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Development of an Application Program of Ultraviolet-visible Spectrum Measurements

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ABSTRACT: To measure digitalized ultravoilet-visible absorption spectra of polynucletide solutions, an application program was developed under a LabWindows/CVI environment that is a software development system for describing source code in C language. There were no information about RS232C communication protocol and commands for controlling the spectrophotometer whereas the software should control communication between RS-232 devices, a personal computer and a Shimadzu UV-2500 ultraviolet-visible spectrophotometer. This puzzling problems were solved by the monitor of communication between the RS-232 devices. The protocol and commands were decoded and subsequently were confirmed by running a program for a check on the communication. The software developed in this study has graphical user interfaces and is easy to operate. It will be available for all measurements of ultravoilet-visible absorption spectra.

KEY WORDS: Thermal Denaturation / Absorption Spectrum / Laboratry Automation

I. INTRODUCTION

Polynucleotides would form secondary structures in aqueous solutions under proper conditions.[1-3] Hyperchromism effect and/or hypochromic effect can, then, be observed for ultraviolet (UV) absorption spectra of the solutions.[2] Thus the changes in absorbance clearly reflect the secondary structures. When a temperature of an DNA solution increased, the DNA duplex disassociates cooperatively into two molecular chains at an intrinsic temperature and its absorption increased in an ultraviolet region.[2, 4, 5] The temperature is called for a melting temperature T_m that characterizes thermal stability of the secondary structure. Melting profiles that are the temperature dependence of absorbance values, are available for an investigation of thermal stability of the natural polynuleotides such as DNA molecules and the synthetic polynucleotides. This is because that the changes in the secondary structures are detectable by the spectropcopic method.[6-14] In the case of the synthetic polynuleotide solutions, the conformational changes also induces the increase or decrease in absoption of light dependent of its wavelength.[15, 16] Thus the melting profiles can be used to distinguish between several types of the structures in the solutions.

Thermal changes in the UV absorption were measured at 280 nm, at 280 nm, and at several mole fractions of Poly(adenylic acid) (abbreviated as Poly(A)) and Poly(uridinylic acid) (abbriviated as Poly(U)) by Ojima *et al.*[16] There appears two cooperative meltings at about 55℃ and at about 65℃. The former corresponds to a melting of a helix Poly (A)·Poly(U) and the latter corresponds to a melting of a triplex Poly(A)·Poly(U)·Poly(U). It is clear

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from the melting profiles at 256 nm and at 280 nm that more than one duplex/triplex are formed in the solution. They can, therefore, be distinguishable from each other. If absorption spectra are measured at various temperatures, further information about the secondary structure will be given. Spectrophotometers would be used to measure the melting profiles but, as it is, they can not be employed for this purpose.

To measure melting profiles and also spectra of polynucletides at various temperatures, we had developed a system composed of a spectrophotometer Shimadze UV-250 and an application program which enables a personal computer, equipped with a GPIB (general purpose interface bus) interface board, to communicate with two GPIB devices, the spectrophotometer and a cell-temperature controller.[13] However, the source code of the application program is written in N88BASIC language. Because of this, it runs only under an MS-DOS operating system and lacks for graphical user intefaces (GUIs).[4, 5, 13, 16]

Recently, pieces of apparatus have come to been mostly controlled by programs attached with them which run under a Windows 95/NT operating system. Even in the systems, spectra can, however, be measured only at a fixed temperature. It should be noted that experimantalist are unable to measure spectra of the solution at more than one temperature, increasing or decreasing temperature. The systems should, therefore, be much improved to measure the spectra at desirable temperatures.

We have developed a system in this series of work with three objectives: (1) to establish communication between a personal computer and ultraviolet-visible spectrophotometer with RS232 protocol, (2) to develope an application program to measure absorption spectra, (3) to controll cell-temperature *via* GPIB bus, and (4) to apply it to polynucletide solutions. In this paper, we will report the first and the second of these four objecties.

II. DEVELOPMENT OF APPLICATION PROGRAM

A. Developmental environment

To develop an application program under Windows 95/98 Operational System, a developmental environment LabWindows/CVI was purchased from National Instruments Corp., USA. It contains an interactive enviroment for editing, compiling, and debugging programs. Under the developmental environment, only C language can be used in describing code of software and also many useful functions, GUIs, and Libraries are available for scientific purpose. In the present study, GPIB Library, RS-232C Library and User Interface Library are of importance.

B. Apparatus

A ultraviolet-visible spectrophotometer UV-2500PC was purchased from Shimadzu Corp., Kyoto, Japan. It is equipped with RS232C port, but this connector have a pin arrangement different from a general type. Because of this, it should be noted that a special serial cable is required only for joining these two connectors to each other. Most serious problem is that UV-2500PC-control commands is not open to users at all in contrast with a previous spectrophotometer UV-250.

