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DATA ACQUISITION IN CAPILLARY ISOTACHOPHORESIS

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SUMMARY

A computer program was developed for data acquisition in capillary isotachophoresis. The program consists of two modules, one for data acquisition and the other for data analysis. The data analysis module calculates zone lengths and step heights automatically. This can also be done by the operator on a graphics screen. The program was tested on the analysis of both a thirteen-component standard mixture and a more complex sample.

INTRODUCTION

During the last decade, capillary isotachophoresis (ITP) has been increasingly used for complex samples, but an important drawback is that the zone lengths and step heights still have to be measured manually. The few computer programs[1–3] that have been developed are sufficiently accurate for standard mixtures, but for complex mixtures they still have major limitations.

The program developed by Reijenga *et al.*[1] is based on conversion of the conductometer signal to a signal with chromatographic properties. This was done for two reasons: the smaller amount of memory in the computer needed for data storage, and the fact that a commercial chromatographic signal-processing system can process the converted data and calculate the zone lengths (surface of the peak) and the step heights (time of the peak). Disadvantages of this method of measurement are the loss of time information: a zone with a smaller step height than that for the previous zone, *i.e.*, an enforced zone[4,5] cannot be recognized as such, and the fact that a slightly increasing or decreasing zone can be subdivided by the computer program into more zones[6].

Stover *et al.*[2] developed a program based on a Hewlett-Packard HP-85 microcomputer. In this program, the differentiated conductivity signal is acquired via a 12-bit analogue-to-digital converter (ADC) and the original isotachopherogram can be reconstructed from these differentiated data. A disadvantage of this system is that slightly increasing or decreasing zones will be reconstructed as straight zones. As a consequence, an error may occur in the step-height measurement. In a later version of the program[3] both the analogue and the differentiated signal were acquired. The analogue signal was used for terminator recognition and step-height measurement,

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and zone lengths were determined from the differentiated signal. This program belongs to a system for complete automation of capillary isotachophoresis and will be sufficient for simple isotachophoretic experiments.

A computer program, however, should provide more possibilities for the user, *e.g.*, to manipulate some thresholds, if the automatic data processing fails. This may happen, for example, with complex mixtures. A new program has therefore been developed that possesses, in addition to possibilities for automatic data handling, several routines for manual data analysis, data smoothing and zone-type determination (straight zone, increasing or decreasing zone).

EXPERIMENTAL

The program, written in Turbo Pascal 4.0 (Borland International, Scotts Valley, Ca, U.S.A.), runs on an IBM MS-DOS computer (IBM, Boca Raton, FL, U.S.A.) with a LabMaster ADC (Scientific Solutions, Solon, OH, U.S.A.). The program supports several graphics cards (Hercules, CGA, EGA and VGA).

The program was tested by analysing: a standard sample consisting of thirteen components and a more complex sample, beer ("Bavaria", Lieshout, The Netherlands). This beer sample was taken because the isotachopherogram consisted of both large and small zones.

The zone lengths and step heights were measured both manually on a recorder and by the computer. The analyses were carried out on the laboratory-made equipment described by Everaerts *et al.*[4]. The diameter of the capillary was 0.2 mm and the length was 20 cm. The driving current, delivered by a high-voltage supply (LKB, Bromma, Sweden), was 25 μA. The electrolyte system used for both samples is listed in Table I. The analogue signal of the conductivity detector was digitized by the ADC, which was connected with the IBM computer. The analogue signal was also plotted on a BD41 line-feed recorder (Kipp & Zonen, Delft, The Netherlands) for the manual determination of the step heights. The electronic differentiated conductivity signal, plotted on the recorder, was used for the manual determination of the zone lengths.

TABLE I

ELECTROLYTE SYSTEM FOR THE ITP ANALYSIS OF THE TEST MIXTURE (FIG. 4) AND BEER (FIG. 5)

Leading ion	Chloride
Concentration	0.01 *M*
pH	6.0
Counter ion	Histidine (Merck, Darmstadt, F.R.G.)
Additive	0.2% Hydroxyethylcellulose (Polysciences, Warrington, PA, U.S.A.)
Terminating ion	Morpholinoethanesulphonic acid (MES) (Sigma, St. Louis, MO, U.S.A.)
Concentration	0.005 *M*

RESULTS

Description of the program

Fig. 1 shows the structure of the program. It consists of two modules, a data acquisition and a data analysis module.

