

Stage	Number Tested	Acceptance Criteria
L_1	6	No individual value lies outside each of the stated ranges, and no individual value is less than the stated amount at the final test time.
L_2	6	The average value of 12 units (L_1 + L_2) lies within each of the stated ranges and is not less than the stated amount at the final test time; none is more than 10% of labeled content outside each of the stated ranges; and none is more than 10% of labeled content below the stated amount at final test time.
L_3	12	The average value of 24 units (L_1 + L_2+ L_3) lies within each of the stated ranges and is not less than the stated amount at the final test time; not more than 2 of the 24 units are more than 10% of labeled content outside each of the stated ranges; not more than 2 of the 24 units are more than 10% of labeled content below the stated amount at final test time, and none of the units is more than 20% of labeled content outside each of the stated ranges or more than 20% of labeled content below the stated amount at final test time.

Table 10 Delayed Release (Acid Stage) Acceptance Criteria

Stage	Number Tested	Acceptance Criteria
A_1	6	No individual unit exceeds 10% dissolved
A_2	6	Average of 12 units ($A_1 + A_2$) is not more than 10% dissolved, and no individual unit is greater than 25% dissolved
A_3	12	Average of 24 units ($A_1 + A_2 + A_3$) is not more than 10% dissolved, and no individual unit is greater than 25% dissolved

Table 11 Delayed Release (Buffer Stage) Acceptance Criteria

Stage	Number Tested	Acceptance Criteria
B_1	6	Each unit is not less than Q + 5%
B_2	6	Average of 12 units ($B_1 + B_2$) is equal to or greater than Q, and no unit is less than Q - 15%
B_3	12	Average of 24 units ($B_1 + B_2 + B_3$) is equal to or greater than Q, not more than 2 units are less than Q - 15%, and no unit is less than Q - 25%

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In This Book

This handbook contains full details of the operation of the dissolution testing software for the Agilent ChemStation for UV-visible spectroscopy in both single and multibath modes. It describes the calculations used in the evaluation of the data and for making corrections for lost volume and sample dilution. the manual is designed to enable you to follow good laboratory practice (GLP) guidelines. Using the information in the manual, you will be able to understand the data processing calculations from beginning to end and perform the data evaluation and corrections manually.

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