



Specifications

November 2003

Agilent ChemStation for UV-visible Spectroscopy Systems—Rev. A.10.0x

The Agilent ChemStation for UV-visible spectroscopy provides instrument control, data acquisition, and data evaluation for the Agilent 8453 and HP 8452 (general purpose, advanced and biochemical analysis ChemStation only) diode-array UV-visible spectrophotometers. The ChemStation comprises a Compaq Pentium-based personal computer and

software that runs under the Microsoft® Windows™ operating environment. The software is compatible with Microsoft Windows 2000 and Windows XP. The software is coexecutable with other applications written for the Microsoft Windows operating environment.

Five software modules are available:

- General Purpose Software,
- Advanced Software,
- Biochemical Analysis Software,
- Dissolution Testing UV-visible ChemStation Software (supports Agilent 8453 only), and
- Security Pack for the UV-visible ChemStation.



Agilent Technologies

UV-visible ChemStation Software Overview

General Purpose UV-visible ChemStation Software (G1115AA)

The general purpose software is the core software with instrument control, data acquisition, and a mode for standard data evaluation capabilities. A separate mode allows diagnostic and verification of the HP 8452 and Agilent 8453 spectrophotometers. It is especially designed for ease of use in the routine laboratory or for the occasional user.

It allows the user to

- measure absorbance at up to six wavelengths simultaneously,
- measure spectra and automatically find peaks and/or valleys,
- use a user-definable equation for evaluation using up to six wavelengths, weight, and volume (for example, ratio at two wavelengths),

- do single component quantification, and
- automate the measurement of standards, controls, and samples.

Advanced UV-visible ChemStation Software (G1116AA)

The advanced ChemStation software adds extended spectral processing, advanced single and multi component quantification capabilities to the general purpose ChemStation software.

It allows the user to

- do extensive interactive mathematical operations with spectra,

- compare and compose spectra,
- define up to four equations simultaneously,
- do up to four data analyses or one main and up to three confirmation analyses in parallel,
- do multicomponent analysis with calibration based on pure or mixed standards,
- customize the software with

- macro programming,
- optimize the parameters for quantitative analysis with specific algorithms,
- configure reports, and
- set up a complex sequence of automated analyses.

Biochemical Analysis UV-visible ChemStation Software (G1117AA)

The biochemical analysis ChemStation software adds single cell kinetics, multicell kinetics and thermal denaturation (DNA melt) capabilities to the general purpose software.

Kinetics allows the user to

- measure time traces at up to six wavelengths on one cell,
- measure time traces on up to

seven cells in parallel (multicell option),

- correct for background absorbance,
- evaluate the reaction rate, and
- do simple mathematical operations on time traces.

Thermal denaturation allows the user to

- measure temperature traces with user-definable ramps (mul-

- tiple up and down slopes),
- determine transition temperatures (T_m) by first derivative or average absorbance methods,
- calculate a result by an equation based on T_m (for example, % GC), and
- correct for thermal expansion and molarity of the solvent.

Dissolution Testing UV-visible ChemStation Software (G1118AA)

The dissolution testing ChemStation software adds single- and multi-bath dissolution testing capabilities to the general purpose software. It controls only the Agilent 8453 spectrophotometer.

It allows the user to

- measure absorbance on up to 24 vessels offline,
- or measure absorbance on up to six vessels, one blank and one control with an automated online sampling system including time control of the sampling,
- evaluate dissolution profiles,
- control/monitor dissolution baths (bath driver required),
- set up complex pre- and post-run sequences including opera-

tor guidance,

- use the data analysis features of the advanced ChemStation software,
- correct results for volume changes,
- re-evaluate saved data,
- use four independent methods in case of multi-bath dissolution testing,
- configure customized reports, and
- print combined reports on up to four dissolution runs (4 x 6 samples), including statistics according to the acceptance tables.

Security Pack for the UV-visible ChemStation Software (G1813AA)

The security pack modifies the advanced and dissolution testing mode to support the requirements for electronic records and signatures (21 CFR part 11).

It modifies the ChemStation to

- provide access control including user setup and password administration
 - prevent loss of raw and meta data as well as their unauthorized modification
- adds versioning on the ChemStation level to store all versions of a method to a new file,
 - follow the detailed requirements for electronic records and passwords given by the FDA 21 CFR, Part 11, and
 - allows to use existing global groups and accounts for access control and user management.

Computer

The ChemStation software requires an IBM-compatible personal computer with the following minimum specifications:

- An Intel Pentium™ III processor, 600 MHz.
- At least 128 MB RAM.
- A CD-ROM disk drive.
- A hard disk with a free capacity of at least 4 GB.
- A Microsoft Windows compatible pointing device.
- A super VGA or ultra VGA 15 inch color monitor and inter-

face capable of at least 800×600 pixel resolution.

- 256 colors and 1 MB Video RAM, small fonts are recommended.
- A LAN interface or an appropriate GP-IB interface.

The ChemStation software has been tested on Compaq Pentium-based personal computers that conform to the above specifications. Although the software is designed to be compatible with

other IBM-AT compatible hardware, Agilent Technologies will not necessarily accept responsibility for defects reported on such hardware.

Printers

The ChemStation software operates with any Microsoft Windows compatible printer directly connected to the computer through a Centronics or serial interface or a Microsoft Windows compatible printer connected through a Local Area Network (LAN). The recommended printers are the HP DeskJet printer family (for lower-volume applications) or the HP LaserJet printer family. The color

print capability of the DeskJet printers is supported on the appropriate models. Dot matrix printers, plotters and color printers should not be used if printing performance is a system requirement. Printer performance enhancement hardware should operate correctly with the system but is neither supported nor recommended.

Operating System

The ChemStation for requires Microsoft Windows 2000 or Windows XP.

