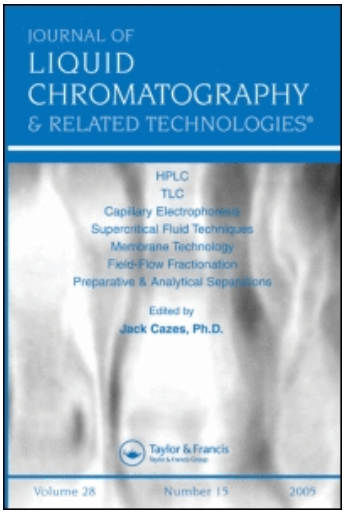

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Ernst, Ulrich P. , Hsu, James T. and Chou, F. Edward(1992) 'Automation of Countercurrent Chromatography (CCC) Via Personal Computer', Journal of Liquid Chromatography & Related Technologies, 15: 15, 2677 — 2690
To link to this Article: DOI: 10.1080/10826079208016341
URL: http://dx.doi.org/10.1080/10826079208016341

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(15&16), 2677-2690 (1992)

AUTOMATION OF COUNTERCURRENT CHROMATOGRAPHY (CCC) VIA PERSONAL COMPUTER

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ABSTRACT

The automation of a commercially available high speed countercurrent chromatography (HSCCC) instrument is described. The prototype system incorporates a personal computer to carry out on-line monitoring and control of the essential operating parameters. The benefits of automated control and monitoring include reduced instrument supervision, improved documentation means and operating safety. Two sample separations have been performed in order to highlight the basic features of the automation and software package.

INTRODUCTION

Since the initial development for the modern design basis of countercurrent chromatography (CCC) instrumentation by Ito and co-workers in the early '70's, there have been a number of steady design improvements made to the instrument design and a wide variety of

operating approaches and applications have been successfully formulated [1]. Significant improvements to CCC design include 1); the elimination of the need for rotating seals in the apparatus [1] by utilization of flexible tubing 2); the implementation of multilayer coiled tubing columns [2], which yielded increased column length, and hence, improved separation efficiency 3); the utilization of multiple columns in series which further extended separation efficiency while simultaneously eliminating the need for counterbalancing of the single column coil [3].

The last several years especially, has seen the evolution of a wide array of different designs all based on the fundamental principle of the CCC concept and adapted to carry out a specific separation or to fill a particular niche. One of the most significant recent developments has been the adaption of the CCC, which has traditionally been used almost exclusively for preparative scale purposes, to carry out analytical scale separations. Both significant improvements in plate number and separation time have been achieved [4]. This design improvement has greatly broadened the base for potential implementation of the CCC apparatus.

While there has been a significant amount of attention paid to mechanical and analytical improvements for the instrument, CCC technology is still lagging behind conventional column chromatography in terms of simplifying its use by automation of the operation. The current status of the CCC instruments is such that it still renders the instrument as a novel separation tool in the eyes of most potential users. Commercially available CCC units offer very little in the way of process monitoring and control, and in general, must be operated and supervised manually and it is perhaps primarily this lack of

sophistication that has limited its popularity amongst chromatographers [5].

The benefits gained from offering an automated system are several: 1) Since run times can be relatively long (on the order of hours), especially for preparative scale separations, monitoring and control of the primary process variables would largely eliminate the tedium and expense involved in providing constant supervision. 2) For analytical applications, automation benefits the user by providing documentation software which allows the analytical results to be stored, recalled and manipulated (e.g., to make direct comparisons of run-to-run variance). 3) Should the instrument prove potentially useful as a means of fractionating or purifying selected compounds for use in clinical trials, good manufacturing practice (GMP) mandates that the process methodology be thoroughly validated and documented using the best available current technology. Again, this requires instrumentation equipped with the necessary data acquisition and control package for continuous tracking of all pertinent process variables. 4) Concerns for safe operation are accounted for by tracking pressure and temperature within the process on a continuous basis during operation. Should the specified system limits be exceeded, automatic shutdown of the operation is invoked.

EXPERIMENTAL

Several experimental examples have been provided in this work with which to gauge the needs for various aspects of automation for the CCC instrument and accompanying peripheral equipment. This section describes the equipment, reagents, phase system preparation and outlines the hardware configuration for the prototype data acquisition and control package developed.

