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Note

Automated data acquisition and control system for isotachophoresis

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Isotachophoresis (ITP) has proved to be a valuable technique for quantitative determinations of ionic compounds[1,2]. Until very recently commercial instrumentation provided only strip chart output of the signal. Measurements of zone lengths and heights had to be made manually. To reduce the total analysis time and to provide more reliable measurements, we have developed an automated data acquisition and control system for an LKB (Bromma, Sweden) isotachophoresis analyzer.

Several automated data handling systems have been proposed. Mulder and Zuska[3] presented timing circuits for recording lengths of ITP zones. The most complete system to data was proposed by Reijenga and Kroonenberg[4], in which the signal from the conductivity detector was digitized by an 8-bit analog-to-digital (A/D) converter interfaced to a microcomputer. A unique feature of this system is that the data is stored as time counts *versus* conductivity, with the number of counts being proportional to the length of the zone at that conductivity. Finally, Shimadzu (Tokyo, Japan) has recently introduced a data reduction unit for its IP-2A analyzer.

None of these systems completely fulfills our needs for data acquisition, reduction and instrument control. The Shimadzu instrument prints information concerning zone number, length and height, but offers no way of correlating zone numbers with strip chart traces. Reijenga and Kroonenberg's conversion scheme results in some time based information being lost, *e.g.*, the order in which the zones migrate.

The system presented here is based on a Hewlett-Packard (Corvallis, OR, U.S.A.) HP-85 microcomputer interfaced to an LKB 2127 analyzer. Software has been written to acquire differential conductivity data, calculate zone lengths and heights, print out a record of the differential and reconstructed analog signal with zones labeled, and control the current and recorder chart drive of the 2127.

EXPERIMENTAL

Data acquisition and instrument control

A block diagram of the major hardware components of the system is shown in Fig. 1. The signal is digitized using a 12-bit A/D converter (Datel Intersil ADC-EH12B2, Mansfield, MA, U.S.A.). The HP-85 is equipped with matrix and input-output (I/O) read-only-memory (ROMs) and a total of 32K random access memory (RAM). Communication with the A/D is accomplished through an HP-IB interface.

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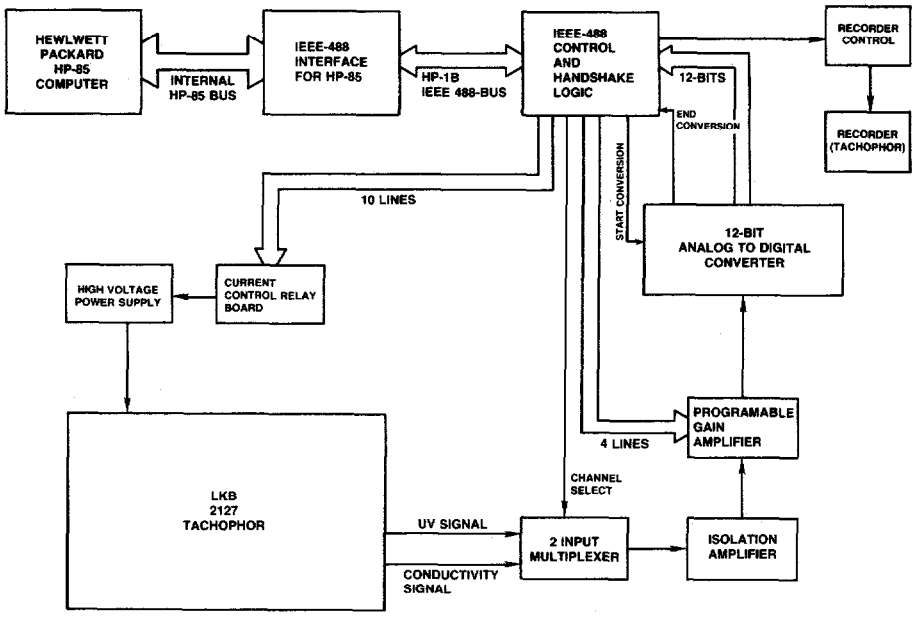


Fig. 1. Block diagram of isotachophoresis data acquisition and control system.

A two input multiplexer is used to allow sequential reading of either the differential conductivity or UV signal. Additional output lines are used to control the current setting and recorder. A relay board has been built into the 2127 power supply to allow the system to set the current from 0–450 μA in 50 μA increments. A TTL high-low signal is sent to the recorder to start the chart drive during data acquistion.

All software for data acquisition, reduction and experiment control is written in BASIC. Three internal timers are available with the HP-85. TIMER No. 1 is used to control the acquisition rate and can be set to the nearest msec. On timer interrupts, one differential signal data point is acquired and stored in an 8000 element integer array. An array pointer index is incremented and the program waits for the next interrupt. Data acquisition and index incrementing take 30 msec so the maximum sampling rate is 33 points/sec. TIMER No. 2 is used to stop data acquisition when the data array is full. The length of time for data acquisition can be set using TIMER No. 3.