Data acquisition module (Fig. 1A)

This is for the regulation of the data collection of the analogue signal of the conductivity detector by the ADC and for writing the data to floppy disk. This module consists of three parts:

"Measure". This starts the data acquisition. The sampling frequency can be varied from 1 to 30 000 Hz. For most routine analyses a sampling frequency of 40 Hz will be satisfactory. During the measurement a real-time plot is displayed on the

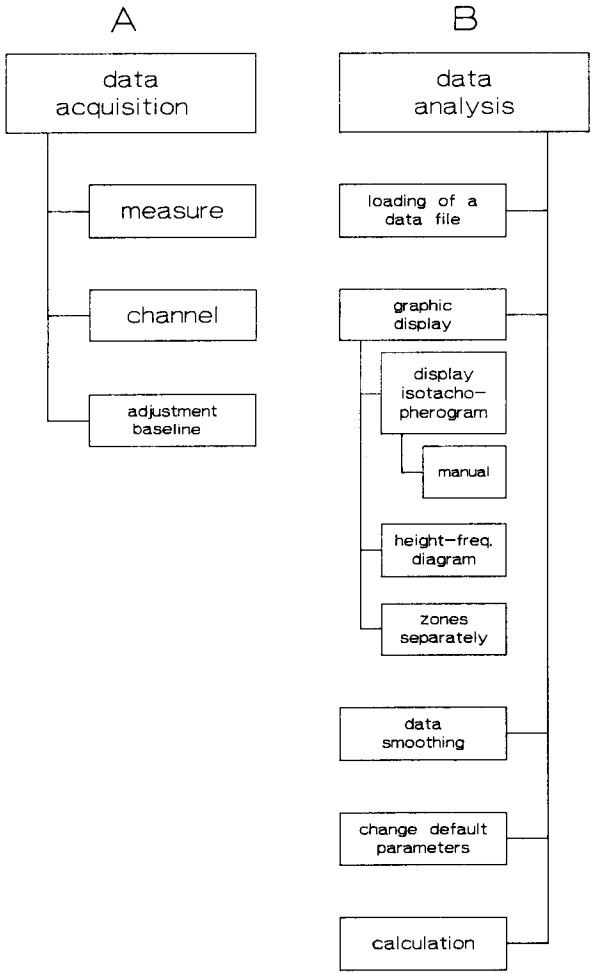

Fig. 1. Outline of the computer program.

screen. After the measurement the user is asked for a name under which the datafile has to be saved.

"Channel". This changes the channel number.

"Adjustment baseline". This adjusts the base line above zero.

Data analysis module (Fig. 1B)

This is for the automatic or manual calculation of the step heights and zone lengths. This module consists of six parts:

Loading of a datafile. This part serves for the loading of an earlier saved datafile from the floppy disk into the RAM of the computer and a first rough definition of the zone borders and a zone-type determination. For the determination of the zone borders the isotachopherogram is transformed into a height-frequency diagram as described[1]. The values which exceed the noise level are indicated as zones, provided that the distance between two zones is larger than the value of the parameter "minimal zone distance". In Fig. 2 zones 1, 2 and 5 are recognized by the program as separate zones, whereas zones 3 and 4 are linked. The peak between 1 and 2 is not identified as a zone at this noise level. Finally, a routine "zone-type determination" attributes to each zone a zone-type number: 1 for a straight zone, 2 for a decreasing zone, 3 for an increasing zone and 4 for a small zone.