Equipment

Three commercially available high speed countercurrent chromatography (HSCCC) instruments (models HSCCC 800, HSCCC 1000 and HSCCC 3000) courtesy of Pharma-Tech Research Corp., Baltimore, MD, USA were utilized to complete the experimental portion of this work. An analog driver interface board for drive motor control was obtained from Bodine Electric Co., Chicago, IL, USA. HSCCC shaft speed was monitored using a magneto RPM pick-up and monitoring assembly available from Minarek Instruments (Glendale, CA, USA).

A precision flow pump (model SSI 222C HPLC pump) with frequency signal flow control and built-in pressure transducer was obtained from Scientific Systems, Inc., State College, PA, USA. An Everex 386/33 PC (Everex Corp., Fremont, CA, USA) equipped with 40 MB harddrive and the LAB-PC^{TM} multifunction I/O board available from National Instruments, Austin, TX, USA was utilized to interface with the perquisite input/output signals.

Hardware Configuration

Shown in Figure 1 is a schematic of the essential design configuration adopted in the prototype automated system. The essential elements of the automated package includes a personal computer (PC) equipped with the multifunction I/O board, a power controller box which houses the driver interface and a power relay switch, a high precision pump equipped with built-in pressure transducer and internal flow control feedback and a CCC unit and driver motor. The power controller contains a REMOTE/LOCAL toggle to enable switching between fully automated and manual operation of the pump and CCC unit. For automated operation the following run parameters are continuously controlled and/or monitored: RPM, flow rate, pressure, temperature

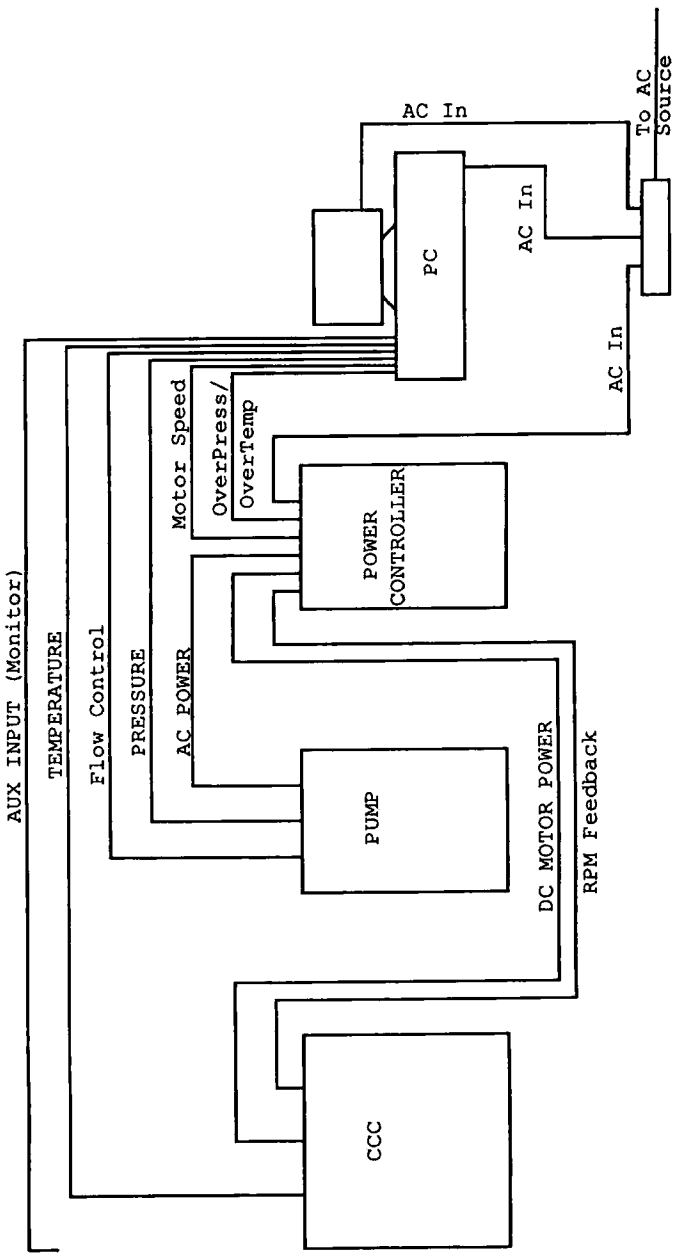

Figure 1. Schematic layout of AHS-CCC electrical connections.

and an auxiliary input for monitoring the desired detector (or integrator) output. The multifunction I/O board is equipped with multiple analog and digital I/O channels and can be suitably modified for additional inputs and outputs, e.g., for documenting event markers of sample injectors and fraction collectors.