Normal ITP runs are made at a high initial current which is reduced just prior to conductivity detection. Since the UV detector is mounted before the conductivity detector in the capillary, a change in the UV signal can be used to trigger current reduction and start data acquisition and the recorder chart drive. Alternatively, the current reduction can be timed using TIMER No. 3.

At a maximum sampling rate of 33 points/sec the HP-85 can store 4 min of data. For longer run times, a lower sampling rate must be used. Our normal sampling rate of 10 pts/sec allows sampling times up to 13.3 min. For a moderate zone length of 2 min this gives an accuracy of 0.1%.

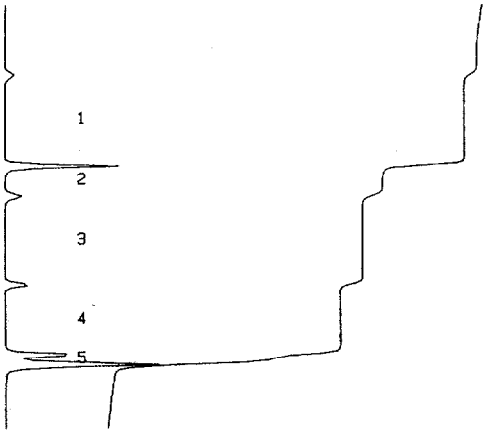
Data reduction

At the end of data acquisition, the raw differential data is stored in the 8000 element array. Data analysis consists of a peak-picking algorithm which identifies the array elements corresponding to zone boundaries. Since the data acquisition occurs at fixed and reproducible time intervals, element numbers of the differential conductance array are proportional to time. The algorithm steps through the array testing for maxima. If a maximum is found, it is tested against an operator-selected peak height parameter to reject non-significant maxima. After testing each data point, the previous conductivity value is added to the summation of earlier points and stored back in the original array. In this way the analog conductivity signal can be reconstructed from the differential data during analysis.

Both signals are plotted on the HP-85 graphics screen and dumped to the internal printer. The graphics screen is scaled so that the printed data will correspond to the length of the strip chart recording at 0.5 mm/sec. The raw differential data is treated by pages with each page being 200 sec of data. Each page of differential data is plotted, the analysis is performed and the reconstructed analog signal is plotted. That screen is dumped to the printer and the program moves to the next page of data. At the end of data analysis zone lengths and heights are calculated and printed.

Materials and methods

Isotachophoresis was done using an LKB 2127 Tachophor modified for cur-



ZONE	HEIGHT (0-1000)	LENGTH (SEC)
1	50	38.7
2	272	13.3
3	327	38.4
4	388	29.9
5	574	4.1

Fig. 2. Example of output from the automated data acquisition system for isotachphoresis. Zones correspond to 0.6 μg each. (1) Sulfate, (2) EDTA, (3) acetate and (4) phosphate. Zone 5 is an electrolyte impurity. Sampling rate, 10 points/sec; gain, 20 ×; initial current, 150 μA; final current, 50 μA; sensitivity, 10; UV start, manual stop.

rent control and data acquisition as described above. A 230 mm capillary of 0.5 mm I.D. with an LKB 2127-140 conductivity detector was used at 25°C. Chemicals used for the electrolytes were obtained from Sigma (St. Louis, MO, U.S.A.) except for hydroxy propyl methyl cellulose (HPMC) which was obtained from Aldrich (Milwaukee, WI, U.S.A.). Sodium or potassium salts for the anion mixture were obtained from Fischer Scientific (Pittsburgh, PA, U.S.A.). All chemicals were used as received.

RESULTS AND DISCUSSION

Fig. 2 shows the output from the HP-85 for an automatically recorded isotachopherogram. Run parameters are printed as a header followed by a trace of the conductivity signals and a listing of zone lengths and heights. Zone numbers are also labeled on the differential trace for easy correlation with strip chart recordings.

The mixture in Fig. 2 is an example of the types of anions that can be determined simultaneously by isotachophoresis. The leading electrolyte was 0.2% HPMC, 10 m*M* hydrochloric acid + 1-histidine to pH 6 and the terminator was 10 m*M* N-morpholinoethanesulfonic acid + tris(hydroxymethyl)aminomethane to pH 6. The initial current of 150 μA was reduced to 50 μA detection current using a UV trigger.

We have found automated data acquisition and control to be of great assistance for isotachophoretic analysis. Measured zone lengths are obtained upon completion of each run. By timing or UV-triggering current reduction and chart drive, the problem of missed zones is eliminated. The HP-85/LKB 2127 interface was designed and built in our laboratories specifically to include current control. If data acquisition alone is desired, off-the-shelf components such as a microcomputer and a digital multimeter could be used for automatic data recording and analysis. Additional capabilities including treatment of UV data, quantitative calculations and isotachopherogram library storage make automation of isotchophoresis equipment very attractive.

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