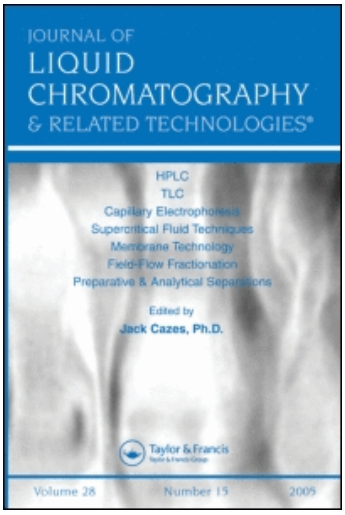

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A Computer Controlled High Voltage Equipment for Capillary Electrophoresis and Electrochromatography

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A COMPUTER CONTROLLED HIGH VOLTAGE EQUIPMENT FOR CAPILLARY ELECTROPHORESIS AND ELECTROCHROMATOGRAPHY

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ABSTRACT

An equipment is designed and evaluated for the separation of biomolecules by capillary electrophoresis and electrochromatography. The major components of the equipment are: (a) a high-voltage dc power supply with reversible polarity output, (b) a computer controlled power supply and data acquisition interface using an IEEE-488 General Purpose Instrumentation Bus, and (c) a chromatography system

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(elution pump, sample injector, capillary and chromatography columns, temperature controller, and effluent monitor). The software written in *Microsoft C* for the control of this equipment has three distinct features: one, precision control of the high voltage, two, generation of high-voltage waveforms, and three, constant monitoring of the set voltage and current values.

The equipment is versatile and applicable for liquid chromatography and capillary electrophoresis utilizing columns and capillaries of different dimensions and properties. It performs data acquisition in real time on two channels and controls the power supply at the same time. The novelty of the equipment lies in the availability of a wide variety of high-voltage waveforms which can be used to control the heat and reduce the band spreading in chromatography. The use of this equipment has been studied for the separation of a mixture of deoxymononucleotides on a chromatography column, while applying a bidirecional electric potential.

INTRODUCTION

With the emergence of capillary electrophoresis as a powerful analytical technique [1-3], efforts have been made to enhance the electrophoretic separations by minimizing the band broadening effects of Joule heating [4,5]. The lack of good capillary electrophoresis equipment has been a limiting factor in this quest. We have developed a computer-controlled high voltage chromatography equipment that can be used for capillary electrophoresis [1-3] and electrochromatography [5-12]. This equipment is capable of generating different voltage waveforms which can limit Joule heating and therefore the band broadening.

MATERIALS

The different components used for the development of a high voltage chromatography system included: (1) A high voltage power supply with features of programmable, reversible-polarity output applied high voltages to the chromatography columns (specifications: output voltage 0 to 30 kV with a resolution of 0.2 V, output current 0 to 4.5 mA; Bertan Associates, Hicksville, NY, model 210-30R). (2) An IEEE-488 General Purpose Instrumentation Bus (GPIB) interface controlled the high voltage power supply using an IBM PC 386 compatible (specifications: 0 to -5 V dc programming signal output for control of the power supply, an IEEE-488 GPIB to communicate with the PC; Bertan Associates, Hicksville, NY, model 200-C488). (3) An IEEE-488 GPIB interface card connected the PC to the GPIB (AT-GPIB with NI-488 MS-DOS Handler; National Instruments, Austin, TX). (4) QuickC (ver-

sion 2.0) and C Language (version 5.0) compilers were used to develop the power supply control software (Microsoft QuickC (version 2.0) and C compiler (version 5.0); Microsoft Corporation, Seattle, WA). (5) The C program was linked to a language interface library for Microsoft C to control the GPIB (National Instruments, Austin, TX, NI-488, revision E3). (6) The software that controlled the high voltage power supply and acquired and analyzed the chromatographic data was developed and executed on an IBM-compatible 386 personal computer (16 MHz clock, 2 Mbytes RAM, 80 Mbyte hard disk, VGA, Logitech mouse; Compuadd, Austin, TX). (7) A data acquisition instrument and software were employed to collect, display, analyze, and print chromatograms (dual-channel, analog-input data acquisition instrument with GPIB interface and data collection and analysis software; Perkin Elmer Nelson Systems, Cupertino, CA, model 900 Series Interface and Software 2600). (8) A dual-pen strip chart recorder plotted chromatograms in real time (Linear Instruments, Irvine, CA, model 290). (9) An isocratic elution pump provided a constant flow rate (specifications: flow rate 0.1 to 9.9 mL/min, maximum pressure 6000 psi; Waters Associates, a division of Millipore, Milford, MA, model 501). (10) A low-pressure, 4-port switching valve was used to connect the four buffer reservoirs to the inlet of pump (Hamilton HVXD4-5 valve; PJ Cobert Associates, St. Louis, MO). (11) A sample injection valve was used to apply samples to chromatography columns (Rheodyne, Cotati, CA, model 7125). (12) A pair of platinum wire electrodes (0.02 in. diameter) applied electrical voltage to the chromatography column. (13) A dual-wavelength chromatography detector was employed to monitor the column effluent (wavelengths 254 and 280 nm, flowcell volume 20 μL; Altex-Beckman, San Ramon, CA, model 152). (14) A constant temperature circulator cooled the chromatography columns (temperature range -20°C to 100°C; Fisher Scientific, Pittsburgh, PA, model 900).