Feedback control of the CCC motor is required to maintain speed of the CCC unit at the desired setting. A constant feedback loop is produced by first amplifying and then comparing the measured output of the magneto pick-up to the set value.

Temperature in the system is monitored at the load-bearing points within the apparatus. In this manner, the potential hazards caused by worn bearings can be mitigated. For purposes of safety, the power controller houses a power cutout relay, by which power to both pump and the CCC unit may be interrupted in case the pressure or temperature in the instrument exceeds preset operating limits.

Reagents

Chloroform of chromatographic grade was obtained from Fischer Scientific, Fair Lawn, NJ, USA. Reagent grade acetic acid (glacial) and hydrochloric acid were obtained from Fischer Scientific as well. Potassium phosphate (monobasic) and polyethylene glycol, MW ≈ 3400, (PEG-3400) was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI, USA. Bovine serum albumin (BSA), Lysozyme (LYS) and DNP-amino acid standards were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Phase Systems

Two phase systems were made up as follows for the different examples used in this work. For separation of the DNP-amino acids, the two phase system was composed of chloroform-glacial acetic acid-0.1M HCl

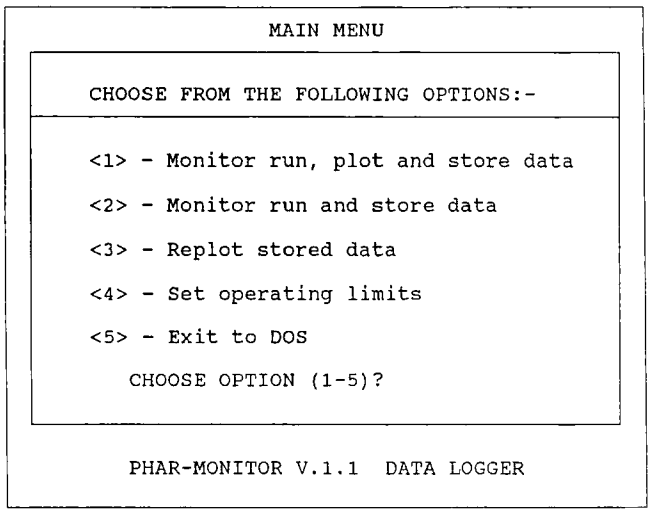

Fig. 2. Main Menu Options.

in a 2:2:1 volumetric ratio. For the BSA/LYS separation, the aqueous two-phase system was made from 1M KH_2PO_4 at pH 7 and PEG 3400 at 10% (w/v) in deionized H_2O. All solvent systems were thoroughly equilibrated by repeated shaking and settling prior to separation and subsequent use.

Control Software

The essential features of the **PHAR-MONITOR** software program developed to perform the overall monitoring and control of the complete system is described here. The program, written in BASIC, may be accessed by typing **CCC** at the appropriate directory prompt depending on the configuration (with or without a harddrive) of the PC available. As is shown in Figure 2, the main menu lists five options. Option **<1>** provides for full monitoring and control of the instrument along with on-line plotting and data storage of user selected process

variables. Option **<2>** provides the same with the exception that no on-line plotting is offered. This option may be utilized for operation where on-line monitoring cannot directly be applied. Option **<3>** allows the user to retrieve stored run data for purposes of comparison or further examination. Option **<4>** allows the user to specify operating limits for pressure and temperature as well as the full range signal output of the detector/integrator being utilized. Option **<5>** escapes the program and returns the user to the DOS prompt. The scope of the individual options is outlined in more detail below.