Common ChemStation Software Features

System Configuration

The instrument system is configured with the configuration editor program for instruments and controlled through GPIB (HP 8452 or Agilent 8453 spectrophotometer and Agilent 89090A Peltier tem-

perature controller) or LAN (Agilent 8453). It allows the user to define the connected instruments, the GP-IB addresses, the directories for data, methods and automation files, and the color

definitions for the ChemStation. Other accessories, which are controlled through the GP-IO interface of the HP 8452 or Agilent 8453, are configured from within the ChemStation software.

Analytical Instrumentation

The ChemStation software controls and acquires data from all versions of the HP 8452 (not valid for G1118AA) or Agilent 8453 spectrophotometers and the Agilent 89090A Peltier temperature controller. All control and data acquisition for these instru-

ments is performed through the GP-IB interface system (IEEE-488) or LAN (8453 only). The 89068C/D sipper/sampler accessories are controlled through the general purpose GP-IO interface of the HP 8452 or Agilent 8453.

The Agilent 89075C/D and G1120A multicell transport accessories use a dedicated interface on the HP 8452 or Agilent 8453. The XY Autosampler and the Gilson models 221 and 222 Autosampler are controlled through the RS-232C interface of the PC.

Data Analysis - Graphic

Data is displayed in graphic windows together with information about the samples in table windows. A combination of windows is called a view. In a view the

windows are linked so that a change in one window automatically updates the other windows. 'Zoom', 'cursor', and 'tabulate data' functions are all available through

point and mouse click. The Microsoft Windows features of 'cut/copy' and 'paste' can be used to move data within the application or transfer data to other applications.

Data Analysis - Reporting

A variety of reporting options are provided from simple copies of windows to a fully-detailed GLP-compatible report.

Printing Windows – any graphic or tabular window can be selected and printed. The hard copy includes a header with date, time, and number/title of the window printed.

Reports – All reports include a header on each page which identifies the report by time, date page number, and a single line for a user-defined constant text. A print preview is available for all reports. The report device and output format for a file may also be specified. When a file is specified as the

destination, the software supports ASCII and Windows metafile (WMF) output formats. The ASCII files may be used by word-processing software and can be parsed into spreadsheet software. The WMF format is useful for transferring graphic information into word-processing software.

Methods

The analytical method, which is stored in a single file on disk, fully describes how a particular

analysis is performed. It contains all the parameters for sampling system, instrument control, task

selection, data acquisition, and data analysis.

Instrument Control and Data Acquisition

The integration times of the HP 8452 or Agilent 8453 spectrophotometers can be varied from 0.1 to 25.5 seconds. Full or partial spectra can be acquired as single measurements. Full diagnostics and verification of the HP 8452 or Agilent 8453 can be performed. All features of the Agilent 89090A Peltier temperature controller can be set from the software. They include 'set temperature', 'stirrer on/off', stirring speed, and temperature units (C,F,K). Data that is read from the Peltier include actual temperature and external sensor temperature.

If a Peltier is configured the temperature information is automatically appended to each measured spectrum. The status of all instruments is continually monitored and can be displayed in status windows. On acquisition, spectra are automatically labeled with all information that is directly available to the system, that is, data, time, operator name, HP 8452 instrument number, or Agilent 8453 serial number, cell path length, and temperature if the Agilent 89090A Peltier is being used. Additional information such as sample name, comment and

sample properties such as component names or concentrations may be entered by the user as required. All this information is automatically stored with the spectra when they are saved to disk.

	G1115AA General Purpose	G11166AA Advanced	G1117AA Kinetic	G1117AA Thermal Denaturation	G1118AA Dissolution Testing
Supported spectrophotometer	HP 8452A, Agilent 8453	HP 8452A, Agilent 8453	HP 8452A, Agilent 8453	HP 8452A, Agilent 8453	Agilent 8453
Type of data acquired	Absorbance mode with or without variance	Absorbance mode with or without variance	Absorbance mode without variance	Absorbance mode without variance	Absorbance mode with variance
Agilent 89090A Peltier temperature controller	Supported requires GP-IB	Supported requires GP-IB	Supported for single cell kinetic, requires GP-IB	Supported requires GP-IB	Not supported
Agilent 89068C/D sipper	Supported	Supported	Not supported	Not supported	Supported
Agilent 89072A autosampler	Supported	Supported	Not supported	Not supported	Supported
Agilent 89079A valves	Not supported	Not supported	Not supported	Not supported	Supported
Agilent 89075C/D multicell transport	Supported	Supported	Supported	Not supported	Supported
Agilent G1120A multicell transport	Supported	Supported	Supported	Not supported	Supported
Agilent XY autosampler	Supported	Supported	Not supported	Not supported	Supported
Gilson 221/222	Supported	Supported	Not supported	Not supported	Supported

Table 1

Matrix of supported Agilent ChemStation for UV-visible spectroscopy modules and accessories

Good Laboratory Practice

The ChemStation has a number of features designed to help users validate their spectrophotometer and the ChemStation itself.

System and method validation features include

- A *Declaration of Validation* that is shipped with each ChemStation. It documents the software development and testing steps executed as part of the development cycle. The development process is available for regulatory agencies to review at Agilent Technologies, Waldbronn site.
- A *Verification Procedure* for the HP 8452 and Agilent 8453 built in the ChemStation software. This guides the user through a verification procedure equivalent to that performed at the final test in the factory to verify that the instrument is performing within specifications. In addition, the verification test can be configured by the user by selection from a list. It includes reporting and

disk storage of verification data.

- A *Software Validation Kit* for revalidation of the ChemStation by the user. It is designed to check for the correct installation and operation of the data analysis and reporting parts of the software by comparing results generated when the test is executed against pre-recorded known values.
- The *Understanding Your ChemStation* manual documents the steps and algorithms used by the ChemStation during data analysis.

Features to ensure data quality and data integrity include

- Global methods. The complete instrument and data analysis specification is stored in one place. Methods can be protected by a password.
- Two levels of operation. An operator level allows methods to only be recalled, if the operator specific password is given, and used - they may not be

saved again. At the manager level, which is accessible with a password, methods can be created, edited and saved.