Chromatography Columns and Chemicals. A reversed-phase column (C-18 bonded, 5 μm beads, 4.6 mm 25 cm; Alltech Associates, Deerfield, IL), was employed for the evaluation of the equipment. All PEEK tubing, Tee connectors and Fingertight fittings were from Upchurch Scientific, Oak Harbor, WA. The chemicals used for preparing buffers were: succinic acid (Sigma Chemical Company, St Louis, MO), sodium azide (Fisher Scientific Company, Fair Lawn, NJ), an artificial mixture of deoxymononucleotides (Sigma Chemical Company, St Louis, MO).

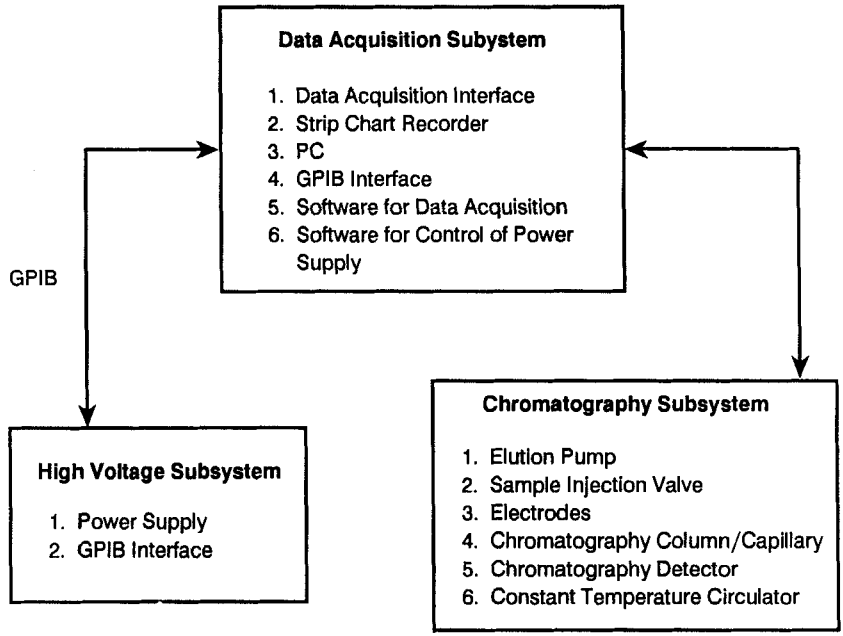

FIGURE 1. The High Voltage Chromatography System.

RESULTS

The computer-controlled high voltage chromatography system designed here consisted of three major components: a chromatography component, a high voltage facility, and a data acquisition and retrieval component. The three components are outlined in Fig. 1.

The Chromatography Subsystem

This component consisted of an isocratic pump, a sample injection valve, a pair of platinum electrodes connected to a chromatography column, a chromatography column, a dual-wavelength chromatography detector, and a constant temperature coolant circulator (Fig. 2). The eluant path started from the solvent reservoir, to the pump, the injection valve, the first electrode, the chromatography column, the detector flow cell, and the second electrode. A 4-way switching valve connected