Options 1 and 2. By choosing options **<1>** or **<2>**, the user is directed to a second menu which allows the user to specify the necessary run parameters. As shown in Figure 3, a file name for data acquisition, rpm, flow rate, scan interval and which parameters (detector, pressure, temperature, flow rate and rpm) are to be logged are set by this menu. By hitting the **RETURN** key, the system is readied for monitoring and control. At this point, the screen for option **<2>** is in standby mode. With option **<1>**, an additional input is required which prompts the user for the desired on-screen time scale of the plotter (default is 60 minutes). Subsequently, the system is put in standby mode as shown by the example in Figure 4. The automated CCC operation is now readied for use. System equilibration may be accomplished by pressing the **F1** function key (Initiate Equilibration). This activates the pump and uniformly ramps the CCC unit up to constant speed. Simultaneously, this also initiates the on-line monitoring and plotting functions but does not store data. In this manner, unnecessary data is not accumulated. When phase system equilibration has been completed, by pressing the **F2** function key, data acquisition may be activated at the user's convenience, e.g., at the time of sample injection. This function automatically clears the plotter

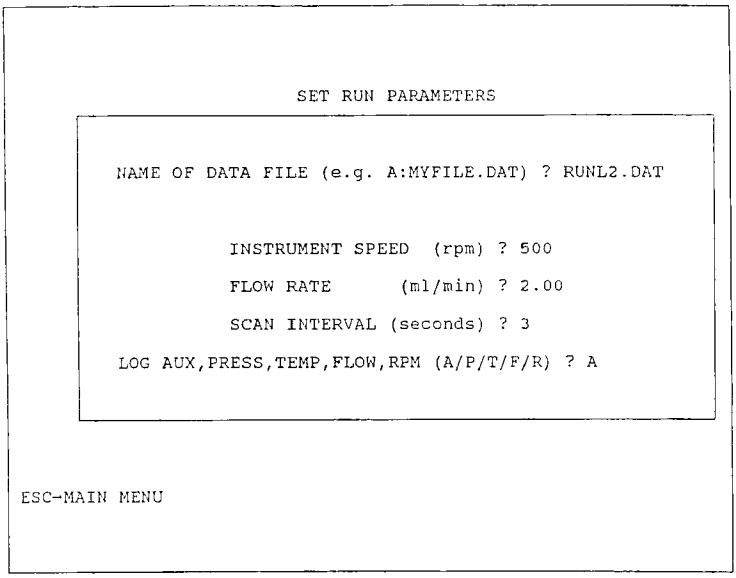

Fig. 3. Run Menu - Setting run parameters.

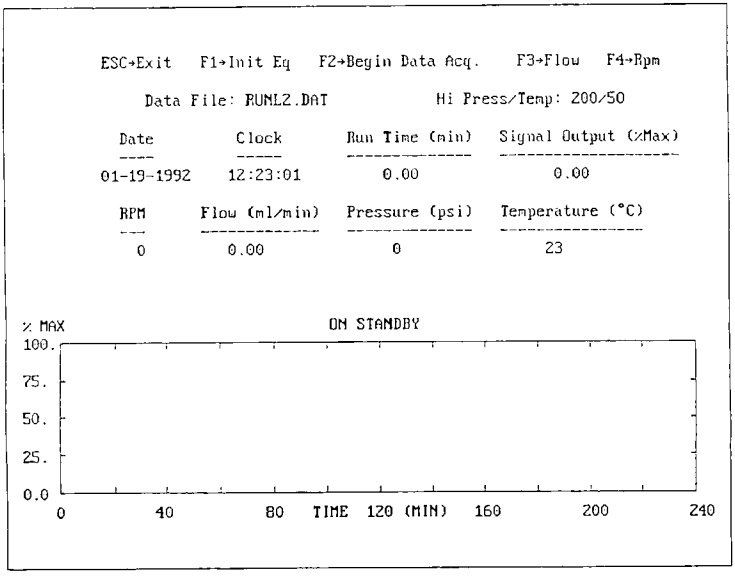

Fig. 4. Standby Mode.

screen and resets the run time to reflect the initiation of an actual sample run. A further feature allows the user to increase or decrease the flow rate or CCC rpm at any time by simultaneously pressing **F3** (for flow rate) or **F4** (for rpm) and the up-arrow ↑ (for increase) and down-arrow ↓ (for decrease). The data acquisition mode may be exited at any time by pressing **ESC**, once to freeze the screen and twice to exit back to the Main Menu.

Option 3. Under option **<3>**, the user may specify multiple files which can be retrieved for comparative purposes. The format is the same as for the Run Menu with the exception that the only inputs required are the name of the storage file(s) and the pertinent variables which are to be examined (i.e., detector, pressure, temperature, flow and rpm).