- Each spectrum is stamped with date and time of measurement, the operator name, and the identity of the HP 8452 or the serial number of the Agilent 8453 spectrophotometer on which the measurement was made.
- Data files are stored in binary format which is not editable outside the ChemStation and essential data such as the raw spectral data is not editable within the ChemStation.
- All reports have date and time stamps and traceable page numbering (page x of y pagination style).
- Data security in the PC environment can be achieved through password protected PCs, software locks built into Microsoft Windows and secure (password-protected) networks.

Networking

The ChemStation is compatible with Microsoft 2000 and Windows XP.

The offline version of the ChemStation is ideally suited to work on a networked PC enabling a user in an office to reprocess

data at leisure without impacting the efficiency of the laboratories' instrumentation.

Data Archiving

XML companion files can be automatically generated to support the information transfer to a NuGenesis database. This way,

archiving can be done completely automatically.

General Purpose UV-visible ChemStation Software (G1115AA)

Software Design

The general purpose ChemStation software is designed around a task model. Internally, the general purpose ChemStation software uses the data model of the advanced UV-visible ChemStation software, which is based on a structure called registers. These are multipurpose memory structures that can hold any type of analytical data and information. The selection of a task controls

the user's view on the internal data structure and user's access to the parameters relevant for the task specific data processing.

The software can be operated either by the menu, toolbar or the pictorial user interface. The pictorial user interface includes a pictorial representation of the instrument and sampling system with point-and-click operation.

The general purpose ChemStation software allows the user to use the following data types:

- absorbance,
- transmittance, and
- first to fourth derivative.

An *Execute Advanced Method* mode allows the execution of any method developed using the advanced ChemStation.

Data Analysis - Spectral Processing

Spectra may be interactively processed by the user with the following functions:

Absorbance – converts a transmittance spectrum to absorbance.

Transmittance – converts an absorbance spectrum to transmittance.

Scalar Add – adds a user-specified constant value (0.00001-999999) to the selected spectrum.

Add – adds two selected spectra together.

Subtract – subtracts the second selected spectrum from the first selected spectrum.

Scalar Multiply – multiplies a spectrum by a user specified constant (0.00001-999999).

Derivative – calculates the derivative of the selected spectrum using the Savitsky-Golay coefficients. The user can select the order of derivative (1-9), the polynomial degree (1-7) and the number of points (3-99) used.

Spline – performs a curve fit to the data points of the selected spectrum and interpolates points between the measured points. The number of interpolated points is selected by the user (1-99).

Internal Reference – performs a constant background correction on the selected spectrum. The user defines the wavelength or the wavelength range to be used to determine the level of the correction.

Except where the function requires a specific number of spectra, single or any number of selected spectra can be processed simultaneously.

Multiple data processing steps can be performed interactively on any spectra.

Data Analysis - Fixed Wavelength(s)

Data at up to six wavelengths can be extracted from the spectrum.

Background Correction – allows the user to set up a background correction function. A single reference wavelength, an average of a wavelength range or three-point drop-line can be speci-

fied in the selection box. If three-point drop-line is selected, a linear reference is used. The absorbance values at the specified wavelengths and activated background correction are displayed as raw and background corrected values in a results window.

Data Analysis - Spectrum/Peaks

Peaks/Valleys – finds up to the specified number of peaks and/or valleys in the spectrum. The last measured spectrum is annotated with the peaks and valleys. The highest peaks of all spectra are

displayed in a results window. Peaks and valleys of a selected spectrum can be displayed in separate peak - and valley windows sorted in order of their amplitude.

Data Analysis - Ratio/Equation

Equation – allows the user to enter an equation for the evaluation of data.

The variables which have been entered for the samples are:

- up to six specified wavelength values, and
- weight and volume.

Mathematical functions include +, -, ×, /, log, ln, exp, sqr, sqrt.

Results are displayed in a window with the values at the specified wavelengths and the equation results with user-entered column name and units.

The results are automatically corrected for the pathlength of the cell and the dilution factor. A post-sample measurement prompt for the sample name, weight, volume, and dilution factor can be activated.

Data Analysis - Quantification

Calibration Standards – a virtually unlimited number of standards can be used for the calibration. The only limit is the memory of the computer.

Concentration of Standards – can be entered directly by the user or calculated from the given weight, volume, and purity from the software.

Calibration curves – a choice of four calibration curves is available:

- linear, forced zero (Beer-Lambert's law),
- linear,
- second order, forced zero, and
- second order.

Calculation Methods – the fit of the calibration curves to the standard is done using a least squares method.

Calibration Diagnostics –

Graphic tools are:

- a plot of the processed spectra from which the calibration data has been extracted, and
- a plot of each data point and the fitted calibration curve.

Calibration curve statistics are:

- the standard deviation for each calibration coefficient,
- the standard deviation of calibration, and
- the correlation coefficient.

Statistics for each standard are:

- the % error (the residual expressed in %).

The analysis results are automatically corrected for the pathlength of the cell and the dilution factor.

A post-sample measurement prompt for the sample name, solvent, dilution factor, and comment can be activated.

A post-standard measurement prompt for the sample name, solvent, analyte concentration, and comment can be activated.

Automation

Automation comprises an automation table with up to three standards (only for quantification), two controls and a user-defined number of samples (only limited by the sampling device). For the controls, a maximum error can be specified.

The automatically generated result report can include statistic on the samples (average, standard deviation, % RSD, minimum and maximum value). Automation normally, but not necessarily, involves automated sample introduction as well as measurement and evaluation.

The following sample handling devices can be controlled:

- manual (system prompts user for the samples),
- Agilent 89068C/D sipper (system prompts user for the samples),
- Agilent 89072A autosampler,
- Agilent XY autosampler (G1811A),
- Agilent 89075C/D multicell transport,
- Agilent G1120A multicell transport, and
- Gilson 221 and 222 autosamplers

Verification and Diagnostics

The general purpose ChemStation software has a *Verification and Diagnostic* mode to allow the user to verify and diagnose the correct operation of the HP 8452 or Agilent 8453 spectrophotometer.

For the HP 8452, a *Self-Test* checks

- spectrophotometer electronic components.