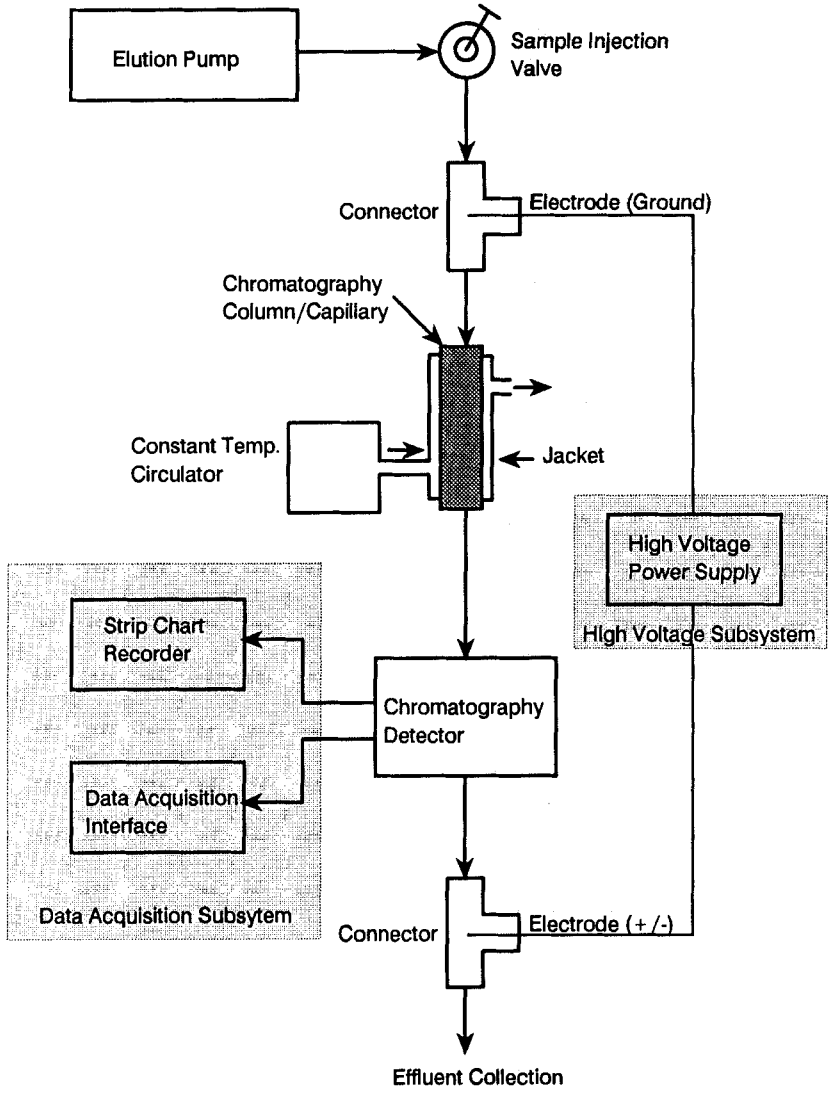

FIGURE 2. The Chromatography Subsystem.

the elution pump to four solvent reservoirs. All connections between the inlet of the pump and the reservoirs were made with a teflon tubing (1/8 in. I.D.). A PEEK tubing (1/100 in. I.D.) was used for all high-pressure connections because it can withstand pressures up to 6000 psi.

The electrodes were housed in Tee connectors to establish electrical contact with the solution flowing through the column. One arm of the Tee was connected to an electrode, the second attached to the injection valve, and the third coupled to the top of the chromatography column through the PEEK tubing. The platinum wire electrode was sheathed in a PEEK tubing to obtain a union with the Tee capable of withstanding pressures up to 3000 psi. The free end of the electrode was soldered to the output cable of power supply and the soldered joint was sheathed in an insulating rubber sleeve and enclosed in a hollow glass tube. The two Tee connectors were mounted on plexi-glass pieces attached to rods machined from Nylon 44 (3/4 in. diameter). The column was installed vertically with an ordinary aluminum clamp at the ground potential. The clamp jaws were sheathed with a special rubber insulating material to prevent high voltage arcing between the outer surface of the column and the clamp.

The high voltage could be applied to the chromatography columns in two ways: one, with the top end of the column at a high potential and the bottom end at the ground potential, and two, with the voltage to the two electrodes reversed. In the first method, a voltage gradient appeared across the short tubing connecting the sample injection valve and the top of the column because both, the pump and sample injection valve were mounted on a steel chassis. Similarly, in the second method of applying voltage, the voltage gradient was built across the tubing connecting the chromatography detector and the bottom electrode because the detector's flow cell assembly was encased in a steel casing at the ground potential. In both the cases, the desired voltage gradient was not set up across the column length because the pump, the sample injection valve, and the flow cell were all at the ground potential. This problem was overcome in two steps. The flow cell assembly was modified to electrically insulate the flow cell from its steel housing; all the mounting plates in the flow cell were machined from fiber-based phenolic sheets rated to insulate up to 70 kV. A common system ground was established and the top electrode was connected to it. Since the high voltage output of the power supply was referenced to its chassis ground, and hence relative to the common system ground, the top electrode was always at the ground potential. Consequently, the

bottom electrode was at a corresponding positive or negative potential relative to the top electrode when voltage was applied.

The ionic conductivity of the column matrix and the elution buffer essentially determined the total current resulting from the application of high voltage. The current value typically ranged from tens of μA to a few mA for voltages in the kV range. Larger values of current resulted in higher electrical power dissipation, producing high temperatures in the column. For electrochromatography, a constant temperature coolant circulator (4°C) was provided to dissipate the heat quickly and uniformly. Since the coolant was in direct contact with the outer surface of the column at a high potential, a mixture of very high resistivity (75% methanol and 25% water) was used for the coolant.