Personal computer (PC) Frontier High-grade LX was purchased from KOJIRO Corp., Tabuse, Japan. It has a Pentium II (300 M Hz) processor, two standard RS232C ports, and ram memory of 191 M bytes which is large enough to run a LabWindows/CVI system under Windows95 (version 4.00.950 b) operating system.

C. Communication specification

As mentioned in the above, two major problems exist. One is that there are no information to configure the serial port parameters. The other is that all commands to control the spectrophotometer is kept closed. Establishing communication between the two RS-232C devices seemed to be difficult because of the many possible configurations. To clarify the device requirements of the spectrophotometer, such as baud rate, parity, number of data bits, and number of stop bits, the RS232C communication protocol was examined by a monitor program. Fortunately, it was found that the protocol does not use two types of software- and hardware-handshaking, and the baud rate is 9600 baud, parity is odd, the number of data bits are 7, and the number of stop bit is 1.

Table I shows configuration parameters of the serial port of the PC used in the present study.

TABLE I. Configuration parameters of serial port of personal computer

Port Name	Baud Rate/baud	Parity	Data Bits	Stop Bit
COM 2[a]	9600	odd	7	1

[a] While the other parameters should be fixed in the present system, only a serial port name can be changed from COM2 to COM1 in the application program. Two types of software handshaking and handware handshaking are not used for the present program.

These parameters must match between the two parties of communication. In this study, the part of source code related to the configuration was written as the parameters are set for the port of the personal computer by a function OpenComConfig in the RS-232C Library of the LabWindows/CVI. It should be emphasized that while many graphical user interfaces are available for the development, fundamental understanding of C language is needed for the program development.

III. DECODING CONTROL COMMANDS AND DATA

A. Monitor of commands

The commands which are defined for the spectrophotometer were decoded by monitoring message queues between RS232C devices. All commands classified into two types. One controls flows of data. The other is a set of data coded in hexadecimal or controls the spectrophotometer. The set of data necessarily contains an ascii character with which they are checked to exactly receive themselves from the spectrophotometer or to exactly send themselves to it. The former should be given in a set of hexadecimal characters but a function ComWrtByte in the RS-232C Library can write only one byte. We cannot use this function for the purpose. Thus the problem has been solved by an initial definition of sets of hexadecimal characters in a header file of the program. For example, a set of two characters/x1b/x04 is defined in the header file uv_ini.h and can be placed in the output queue before it is used in the application program. By doing so, users can start to communicate between the spectrophotometer and the PC. The other flow-control-commands can be used in a similar manner as above.

B. Expression of numerical data

All data are treated as a set of hexadecimal characters during measurements. Numerical data should, therefore, be expressed in decimal system to calculate absorbances and wavelengths from them. Let an absorbance being $(1271)_{16}$ in hexadecimal. The hexadecimal set is expressed in decimal as follows:

$$(1217)_{16} = 1 \times 16^3 + 2 \times 16^2 + 1 \times 16^1 + 7 \times 16^0$$
$$= 4631$$

Divide the number by a factor of 16^3, the absorbance is given as follows:

1.130.

For negative values, these complements are used in calculations.

In the case of wavelengths, let a wavelength value being $(1f40)_{16}$ in hexadecimal. It can be expressed in decimal as

$$(1f40)_{16} = 1 \times 16^3 + 15 \times 16^2 + 4 \times 16^1 + 0 \times 16^0$$
$$= 8000.$$

It should be noted that the wavelength in angstrom can be calculated from the hexadecimal value without any division.

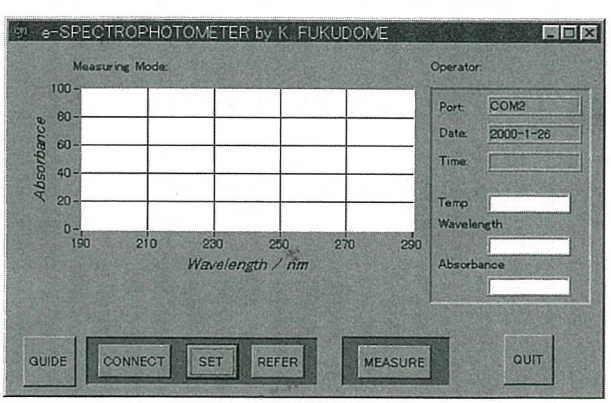

FIG. 1. Main window. The main window appears when the project about the application pragram begins running. There are seven buttons, a graph control to display the acquired spectra, and string controls to display the configration parameters and experimental values. Users can select a menu to procceed with a series of work by clicking on a button such as the GUIDE button.