Option 4. As illustrated in Figure 5, option **<4>** allows the user to define maximum operating limits for pressure and temperature as well as define the full-scale voltage output for the detector with the capability for setting the full scale range at 10mV, 100mV or 1V. Default values are 200 psi, 50°C, and 100mV. The default values may altered by accessing option **<4>**. The user is automatically sent back to the Main Menu upon completing entry of all three parameters.

Experimental Procedure

For each experiment, the CCC instrument used for carrying out the particular separation was manually loaded with stationary phase by placing the power controller in LOCAL mode. The power controller was returned to REMOTE and column coil equilibration was performed until a steady baseline signal following mobile phase breakthrough was obtained. This was accomplished by implementing option **<1>** using the Initiate Equilibration function (F2 key). Upon reaching phase equilibrium in the CCC unit, a 0.5 ml sample was injected and

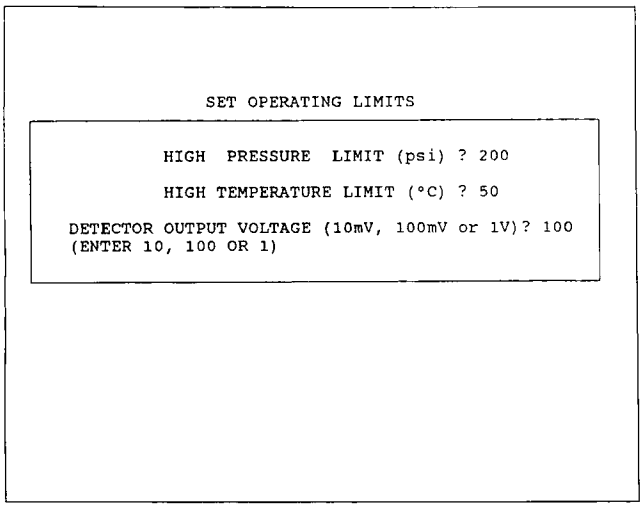

Fig. 5. System Limits Menu - Setting operating limits.

on-line monitoring and data acquisition was initiated. A model 500 Variable Wavelength UV-Vis Detector (Scientific Systems,Inc., State College, PA, USA) was utilized for both runs. The BSA/LYS separation was carried out in a model HSCCC 800 unit (Pharma-Tech Research Corp., Baltimore MD, USA) with a total column volume of 450 ml and the effluent was monitored at 280nm. Separation of the DNP-amino acid mixture was carried out in a model HSCCC 1000 unit with a total column volume of 110 ml and the effluent was monitored at 350nm.

RESULTS AND DISCUSSION

For demonstration purposes, shown in Figure 6 and 7 are the results for the separation of BSA/LYS and DNP-amino acids as obtained from the on-line monitoring software, respectively. Pertinent information which is displayed includes the storage file name, upper

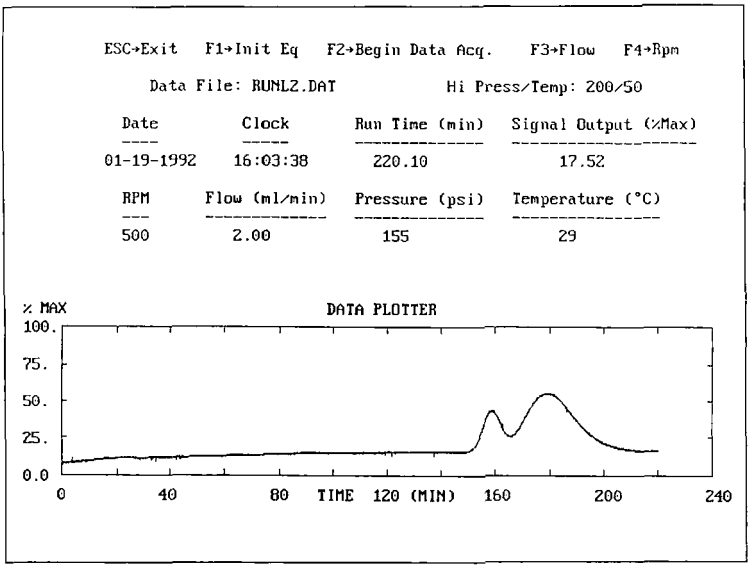

Fig. 6. On-line monitoring of a BSA/LYS separation, BSA (1) and LYS (2), using HS-CCC 800. Sample size 0.5 ml, 5 mg ea BSA and LYS. Solvent system, 1M K_2PO_4/PEG 3400:H_2O (10%w/v), pH = 7.0 (1:1 ratio). Mobile phase, bottom phase. Flow rate 2.00 ml/min. Rotation speed, 500 rpm. Absorbance at 280nm.