High Voltage Subsystem

This subsystem contained a power supply, its GPIB interface, and a high voltage output cable. The power supply had a reversible and programmable high-voltage dc output with a maximum output current of 4.5 mA at 30 kV. The output current was linearly derated at lower voltages according to the relation:
$I_{max} = 1.05 \times 10^{-4} V_{out} + 1.35$ mA.
For example, with the output voltage set to 10 kV, I_{max} was 2.4 mA.

The power supply had a local and a remote mode of operation. In the local mode, the front panel potentiometeric controls adjusted the output voltage between 0 and 30 kV. In the remote mode of operation, the power supply's front panel controls had no effect on the output voltage; the voltage was controlled by an external 0 to -5 V dc analog signal generated by the GPIB interface of the power supply on command from the PC. The GPIB interface was used to program the output voltage, current, and the overload limits of the power supply, and to provide status information to the PC. Only the reversal of output voltage thus required physical commutation within the power supply unit; all other controlling and monitoring were done via the GPIB interface. This arrangement offered a great degree of flexibility for the control of the high voltage subsystem.

The high voltage output of the power supply was available at the double-shielded interlocking connector. A highly insulated coaxial cable connected the output of the power supply to the platinum electrodes. The shield (outer conductor of the cable) was chassis ground, which, for safety purposes, was also the common system ground. The inner conductor was either at a positive or a negative potential

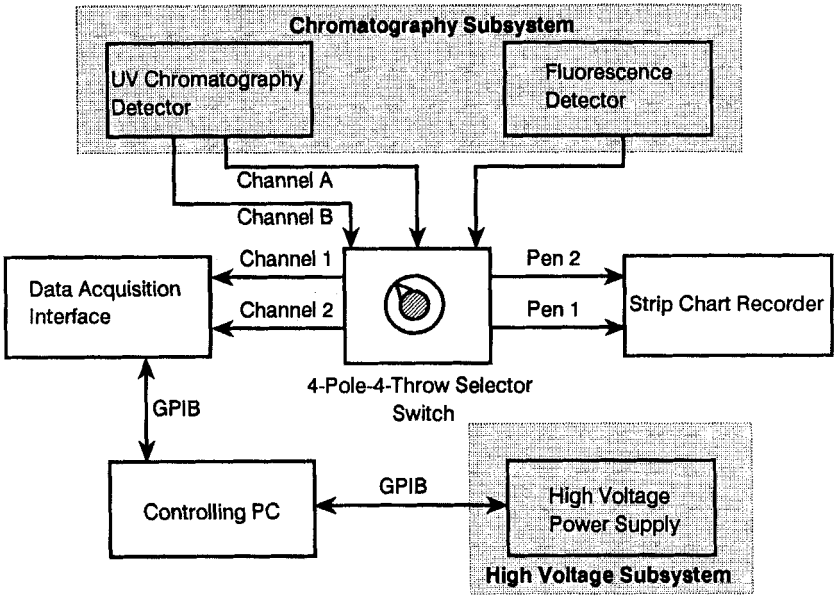

FIGURE 3. The Data Acquisition Subsystem.

with respect to the system ground. The output voltage was reversed by turning the power supply off, removing its top cover, and reversing the high voltage connectors. The power supply had a built-in safety system that switched off the unit for reversing the output voltage.

Data Acquisition Subsystem

The data acquisition interface, strip chart recorder, the PC, and the data acquisition software are the components of this subsystem (Fig. 3). The 10 mV signal outputs of the absorption (UV) and fluorescent detectors were used by the strip chart recorder and the data acquisition interface. With three output channels available (two from the UV detector and one from the fluorescent detector) and the data acquisition system capable of processing data from only two channels simultaneously, a 4-pole-4-throw switch was used to select the two channels to be processed. Since the input impedance of the data acquisition interface was 20 MΩ, the

data acquisition interface and the strip chart recorder were connected in parallel to the 10 mV output available from the selector switch. All signal cables connecting the selector switch to the strip chart recorder and the data acquisition interface were twin-conductor shielded cables. Ground loop currents were minimized by connecting the cable shields only at the detector ends and not at the strip chart recorder and the data acquisition interface ends.

The strip chart recorder plotted the two input signals as a function of time. The user could view the chromatography peaks as they eluted in real time, while simultaneously, data was collected by the data acquisition interface for a subsequent detailed analysis on the PC. The data acquisition interface had a dual-channel analog-to-digital interface with an on-board memory of 16 kbytes. It had communication ports for an RS-232C serial link and an IEEE-488 GPIB. A variable data sampling rate of 10 ms to 10 s could be programmed. The interface performed real time data collection on a stand-alone basis by using its on-board memory. The time duration of an experiment run was limited only by the on-board memory for data storage since the memory used was a function of the run time length and the rate of data sampling.