For the HP 8452, the

Performance Verification checks

- photometric accuracy,
- wavelength accuracy,
- stray light,
- noise,
- baseline flatness, and
- drift (1 hour, optional).

For the Agilent 8453, a *Self-Test* checks

- spectrophotometer electronic components,
- wavelength calibration,
- dark current,
- lamp intensity,
- noise test (1 min), and
- baseline flatness.

For the Agilent 8453 the *Performance Verification* procedure is configurable by the user and checks

- wavelength accuracy,
- wavelength reproducibility,
- photometric accuracy,
- drift (1 hour),
- stray light,
- noise,
- baseline flatness, and
- resolution.

Diagnostics allows the user to:

- run an instrument self-test,
- check the lamp intensities,
- check the lamp stability,
- check the dark current, and
- check the pump time for a sipper/autosampler system with a flow test.

Disk Files

ChemStation data is stored on disk in binary files with the suffix .SD for samples and .STD for standards. These files are in fact copies of the register structure where the spectral data is held. They contain one or more spectra and all information about the spectra and samples that is available. A file browser lets you view the number of spectra in the selected file and the sample name, date, and time of the first spectrum.

In addition to its own file format the ChemStation can import and

export data in a variety of other file formats.

Import and Export formats are:

- *.WAV files—an ASCII text file format which is used by Agilent 89530A, Agilent 89531A, Agilent 89532A and Agilent 89532Q MS-DOS UV-visible softwares as well as Agilent 89550A and 89551A dissolution testing softwares.
- *.DX files—a JCAMP DX file format which is a general file standard for transferring spectral data between instruments from different manufacturers.

The ChemStation can also import the following:

- *.WIN files which are spectral data extracted from chromatographic data acquired with a diode-array detector by the G2170AA, G2171AA and G2180AA HPLC 3D softwares.
- *.UVL files which are generated by the spectral library feature of the Agilent 3D softwares, mentioned above.

The ChemStation can also export the following

- *.DIF and *.CSV files which ASCII text file formats that can be imported by other applications.

Documentation

The documentation set comprises three manuals and two software components:

- Installation of the system is described in the *Installing Your Agilent 8453 UV-Visible Spectroscopy System* manual.
- Theory of data handling, maintenance, repair, diagnostic, and troubleshooting is described in the *Reference Handbook*.

- Explanations of the ChemStation principles and documentation of all the data analysis algorithms are provided in the *Understanding Your Agilent 8453 UV-Visible Spectroscopy System* manual.
- The software has comprehensive, Windows-style, context-sensitive Online Help.

- *Standard Operating Procedures* for installation, maintenance, and main operation are provided as a Microsoft Write file.

Advanced ChemStation Software (G1116AA)

General

The advanced software adds supplement evaluation capability, automation, and customization to the general purpose UV-visible software. All data analysis fea-

tures of the general purpose software specified above are available.

Software Design

The advanced software is designed around a data model based on a structure called a register. The advanced ChemStation software provides commands and functions to construct, expand, extract and, edit registers if it does not alter primary data. The software uses registers in a structured way to logically define the spectroscopic data analysis

process. Measured spectra are first put in a raw data register. Spectra from this register can undergo one or more spectral processing steps and the results are placed in the processed spectra register. From this register data at specific wavelengths, combinations of wavelengths or wavelength ranges can be extracted and the values are placed into a

used wavelength register. This data can then be evaluated using a user-entered or quantification technique. The results of the evaluation are placed in the results register.

Instrument Control and Data Acquisition

Full spectra or partial can be acquired as single measurements or time-based. The time-based spectra acquisition is limited to a

single cell and equal time intervals. No time traces can be evaluated.

Data Analysis - Spectral Processing

Spectra may be interactively processed by the user with the following function, in addition to those of the general purpose software:

Multiply – multiplies two selected spectra by each other.

Divide – divides the first selected spectrum by the second selected spectrum.

Logarithm – calculates the logarithm (ln) of the selected spectrum.

Exponential – calculates the exponential of the selected spectrum.

Reciprocal – calculates the reciprocal of the selected spectrum.

Smooth – smoothes the selected spectrum using the Savitsky-Golay

coefficients. The user can select the polynomial degree (1-7) and the number of filter points (3-99).

Internal Reference – performs a constant background correction on the selected spectrum. The user defines the wavelength or the wavelength range to be used to determine the level of correction.

Correct Scatter – performs a background correction on the selected spectrum using a mathematical model for scattering:

$$A = a\lambda^n$$

where a is a constant and n is the order of scattering. The user defines the wavelength range for the fit to determine a and n .

Sum Up – adds two or more selected spectra together.

Peaks/Valleys – finds the peaks and valleys in the selected spectrum and displays a peaks window, a valleys window and a spectral window with the peaks and valleys annotated. Peaks and valleys are listed in order of amplitude. The user can enter the threshold level and the number of points required to detect a peak/valley. Except where the function requires a specific number of spectra, single or any number of selected spectra can be processed simultaneously. Multiple data processing steps can be performed interactively on any spectra or can be programmed, using a simple table, to be done automatically.

Match factor - compares two spectra selected by performing a linear regression on the absorbances at each wavelength of the two spectra.

Data Analysis - Spectra Evaluation

Compare (Regression) – compares two spectra selected by performing a linear regression on the absorbances at each wavelength of the two spectra. It generates a match factor, a plot of the absorbances of one spectra against the other, and a residual spectrum. The user can specify the threshold amplitude value for the comparison and a wavelength

shift for comparing spectra from different spectrophotometers.

Compare (Normalization) – compares two spectra by internally referencing them at a user selected baseline point, normalizing them at a user selected wavelength, and subtracting the normalized spectra to show the difference spectrum. It generates a

match factor and a plot of the residual spectrum.

Compose – allows the user to combine several selected spectra together to generate synthetic mixtures. The amount of each component is selected by the user.