IV. APPLICATION PROGRAM

A. Main panel window

A main window is shown in Figure 1. The window contains a graph area, an area of information on a port name, a date, a time, a wavelength, and an absorbance, and buttons to open dialog boxes. Users should initialze the spectrophotometer to set parameters to it whenever they measure spectra of samples. For this purpose, there are seven buttons in the window. The details are described below.

B. GUIDE dialog box

Figure 2 shows the GUIDE dialog box. When the GUIDE button is pushed in the main window, the GUIDE dialog box appears and opreration procedures are shown in it. Beginner users can operate the application program, following the procedures. It should be noted that this dialog box has no function.

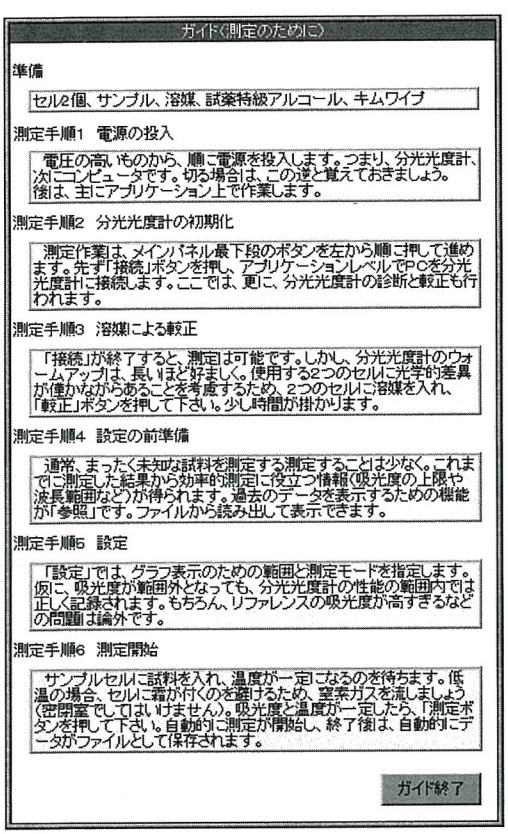

FIG. 2. GUIDE dialog box. This dialog box appears when the GUIDE button is selected in the main window. The dialog box has no function and only shows procedures to use. Beginner users would measure spectra of their samples without mistakes in operation under the guidance, written in Japanese, in the dialog box.

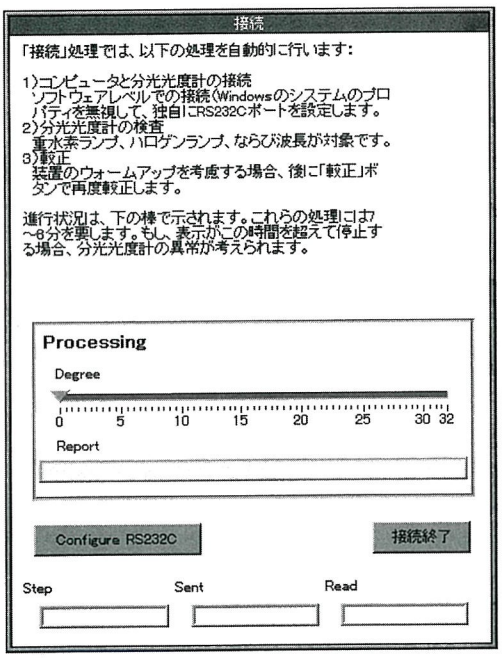

FIG. 3. CONNECT dialog box. This dialog box appears when the CONNECT button is selected in the main window. Once the dialog box is opened, the program start to test two lamps and wavelengths for the spectrophotometer and to initialize it. A degree of proccesing is shown in a slide control of the box. A new, additional dialog box appears only when the CONFIGURE RS232 button is selected in the CONNECT dialog box. To return from this box to the main window, click on the QUIT button in the CONNECT dialog box.

C. CONNECT and CONFIGURE RS232 PORT dialog boxes

To establish communication between RS-232C devices, the PC and the spectrophotometer, many parameters should be configured to them. These configuration parameters are dependent on the spectrophotometer and should be exactly specified. Thus the spectrophotometer is automatically checked by self-check function and then initialized itself after the communication is established. To do so, the CONNECT button should be selected in the main window. Subsequently the CONNECT dialog box appears as shown in Figure 3 and operation mentioned in the above can automatically be performed by using functions in RS-232C library of LabWindows/CVI. Its process is displayed by use of an slide of GUI. After establishing communication between the devices and initializing the spectrophotometer, CONNECT dialog box can be closed.