A run method was downloaded from the computer to the data acquisition interface before the start of an experiment. The method programmed the length of the experimental run and the data sampling rate. The downloading of a method before the start of an experiment made the computer available for power supply control while data was being collected by the data acquisition interface. Therefore, the data acquisition interface collected and stored the data independent of the computer. After the completion of a run, and before the start of the next run, interaction with the computer was required to obtain the collected data, thus making the data acquisition interface ready for the next experiment. All communication between the computer and the data acquisition interface were carried over the GPIB. The GPIB was chosen over a RS-232C serial link to allow for future expansion of the data acquisition system consisting of several data acquisition interfaces, all connected by the common GPIB to the PC.

Analysis of chromatography data was performed using a commercially available software (Software 2600, PE Nelson). This software created run methods, downloaded them to the data acquisition interface, and retrieved data from the interface upon completion of an experiment. Once the data was stored on the PC,

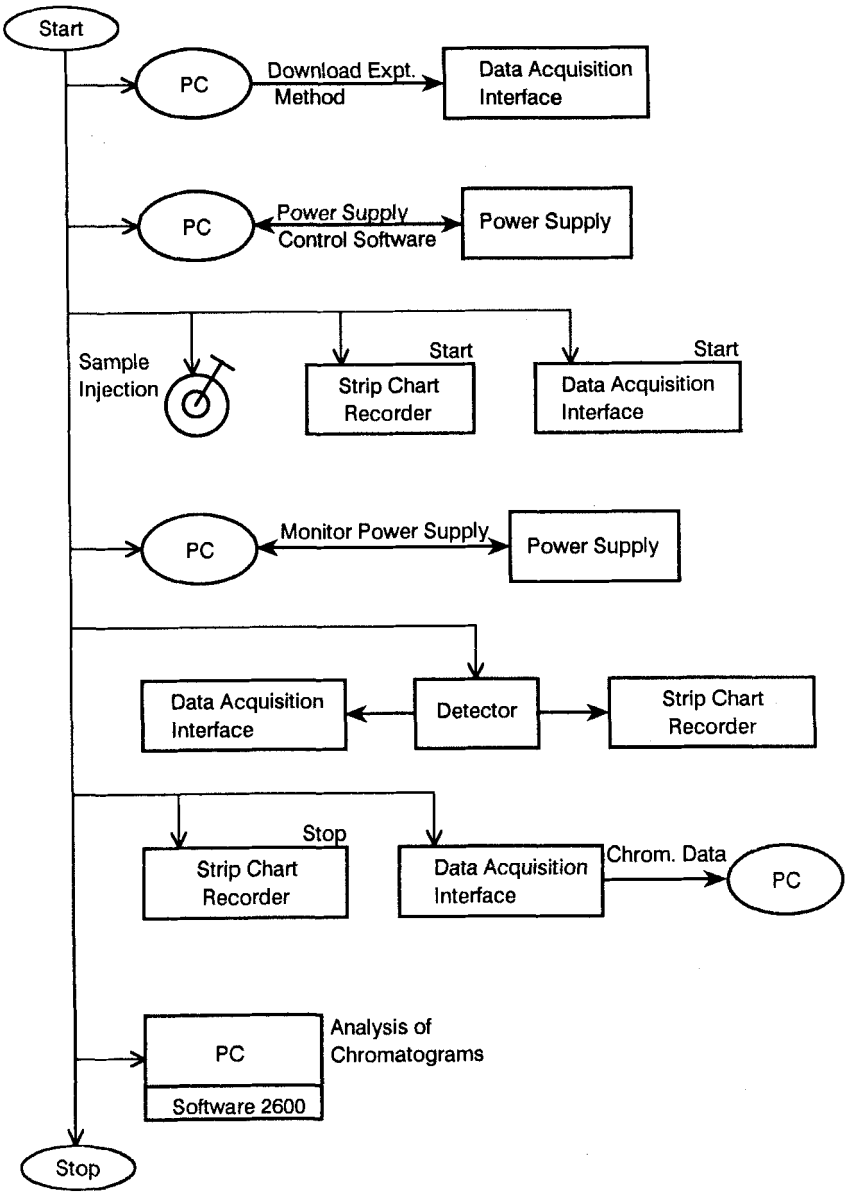

FIGURE 4. The Protocol of an Experiment.