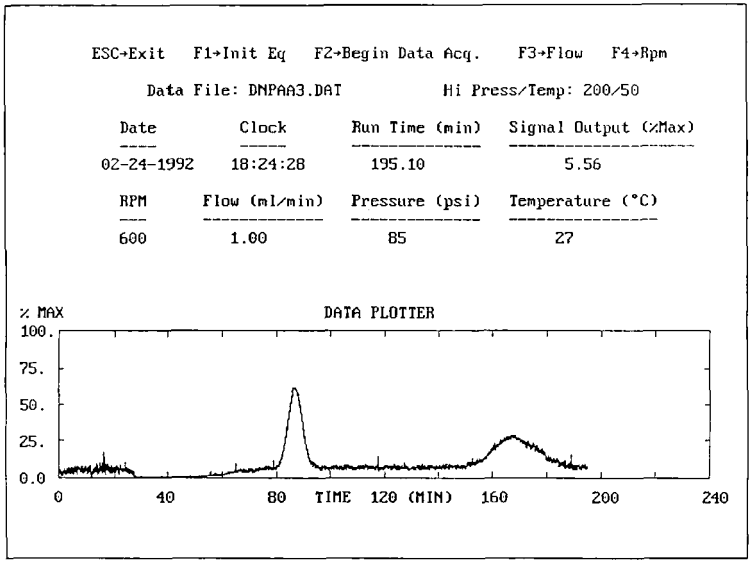

Fig. 7. On-line monitoring of a DNP-Ala and DNP-Asp separation, DNP-Ala (1) and DNP-Asp (2), using HS-CCC 1000. Sample size 0.5 ml, 2.5 mg ea DNP-Ala and DNP-Asp. Solvent system, $CHCl_3$/CH_3COOH (glacial)/0.1M HCl (2:2:1 ratio). Mobile phase, top phase. Flow rate 1.00 ml/min. Rotation speed, 600 rpm. Absorbance at 350nm.

pressure and temperature limits, date and clock time, total run time in minutes from the point at which the sample was injected, signal output of the detector as a percentage of maximum relative signal strength, rpm of the CCC unit, flow rate in ml/min, pressure in psi and temperature in °C.

Peaks 1 and 2 in Figure 6 were determined to be BSA and LYS respectively, from additional runs using pure samples. Peaks 1 and 2 in Figure 7 were determined to be DNP-Ala and DNP-Asp respectively, in the same manner. Data acquisition in each case, was terminated following elution of the second peak. Additional interpretation of the peaks by enumerating them in order of elution was done manually here for purposes of clarification and is not provided by the software.

CONCLUSIONS

Automation of CCC instruments represents an important aspect in the refinement of this particular separation tool by providing a much needed upgrade in the user friendliness of the operation. 1) It eliminates the need for continual supervision of the instrument. The tedium and/or labor costs associated with the long run times, which, in some instances are required to effect proper separation, can to a large extent be alleviated by providing for continuous monitoring and control of the process. 2) The reliability and run-to-run reproducibility of the CCC instrument can be ascertained through the continuous sampling and documentation of the pertinent process variables which is provided by automation. 3) Continuous monitoring of pressure and temperature with in-built power relay cutouts translates into improved safety for instrument operation especially under conditions of heavy use. 4) Finally, automation yields improved process efficiency and flexibility especially for analytical and preparatory-scale work by

facilitating unsupervised operation, e.g., in conjunction with a timed production schedule involving overnight operation.

While the primary benefits associated with automation of the CCC instrument have been listed above, it is also important that this can be accomplished in a cost-effective manner by using a personal computer-based system. To varying extents, most laboratories already use personal computers for data acquisition and control of analytical equipment. These can be inexpensively modified by incorporating the multifunction I/O board to accomplish the task of automating the CCC operation as is deemed practicable.

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