Data Analysis - Equation

Equation – allows the user to enter up to four equations for the evaluation of data. The variables are the specified wavelength values and any information (for example, dilution, weight) about

the samples that has been entered. The results can be corrected by the path length of the cell and/or a dilution factor.

A post-sample measurement prompt for the sample name, solvent, analyte concentrations, dilution factor, and comment can be activated.

Data Analysis - Used Wavelengths

Data from single and multiple wavelengths can be extracted from spectra after any spectral processing steps using the following functions:

Single – extracts the value at a single, user-specified wavelength.

Range – extracts the average value over a user-specified wavelength range.

List – extracts the values at any number of user-specified wavelengths. In addition the average of all the values is calculated.

Analytical Function – allows the user to set up a complex multi-wavelength function. The user can specify the average of any combination of single wavelengths and/or wavelength ranges as the analytical value plus one or two reference wavelengths or wavelength ranges. If one wavelength or range is used, a constant value is used as the reference. If two wavelengths or ranges are selected, a linear reference is used (the so called three-point correction).

The user may choose to subtract, multiply or divide the analytical value by the reference value. The extracted values can be reported as results in their own right or passed on for further evaluation.

Single Component Analysis

Calculation Methods – the fit of the calibration curve to the standards can be done using a least squares method (each point given equal weighting) or by the maximum likelihood method (each point weighted by the standard deviation of the entered calibration standard concentrations and the standard deviations of the measurement).

Calibration Diagnostics – in addition to the calibration diagnostics of the general purpose software there are extensive graphic and statistical diagnostic tools to provide additional help in the optimization of the calibrations.

Graphic tools are:

- confidence intervals of the calibration curve.

Calibration curve statistics are:

- % uncertainty, and
- relative fit error (maximum likelihood calculation only)

Statistics for each standard are:

- residual (difference between the standard value and the calibration curve value),
- 95 % confidence interval,
- leverage,
- studentized residual, and
- Cook's distance

A post-standard measurement prompt for the sample name, solvent, analyte concentrations, and comment can be activated.

Analysis Result Diagnostics – each quantitative result is displayed together with its 95 % prediction interval. The results will also be corrected by the path-length of the cell and a dilution factor. A post-sample measurement prompt for the sample name, solvent, dilution factor, and comment can be activated.

Multicomponent Analysis

Calibration Standards – a virtually unlimited number of standards can be used for the calibration. The only limit is the memory of the computer.

Spectral Data – calculations can be performed by using multiple individual wavelengths (at least one per component), a single continuous wavelength range, or multiple discontinuous wavelength ranges.

Calibration Curve – a linear, forced-zero (Beer-Lambert's law) type of calibration curve is used for each selected wavelength.

Calibration Types – the calibrations may be performed using pure standards or mixtures of standards. Multilevel standards may be used to calculate an average response at different concentrations.

Calculation Methods – the fit of the calibration curves to the standard can be done using a least squares method (each point given equal weighting) or by the maximum likelihood method (each point weighted by the standard deviation of the entered calibration standard concentrations and

the standard deviations of the measurement).

Calibration Diagnostics – after calibration the standards are quantified using the calibration matrix to provide diagnostic data. Extensive graphic and statistical diagnostic tools are provided to aid in the evaluation and optimization of the calibrations.

Graphic tools are

- a plot of the residual spectra (the difference between the measured standard spectrum and the estimated spectrum from the evaluation), and
- a plot of the pure component spectra (when calibrating with pure standards they are the spectra of the standards, when using multilevel standards they are the average spectra and when using mixture standards they are the deconvoluted component spectra).

The calibration statistic is

- the standard deviation of calibration.

Statistics for each standard are

- the standard deviation of the residual,
- the relative fit error (maximum likelihood calculation only),

- the independence of standards, and
- the standard deviation for each component.

A post-standard measurement prompt for the sample name, solvent, analyte, concentrations, dilution factor, and comment can be activated.

Analysis Result Diagnostics – are both graphic and statistical.

The graphic diagnostics are

- an overlay plot of the measured spectrum, the estimated spectrum and the estimated component spectra, and
- a plot of the residual spectra (the difference between the measured and estimated spectra).

Statistical diagnostics are

- the standard deviation of the residual,
- the relative fit error (maximum likelihood calculation only),
- the independence of standards, and
- the standard deviation for each component.

A post-sample measurement prompt for the sample name, solvent, dilution factor, and comment can be activated.

Data Analysis - Extended Analysis

Multiple Analyses – up to four equivalent data analyses can be performed in parallel. A data analysis can comprise spectral processing, wavelength value extraction, and evaluation steps. If multiple data analyses are selected, the results for all analyses for all components are

collected and displayed in a single summary table.

Confirmation Analysis – up to three confirmation analyses can be specified in addition to the main analytical analysis. Confirmation analyses can comprise any spectral processing,

extract wavelength, and evaluation steps. The results from the confirmation analyses are compared with the main analytical results for each component and if the confirmation results deviate by more than a user entered tolerance (expressed in %) the out of tolerance results are flagged in a summary table.

Customizing

The columns of Tabular View of the sample/standard table can be configured by the user.

The advanced ChemStation software can be customized. These commands may be grouped to automatically execute a specific function. Such a group of commands is called a macro. Users writing macros may define their own variables, build in conditional

or looping constructs, perform physical I/O including file handling and user interaction, nest their macros and schedule and exchange data with other Microsoft Windows applications.

As well as using simple scalar or string variables data may be stored as tables. Customizers have

access to all the ChemStation data through standard data structures and may even define their own tables for specialized applications. The user can define menus and dialog boxes and use the Microsoft Windows Dynamic Data Exchange (DDE).

Method Development

Four special utilities are provided to assist the user in developing the best parameters for quantitative analysis.

Evaluate Standards – performs a single component calibration at each wavelength over a user specified wavelength range using user-specified calibration parameters. It determines the correlation coefficient and uncertainty at each wavelength and plots these against wavelength. This helps identify which wavelengths give the best calibration curve for a target analyte.