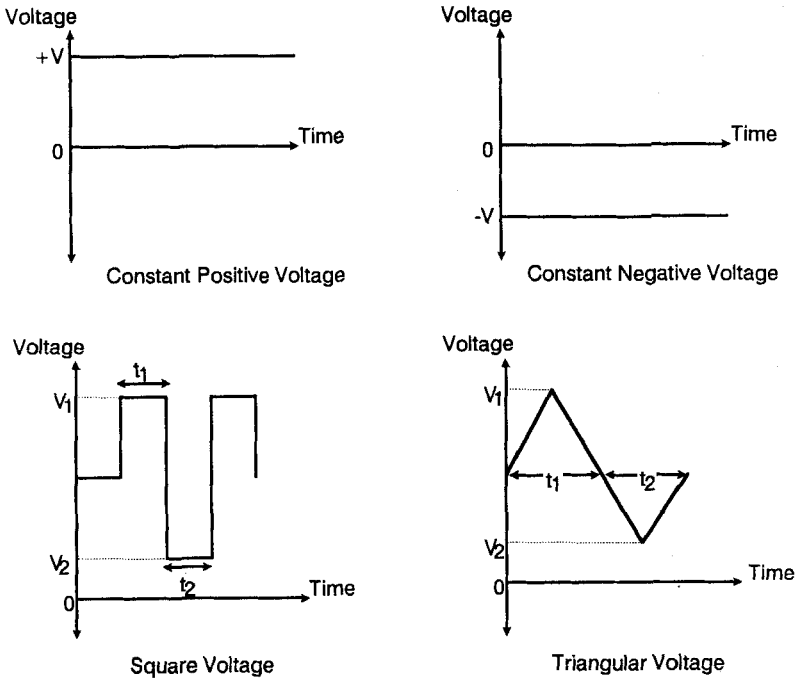

FIGURE 5. Sample Voltage Waveforms Generated by the Power Supply.

Software 2600 was used to plot, analyze and compare the chromatograms. The protocol of a typical experiment is shown in Fig. 4.

Software for Control of Power Supply

A software to control and monitor the power supply functions was developed using a Microsoft C compiler [13]. It was a menu-driven program that accepted the values set by the user, downloaded them to the power supply, and monitored the response of power supply during an experimental run. It also generated different voltage patterns to limit the power dissipation in the chromatography column (Fig. 5). The software programmed the output voltage, the current limit, the waveform patterns, the frequency and duty cycle of the waveforms and the run time. During a run it monitored these programmed parameters by displaying them

on the video screen and storing them in a data file. The software activated the user programmed responses upon detecting an overload condition of the power supply. Thus the software controlled the power supply in real-time.

Limiting Joule Heating and Band Broadening

As an effect of the applied electric potential, some heat is generated, resulting in the increase of temperature of the solutes. If the electrical power dissipated in the column is W,

$W = I^2R = I \times V$

where R = bulk resistance of material in column and I = current passing through column. The consequent increase in temperature [14] is:

$\Delta T = 0.24\ Wr^2/4K$

where r = internal radius of column, and K = thermal conductivity of the medium.

As ΔT increases, the mobility of the solutes increases exponentially. Calculated values [12] show the increase in mobility to be 2% per °C. This increase in temperature results the loss of resolution, the band broadening. Band broadening can be minimized in three different ways: one, the internal radius of columns can be kept very small, to the order of a few mm, two, a constant temperature circulator can be used to maintain the column jacket at a constant, low temperature, and three, pulsed voltage patterns can be employed such that the electrical power dissipated is lower than in the constant voltage case. For example, if a constant dc voltage V_{dc} is applied and a current I_{dc} results, then the power dissipated in the column is:

$W_{dc} = I_{dc} \times V_{dc}$.

If the constant voltage is replaced with a square wave voltage pattern varying between 0 and V volts, then the effective voltage applied is V_{rms} and an effective current of I_{rms} results. The power dissipated in this case is:

$W_{square} = I_{rms}V_{rms}$

Since W_{square} is less than W_{dc}, Joule heating, and hence band broadening can be limited by applying different voltage patterns instead of dc voltage.

Observations

The high-voltage chromatography equipment was tested for electrochromatography. An electric potential was applied to a reversed-phase (C-18) column (Fig. 2) and a mixture of deoxyribonucleotides to the column. The separation was carried

out as described elsewhere (15,16]. Three sets of experiments were performed: (a) no electric potential applied, (b) a 10 kV potential applied while maintaining the positive electrode (anode) at the exit side of the column, and (c) a 10 kV potential applied after reversing the polarity, *i.e.* with anode towards the entry side of the column. In the first case, four distinct peaks were obtained corresponding to the four nucleotides, C, A, G, and T. In the second case, peaks were sharper and less diffused, *i.e.* $W_{1/2}$ to peak height ratio for each peak was approximately 15% less than the corresponding ratio for the experiment carried out without any electric potential. In addition, the capacity factors and plate heights were increased and retention times decreased with the application of the high voltage. In the third case, the results were reversed, *i.e.* peaks were diffused and retention times increased.

Each deoxynucleotide exhibits a net negative charge of −1.0 at pH 5.5. They are resolved because of differences in their hydrophobic character, which offers affinity for the hydrophobic matrix [17,18]. By introducing an electric field, the polar nucleotides exhibit more affinity towards the mobile phase (a mixture of methanol and buffer) and orients towards the opposite charge, *i.e.* the anode located towards the exit port. By reversing the voltage, essentially the same effect is observed, but in the opposite direction of the mobile phase since anode is now located towards the entry port. Application of this equipment for the separation of DNA fragments involving electrochromatography and capillary gel electrophoresis principles is currently under study and the results will be reported elsewhere.