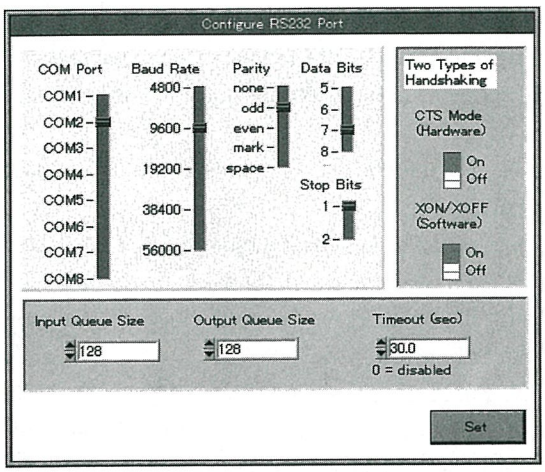

FIG. 4. CONFIGURE RS232 PORT dialogbox. This dialog box appears only when the CONFIGURE RS232 button is selected in the CONNECT dialog box. Users can confirm the parameters of their RS232C port configurations before they measure spectra. It should be noted that default settings are used in the present program if no values are set to the port.

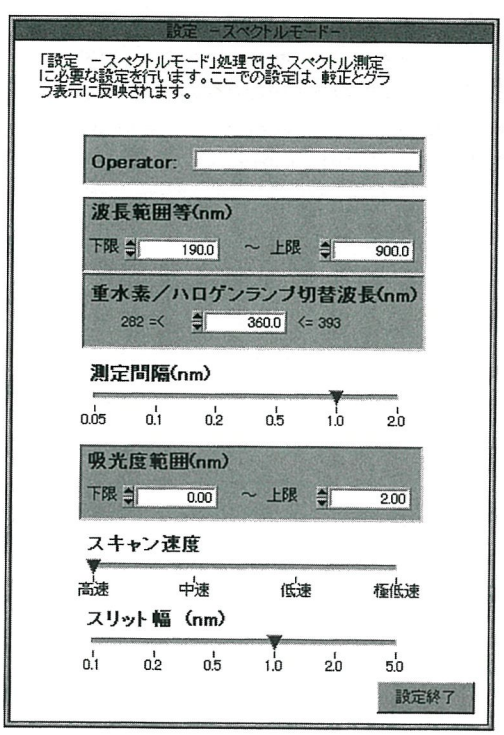

FIG. 5. SET dialogbox. This dialog box appears when the SET button is selected in the main window. Users can procced to next step without any setting. In this case, default settings are used as shown in the Figure. To obtain more accurate data, you can set a slower scan speed and a smaller slit width in nm into the box.

It should be noted that the prameters of the RS232C port can be changed. The CONFIGURE RS232 PORT dialog box is prepared for this purpose. Figure 4 shows the CONFIGURE RS232 PORT dialog box. Default values will automatically be used in the program when no parameters are set to the PC.

D. SET dialog box

Figure 5 shows a SET dialog box. Users can change from default values of parameters, such as a scan speed and a slit width, to desirable values in the dialog box. Default values are automatically set to the spectrophotometer *via* RS232C serial port when no parameters are given in the dialog box.

E. Measurement of spectra

Users can start to measure spectra of samples by clicking on the MEASURE button in the main window whenever they complete all the settings and the sample solution is set into a sample cell. They can display any spectra in the graph of the main window by choosing these data files from the flopy disks and hard disks. To do so, click on the REFER button in the REFER dialog box and load the files. Figure 6 shows the REFFER dialog box. The graph will assists you to compare a measuring spectrum with those spectra in the main window.

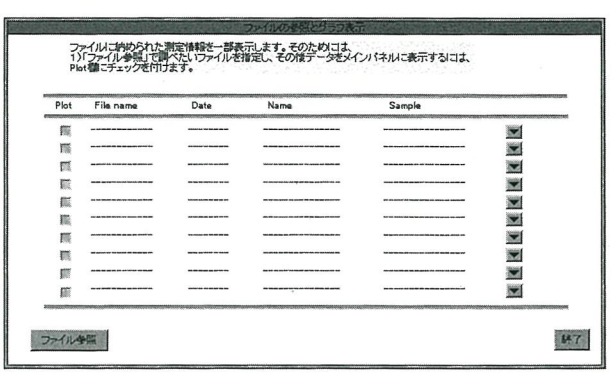

FIG. 6. Refer dialogbox. This dialog box appears when the REFER button is selected in the main window. Users can display any spectra in the graph of the main window by choosing these data files from flopy disks and hard disks. To do so, click on the REFER FILE button in the Refer dialog box and load the files.

V. CONCLUDING REMARKS

In this work, we developed the application program to measure digitalized ultravoilet-visible absorption spectra of polynucletide solutions. We confirm that the application program have run normally and that the spectrophotometer has been operating without any problem. Then the program was amply demonstrated to has graphical user interfaces and to be easy to operate. By obtaining digitized data, these data can easily be analyzed to determine melting temperatures of the sample and to distinguish between several types of highly stranded molecular chains in the solutions. It will also be available for all measurements of ultravoilet-visible absorption spectra.

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