Compare Calibrations – puts the results from two independent calibrations side by side on the screen for comparison. This is useful for comparing, for example, the influence of different spectral processing techniques or different analytical functions on the quality of the calibration curve.

Optimize Wavelength – calibrates at all wavelengths over a user-specified wavelength range using user-specified calibration parameters and then quantifies a user-selected sample at all specified wavelengths and plots the quantification results against

wavelength. This is useful to determine the wavelength(s) which give the best accuracy, that is, selectivity for the target analyte in the presence of the sample matrix.

Test Method – calculates the average and standard deviation of multiple analyses of an identical sample. This gives a measure of the precision of the method. In addition, the Compose Spectra is a useful tool for quantitative method development.

Automation

Automation is set up by entering an automation table as a sequential list of the actions to be performed.

The following actions are permitted in the automation table:

- load method,
- load samples,
- measure blank,
- measure sample,

- measure control,
- measure auxiliary,
- analyze,
- method report,
- calibration report,
- results report,
- store samples,
- store method,
- clear samples,
- clear auxiliary,
- set temperature,

- user macro,
- load standards,
- measure standard,
- calibrate,
- store standards, and
- clear standards.

Information about the samples and standards being measured can be pre-entered in the sample table.

Data Analysis - Reporting

The advanced software allows users to define reports based on *Reports*, which is configurable by the user from a menu of modules.

The method report is configured by selecting from the following options:

- method information (user-entered text describing the method),
- method checklist,
- instrument and accessory parameters,
- data analysis parameters, and
- results report parameters.

The results report is configured from the following options:

- method report,
- sample information,
- a plot of the sample spectra,
- a plot of the processed sample spectra,
- a plot of the wavelength results,
- the data analysis results,

- statistics on the results,
- confirmation analysis results, and
- summary results table.

A calibration report which is configured by selecting from the following modules:

- a plot of the standard spectra,
- a plot of the processed standard spectra,
- a table of the cell path length used for the measurement of each standard,
- the data analysis parameters (wavelengths, calibration type, calculation type, and so on,)
- the coefficients obtained from the calibration,
- the wavelength results used for the calibration (SCA only),
- the analytes calibration table (concentration of each component in each standard),
- a plot of the calibration curve

(SCA only),

- a plot of the residual spectra (MCA only), and
- the diagnostic statistics for the calibration.

The method report has an additional option to include the calibration report which can then be included as part of a full results report.

In addition to these configurable reports, a customized reporting design is included for users who want to define the content of their own reports.

Documentation

The document set comprises one manual and three software component:

- Explanations of the ChemStation principles and documentation of all the data analysis algorithms are provided in the manual *Understanding Your Advanced Software*.

- The *Online Help* system is extended with information related to the advanced software.
- The *Commands* help file documents the syntax and action of all the commands used by the ChemStation.

- For advanced users the *Macro Programming Guide* contains a description of the ChemStation data structures and variables and a guide to customization with practical examples. This manual is provided as an Adobe™ Acrobat file and can be printed on demand.

Biochemical Analysis UV-visible ChemStation software (G1117AA)

General

The Agilent G1117AA biochemical analysis ChemStation software adds kinetics and thermal denaturation capability to the general purpose ChemStation software.

Kinetic Instrument Control and Data Acquisition

For time-based measurements the run, start, and cycle time can be defined. For non-equidistant time intervals, a percent increase of the cycle time after an initial hold time can be defined. Full or partial spectra can be acquired. Automatic gain adjust can be done separately from the reference measurement for special applications. Measurements can be made at any of the 7-cell (Agilent 89075C/D) or 8-cell (G1120A) multicell transports. In case of multicell kinetics, the positions of the blank and sample cells for time-based measurements are defined by the user. All traces and the spectra of one selected cell can be monitored online in a trace monitor and a spectra monitor.

Kinetic Data Analysis

Time Trace Evaluation

Data from single (multiple cell acquisition) or multiple wavelengths (single cell acquisition only) can be extracted from time-based spectra.

Background correction – allows the user to set up the same background correction functions as in the general purpose ChemStation software.

Time Trace Processing

Time traces may be interactively processed by the user with the following functions:

Add – adds two selected time traces together.

Subtract – subtracts the second selected time trace from the first selected time trace.

Scalar multiply – multiplies a time trace by a user-specified constant (0.00001-999999).

Logarithm – calculates the logarithm (ln) of the selected time trace.

Derivative – calculates the derivative of the selected time trace using the Savitsky-Golay coefficients.

Rate Evaluation

Rate type – a choice of four rate calculation types is available:

- initial rate (quadratic fit),
- zero order (linear fit),
- first order, and
- delta AU.

Kinetic Disk Files

Kinetic time-based data is stored on disk in binary files with the suffix .KD. A file browser lets you view the sample name, method information, date, time, number of used cells, run, start and cycle time as well as the spectral acquisition range. In addition to its

own file format the ChemStation can import and export data in a variety of other file formats.

Import and Export formats are:

- Import of *.MKD files – a binary file format which is used by

Agilent 89532K MS-DOS UV-visible multicell kinetics software.

- Time traces and rate data can be exported as *.CSV and *.DIF files which are common ASCII text file formats.

Thermal Denaturation Instrument Control and Data Acquisition

For temperature-based measurements, a temperature ramp with multiple start temperatures, temperatures step, and end temperatures can be defined with the

Agilent 89090A Peltier temperature controller. The temperature can be taken from the cell holder or the optional external probe. Full or partial spectra can be

acquired. The temperature trace can be monitored online.

Thermal Denaturation Data Analysis

Temperature Trace Evaluation

Data from single wavelength can be extracted from temperature-based spectra.

Background correction – allows the user to set up the same background correction functions as in the general purpose ChemStation software.

Transition Temperature Evaluation

The transition temperature can be determined in a user-defined calculation range by absorbance average or first derivative.

For first derivative the sensitivity and smoothing is user-definable.

Equation

Equation allows the user to enter an equation for the calculation of a result from the T_m value. The variables are the transition temperature T_m, the DNA length, and molarity of the buffer, which has been entered about the samples. Mathematical functions include +, -, ×, /, log, ln, exp, sqr, sqrt. The column name and the unit are user definable. The default equation is the calculation of % GC.