DISCUSSION

The capillary electrophoresis technique has been in the forefront of the separation technology in recent years. This powerful method has rapidly achieved the status of a realistic technique for resolving extremely small amounts of samples, such as at nanomolar level. Several capillary electrophoresis systems are available commercially. In comparison to these commercial systems, the high voltage chromatography equipment developed here has many novel features.

The equipment consists of three modular subsystems. All the subsystems are independent modules and their components can be easily substituted to adapt to the needs of a particular set of experiments. For example, depending upon the type of solute molecules being separated, different chromatography detectors, such as a single- or a dual-wavelength detector, a fluorescent monitor, and a diode-array detector can be used without any modification to the rest of the equipment. The

TABLE 1

A Comparison of Different High Voltage Chromatography Equipment.

Systems: *Specifications*	1	2	3	4	5	6	7	8
Voltage, kV	30	30	30	12	30	30	0.3	30
Current, mA	4.5	0.3	0.25	0.2	1	0.3	0.1	0.25
Regulation[a]	prog[b]	V	V, I, P	V, I	V, I, P	V, I	V, I	V
Dual supplies	revers.[c]	yes	yes	yes	yes	revers.[c]	yes	yes
Heat control	circ. cool.[d]	air bath	circ. cool.[d]	none	forced air	forced air	none	forced air
Temp. range, °C	-20 to 100	5 to 60	15 to 50	none	ambient	4 to 38	none	ambient
Capillary length (max.), cm	no limit	38	50	12	1	100	60	none
Price (approx.), $	–	39,000	31,190[e]	17,500	37,500	18,000	53,000[f]	28,000

1. Equipment described in this article.
2. Model 270A, Applied Biosystems, Foster City, CA.
3. Model P/ACE 2000, Beckman Instruments, Fullerton, CA.
4. Model HPE 100, Bio-Rad Lab., Richmond, CA.
5. Model CES 1, Dionex Corp., Sunnyvale, CA.
6. Model 3850, Isco, Lincoln, NE.
7. Model 1200, Microphoretic Systems, Sunnyvale, CA.
8. Model Quanta 4000, Waters Chromatography Division, Milford, MA.

[a]The equipment offers constant voltage (V), or current (I), or voltage and current, i.e., power (P).

[b]The regulation is programmable. The main feature of the equipment is its ability to generate different high-voltage waveforms.

[c]The output voltage is reversible.

[d]Circulating coolant.

[e]Includes software.

[f]Includes computer and printer.

design of the equipment allows it to be used for three different separation methods: (a) capillary electrophoresis, (b) electrochromatography, and (c) HPLC. The equipment uses fused silica and PEEK capillaries with appropriately modified on-column detector for capillary electrophoresis. Both, capillaries and chromatography columns can be employed for electrochromatography. For the HPLC mode of operation, the power supply can be simply turned off. Moreover, the equipment uses chromatography columns and capillaries of different dimensions and characteristics appropriate for resolving different types of biomolecules. Thus, this equipment can be adapted for a wide range of applications.

A General Purpose Instrumentation Bus (GPIB) is employed for communication and control of the equipment. GPIB offers the best choice among the four important factors of performance: ease of operation, high speed of communication, low cost, and upgradability. In comparison to other commonly used communication protocols, such as RS232C, GPIB has a much higher speed of operation and an ability to connect up to 32 devices to one bus driver. Thus the data acquisition interface can easily be expanded from the present two channels to a maximum of 62 channels. Moreover, multiple devices can be connected to a common GPIB, for example, it is possible to develop an integrated equipment where the isocratic pump and constant temperature circulator can be controlled by a PC via the GPIB interface. Such an integrated system can be implemented at a cost much lower than the commercial systems.

In contrast to most of the commercial capillary electrophoresis systems that use a fixed-polarity dc power supply, our equipment employs a reversible-polarity power supply [Table I]. This allows bidirectional application of the voltage with two distinct advantages. One, both positively and negatively charged solutes can be resolved by selective control of the electrophoretic mobility. Two, the reverse polarity allows control over the selection of live electrode, consequently permitting the use of non-insulated flow cells for the chromatography detector. All functions of the power supply, such as the output voltage, the current limits, and the overload responses are programmable by the PC. These features of the power supply provide the maximum flexibility in applying voltage to chromatography columns and capillaries.