Thermal Denaturation Disk Files

ChemStation time-based data is stored on disk in binary files with the suffix .TD. A file browser lets you view the sample name, comment, date, time and the trace range.

Temperature traces can be exported as *.CSV and *.DIF files which are common ASCII text file formats.

Documentation

The document set comprises one manual and one software component:

- The *Understanding Your Biochemical Analysis Software* manual. provides explanations of the ChemStation principles and docu-

ments all the data analysis algorithms.

- The *Online Help* system is extended with information related to the biochemical analysis software.

Dissolution Testing ChemStation Software (G1118AA)

General

The dissolution testing ChemStation software adds dissolution specific automation, evaluation and reporting to the general purpose UV-visible ChemStation

software. The dissolution testing software supports both single- and multi-bath operation. All spectral processing features of the general purpose ChemStation and data

analysis features for single and multi-component analysis of the advanced ChemStation software are available within the dissolution testing software.

Single-and Multi-bath Dissolution Testing Mode

The *Dissolution Testing* mode is a dedicated mode for single bath dissolution testing. It supports offline dissolution testing, manual sampling, sipper or autosampler operation and online dissolution testing automated sampling with the multicell transport based or valve- based sampling systems.

The *Multibath Dissolution Testing* mode is a dedicated mode for online dissolution testing on up to four baths in parallel. It is only compatible with the valve based multibath sampling system in combination with the multicell transport.

Both single and multi-bath valve based sampling dissolution testing have virtually identical functionality.

Instrument Control and Data Acquisition

Only the Agilent 8453 spectrophotometer is supported.

Bath Control

The communication between the ChemStation and the dissolution baths is done via a dissolution bath driver software which is not part of the dissolution testing ChemStation. The ChemStation DDE interface has the flexibility to set and monitor, via the bath driver, all parameter/data which a dissolution bath can have.

The following actions can be controlled by the software (if the bath/bath driver supports the functionality):

- Set/read thermostatted water bath temperature.
- Read minimum, maximum, average and standard deviation of temperature.
- Read individual temperatures of vessels.
- Set/read stirrer speed.
- Read minimum, maximum, average and standard deviation of stirrer speed.
- Control motor drive (on/off).
- Control a tablet drop device.
- Control the lift of the probes for sampling.

- Read individual initial volume in each vessel.

The monitored bath parameters values (for example, temperature, stirrer speed) during the dissolution run are included in the results file and are documented in reports.

For availability and functionality of a specific bath driver, please contact the dissolution bath manufacturer.

Good Laboratory Practice

The UV-visible ChemStation has many features to support GLP (see page 6), for example, the operator/manager level, and the *Verification & Diagnostics* mode (see page 10).

In addition to the GLP features of the general purpose software, the dissolution testing mode has the

following additional features to support GLP requirements:

- predefined methods for USP calibrator tablets to validate the bath,
- documentation of relevant bath parameters through the course of the dissolution run,

- logbook for errors/messages that occur during a dissolution run,
- software validation kit,
- flow test for a selected channel, and
- cross-contamination test for the sampling systems.

Automation - Pre-run

A user-defined sequence of pre-run actions can be executed before the dissolution test starts. These actions can be set as optional and a descriptive message for operator guidance can be given. The following actions are available:

- flow rate test of the peristaltic pump,

- transfer of bath parameters, stirrer speed and temperature settings,
- wash cycle to clean the tubings,
- re-measure Standard,
- measure Blank,
- go to position for multicell transport, valve or autosampler,
- wait for specified time,
- measure Control,

- lift probes of bath,
- lower probes of bath,
- medium test,
- measure spectrum used for capsule background correction,
- message box for operator guidance,
- pump for specified time, and
- user Macro hook for customization.

Automation - Dissolution Run

The time points for sampling and dissolution-specific actions are defined in the dissolution time table. The following actions are available:

- Zero vessels – the measurements at time zero will be subtracted from all subsequent measurements.
- Measure vessels – a complete measurement cycle is executed.
- Pause – the time is frozen to allow, for example, a pH change.
- Set stirrer speed and temperature of the bath (bath parameters).
- Medium volume changes (Add/remove or replace of volume).

- Lift probes of bath.
- Lower probes of bath.
- (Timed) operator message,
- Sampler position to go to a specific autosampler position.
- Measure infinity value.

The start of a dissolution run initiates the following actions besides the user defined pre- and post-run sequence:

- User prompt: Product name, Batch, Comment, Volume, individual tablet weights, operator.
- Wait for 'bath ready'.
- Tablet drop: Visual and acoustic feedback for the user. If the bath supports automatic tablet drop and a multicell based single bath sampling system is used the appropriate command is sent to the bath

A measure vessel cycle consists of the following actions:

- Switch pump on for the defined time.
- Execute measurement cycle: blank, defined number of vessels and control.
- Read bath temperature(s) and stirrer speed.
- Calculate match factor of the sample spectra in respect to the standard spectrum.
- Calculate, store and display the results.
- Store raw spectral data.

Automation - Post-run

After the last measurement cycle the post-run sequence is executed:

- Flow rate test of the peristaltic pump.
- Measure blank.
- Go to position for multicell transport, valve or autosampler.
- Bath parameters (set heater/stirrer).
- Re-measure standards.
- Measure control.
- Switch off the spectrophotometer lamps.
- Switch off bath heater/stirrer.
- Wait for a specified time.
- Message box for operator guidance.
- Lift probes of bath.
- Lower probes of bath.
- Pump for a specified time.
- Wash cycle.
- Print result report.
- Export of data into an ASCII file.
- User macro for customization.

Reporting

The dissolution testing ChemStation software allows users to define reports based on predefined styles, or design their own customized reports.

The predefined reports include:

- header with current date/time, report title and page identifier,
- customized line for lab identification,
- identification of used analytical instrumentation,
- short description of the analytical method,
- calibration information,
- product description,
- dissolution run results,
- a table with QC- limits for each of the components which allows the definition of a minimum and/or maximum % dissolved at user-defined times, and,
- a log book with error messages, pre-run and post-run results.

A separate mode builds combined reports for 6 (stage 1), 12 (stage 2) or 24 (stage 3) samples by loading up to four individual result files.

The statistical evaluation includes a check against the acceptance table criterias for immediate release, extended release or delayed release as defined in USP 23.

A report includes:

- header with current date/time, report title and page identifier,
- information on product, used method and result files,
- combined tabular dissolution run results as % dissolved,
- average, % RSD, minimum and maximum % dissolved for each time,
- overlay of dissolution profiles of all vessels, and
- pass/failed for the acceptance table criterias applying the corresponding stage (stage 1 for 6 samples, stage 2 for 12 samples or stage 3 for 24 samples).

Data Evaluation

The software provides the following types of calculations:

- % dissolved,
- weight dissolved, or
- weight dissolved/weight of tablet.

These types of evaluation are compatible with single- and multi-component analysis. The following options are available

- correction for individual tablet weights in respect to the label weight,

- mathematical correction for volume changes (withdraw, add, remove, evaporation) during a dissolution run, and
- a global factor as a multiplier.

Based on equation

A user-defined equation can be used for calculating results. A standard can be used as the 100 % reference. The absorbance (or derivative etc.) values of the sample/standard,

the actual volume, the number of the cycle and up to eight- user defined variables are available in the equation.

- correction for individual tablet weights with respect to the label weight, and
- a global factor which is a multiplier.

Note: No mathematical correction for volume changes is possible in case of the equation.

Customizing

The dissolution testing

ChemStation software can be customized using the powerful command set. For more details see the *Advanced ChemStation* section in this guide.

Sampling Systems

The dissolution testing software supports both, offline and online sampling systems. The offline sampling systems use the time table only to label the individual sample measurements and generate the dissolution profiles.

- **Manual/Sipper based sampling system**
The operator is prompted for the individual sample, blank, control or standard according to the specification in the method and dissolution time table.
- **Autosampler based sampling system**
Unattended measurement as defined by the dissolution time table and the measurement cycle sequence.

The online sampling systems have a direct sampling link to the bath and sampling is done automatically at the times specified in dissolution time table.

- **Single bath multicell based sampling system**
This sampling system consists of an eight-position multicell transport, multi-channel peristaltic pump, and individual tubing and flow cells for each vessel. Sampling of up to six vessels, a blank and a control sample is possible. A correction for variation in cell path length as well as the absorbance differences of the individual cells is performed. The minimum cycle time of this sampling system is 2.5

minutes

- **Single bath valve based system**
This sampling system consists of a valve controller, eight-port valve unit, a peristaltic pump, tubing to up to eight vessels and one flow cell. The sampling is done sequentially from the vessels. The standard cycle time is 5 minutes, optional 7.5 and 10 min cycles are configurable to allow longer pumping times for special applications.
- **Multibath valve based sampling system**
This sampling system consists of a multicell transport and up to four valve-based sampling systems. The standard cycle time is 5 minutes, optional 7.5 and 10 min cycles are configurable to allow longer pumping times for special applications.

Documentation

The document set comprises one manual and three software component:

- Explanations of the ChemStations principles and documentation of all the data analysis algorithms are provided in the manual *Understanding Your Dissolution Testing Software*.
- The *Online Help* system is extended with information related to the dissolution testing software.

Security Pack for the UV-visible ChemStation Software (G1813AA)

General

The Security Pack for the UV-visible ChemStation is a combination of a specific setup of Windows 2000 or Windows XP security features such as user groups (ChemStation operator and manager), password and account management, file access permissions, and controlled operator activities within the ChemStation software.

ChemStation data can also be saved on a Windows 2000 server with the Agilent Security Service installed.

Access Control

The Agilent ChemStation allows two user groups (ChemStation manager/ChemStation operator) with different permissions in terms of file access, method setup or data modification capabilities. The Windows 2000/Windows XP administrator assigns new users to the respective user groups. During login the ChemStation checks if the user/password is found in one of the user groups of

Windows 2000/Windows XP and sets the respective permissions. All aspects of password handling such as aging, length, session logout or uniqueness is defined by the Windows operating system account policy. During a session, the ChemStation can be locked manually or automatically for unattended operation.

If the software is installed on a networked PC in a domain, the identification code and password of a global account can be used with the Security Pack. In the workstation/server setup global groups are applied and all user management can be done on the domain.

Data Integrity

Access to already stored result and method files is controlled by Windows operating system file management (NTFS) based on the permission specific for the user groups. For example, the operator can only read an existing result file or predefined methods and create new result files once. The measurement sequence is a “closed” environment and

requires the storage of a result file on exit. The result file includes the complete method, all raw data as spectra, including the deleted spectra and a run logbook as well as a signature logbook. A useful feature during an audit is the capability to recall the deleted spectra of a measurement sequence including information on operator, time, date and reason.

Audit Trail

A versioning on the ChemStation level is done by storing all versions of modified methods in a new method files. A logbook with the history of the method is always stored within the method file. In case of result files, reprocessing with changed parameters will be automatically documented

by appending the logbook with information on the actions done during reprocessing. If the result is stored again, the result file includes the changed method, all raw data and spectra as well as a copy of the this logbook.

Electronic Signatures

Results can be reviewed and signed electronically by the manager with the user ID and password. All signatures are documented in the signature logbook with date, time, reason and full name of signer. All signatures have a single line of user definable text for the purpose (e.g. result created, result reviewed).

www.agilent.com/chem

Microsoft® is a U.S. registered trademark of Microsoft Corp.

Windows® is a U.S. registered trademark of Microsoft Corp.

Copyright © 1997-2003 Agilent Technologies
All Rights Reserved. Reproduction, adaptation
or translation without prior written permission
is prohibited, except as allowed under the
copyright laws.

Published November 1, 2003
Publication Number 5989-0334EN