Joule heating greatly affects the reproducibility of chromatography results in capillary electrophoresis [19]. For example, if electrophoretic separation is per-

formed in a capillary exposed to random air currents at the room temperature, there is a sharp difference in the results from one experiment to another [20]. The effect of Joule heating in commercially available equipment is most commonly regulated by circulating cold air in the capillary enclosure in order to uniformly dissipate heat along the entire length of the capillary. Our high voltage chromatography equipment uses a novel technique to limit band broadening. By using different voltage waveforms to limit the effect of Joule heating, our equipment emphasizes on limiting the heat *generated*, whereas, in commercial systems, the heat is *dissipated* after it is evolved [20, 21].

The equipment uses custom-designed software for precise control of the high voltage. The software controls the power supply functions, monitors the programmed parameters during a run, and provides the necessary user interface for control of the power supply. Since the software has been implemented in a modular fashion, the necessary modules can easily be added to manage additional data acquisition interfaces and detectors added to the existing equipment. Moreover, the software can also be easily modified in order to centrally control additional chromatography components, such as a pump, a chromatography detector, and a constant temperature circulator.

Thus, the high voltage chromatography equipment developed here offers the use of high voltage to enhance the chromatographic separations. It can be used to investigate the combined effect of the principles of capillary electrophoresis and HPLC. The equipment is versatile and can be used for capillary electrophoresis, HPLC, and electrochromatography. It uses a computer-controlled power supply to generate high voltage waveforms which limits band broadening. In addition, the equipment is flexible to have ample scope for future additions and expansions.

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REFERENCES

1. Compton, S. W. and Brownlee, R. G., Capillary Electrophoresis, BioTechniq. 6, 5, 432 (1988).

2. Jorgenson, W. J. and Lukacs, K. D., Zone electrophoresis in open-tubular glass capillaries, Analytical Chemistry 53, 1298 (1981).

3. Cohen, A. S., Paulus, and Karger, B. L., High-performance capillary electrophoresis using open tubes and gels, Chromatographica 24, 14 (1987).

4. Eby, M. J., The reality of capillary electrophoresis, Bio/Technol. 7, 903 (1989).

5. Everaerts, F. M., van de Goor, A. A. A. M., Verheggen, P. E. M., and Beckers, J. L., Electrophoresis versus electrochromatography, J. High Resolution Chromatogr. 12, 28 (1989).

6. Rudge, S. R. and Ladisch, M. R., Electrochromatography, Biotechnol. Progr. 4, 3, 123 (1988).

7. Otsuka, S. and Listowsky, I., High resolution preparative electrochromatography for purification of two subunit types of ferritin, Analyt. Biochem. 102, 419 (1980).

8 O'Farrell, P. H., Separation techniques based on opposition of two counteracting forces to produce a dynamic equilibrium, Science 227, 1586 (1985).

9. Antrim, R. F. and Yacynych, A. M., The effect of supporting electrolytes in electrochromatography, Analyt. Let. 21, 1085 (1988).

10. Antrim, R. F., Scherrer, R. A., and Yacynych, A. M., Electrochromatography -- a preliminary study of the effect of applied potential on a carbonaceous chromatographic column, Analyt. Chem. Acta 164, 283 (1984).

11. Tsuda, T., Electrochromatography using high applied voltage, Analyt. Chem. 59, 521 (1987).

12. Tsuda, T., Chromatographic behavior in electrochromatography, Analyt. Chem. 60, 1677 (1988).

14. Cohen, A. S., Najarian, D., Smith, J. A., and Karger, B. L., Rapid separation of DNA restriction fragments using capillary electrophoresis, J. Chromatogr. 458 323 (1988).

15. Singhal R. P. and Landes, J. P., High-performance liquid chromatographic analysis of DNA composition and DNA modification by chloroacetaldehyde, J. Chromatogr. 458, 117 (1988).

16 Singhal, R. P., Landes P., Singhal, N. P., Brown, L. W., Anevski, P. J., and Toce, J. A., High-performance liquid chromatography for trace analysis of DNA and Kinetics of DNA modification, Biochromatogr. 4, 78 (1989).

17. Singhal, R. P., High performance liquid chromatography of transfer RNAs: separation of transfer RNAs from mammalian sources, J. Chromatogr. 266, 359 (1983).

18. Singhal, R. P. and Cohn, W. E., Analytical separation of nucleosides by anion-exchange chromatography, Analyt. Biochem. 45, 585 (1972).

19. Grushka, E., McCormick, R. M., and Kirkland, J. J., Effect of temperature gradients on the efficiency of capillary zone electrophoresis separations, Analyt. Chem. 61, 241 (1989).

19. Kansal A. K., Parkhurst, W. R., and Singhal, R. P., Computer control of a high-voltage power supply for capillary electrophoresis and electrochromatography (to be submitted to this journal, 1990).

20. Isco Applications Lab Staff, High-performance capillary electrophoresis using a modular system, *Isco* Applications Bull. 65 1 (1989).

21. Berry, V., HPCE '90: The second international symposium on high performance capillary electrophoresis, Part I, LC.-GC 8, 485 and 548 (1990).