Graphics display of the results. With three graphics screen plot functions, the determination of the zone borders can be examined. The first function can display the whole isotachopherogram, the inflection points and estimated zone borders. The

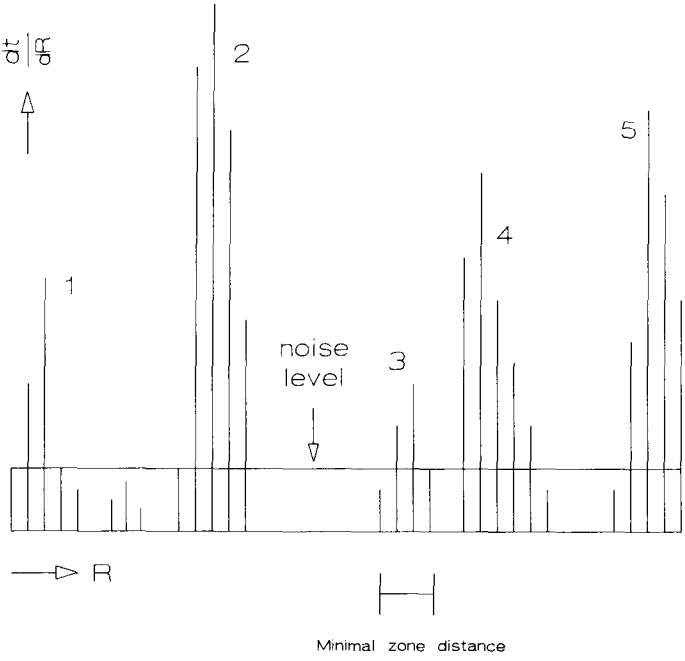

Fig. 2. A converted diagram of a isotachopherogram according to Reijenga *et al.*[1]. t = time; R = electric resistance.

second function plots the height–frequency diagram (Fig. 2). The third function plots the zones separately.

Data smoothing. This part smooths the data when the noise is too high. This routine works with a digital noise filter; the value of a certain point is recalculated from the mean of the surrounding points, where the contribution of each point to the mean value depends on the distance to the original point. In this routine a 25-point filter is used according to the theory of Savitsky and Golay[7,8].

Change default parameters. This part gives the possibility of changing the (default) parameters "noise level" and "minimal zone distance".

Calculation. In this part the step heights and zone lengths are calculated automatically. First, the inflection points are determined. The inflection points are the maxima of the first derivative of the signal. The first derivative is calculated with a Savitsky and Golay filter as described under *Data smoothing.* The parameter "differentiation limit" determines the minimal value that the first derivative must fulfil in order to be recognized as an inflection point. The parameter "differentiation limit" can be entered by the user before the automatic calculation. In flat zone borders, where the first derivative is below this value, no inflection point will be found. The inflection point is estimated from the middle between the temporary zone borders. The zone length is now determined from the distance between two inflection points.

The step height is determined separately for each zone type:

Zone type 1 (straight zone): the mean of all points between the left and right border for which the first derivative is zero.

Zone type 2 (decreasing zone): the highest point between the left border and right zone border.

Zone type 3 (increasing zone): the mean of the step height of the first part (40 points) of the zone to obtain reproducible step heights for zones of different lengths.

Zone type 4 (small zone): the step height of very small zones (fewer than 40 points) is determined from the mean step height of all points between the left and right zone border.

Finally, the results of this part are printed. An example of this is shown in Table II, where a computer output is shown of the analyses of the beer sample (Fig. 5).

"Manual" determination of the zone characteristics. If the zones are not correctly determined in the previous part, then with this part zone lengths and step heights can be determined "manually". This means that the zone lengths and step heights can be determined on the screen.

Starting with the plot procedure "graphic display of results", the whole isotachopherogram is displayed on the screen (Fig. 3). If necessary it is possible to enlarge a certain part of the isotachopherogram. For the determination of the zone lengths two vertical lines can be displayed on the screen. With the cursor keys the lines can be moved. Under the isotachopherogram the distance between the two lines is displayed. For determining the length of a certain zone the lines have to be moved to the inflection points of the zone. The zone length is then displayed under the isotachopherogram. The minimal error of the "manual" zone length determination is 0.025 s (at 40 Hz) with full enlargement of the isotachopherogram.

Analogously the step height can be determined with horizontal lines.

TABLE II

EXAMPLE OF OUTPUT FROM THE COMPUTER AFTER AN AUTOMATIC CALCULATION

Sample, 1 μl of beer (see Fig. 5). Ba6 = sample name; Diff. Limit = differentation limit; Min. Zone Dis. = minimal zone distance; Type = see text; I/Z = method of zone length calculation (I = between inflection points; Z = between zone borders if one or two inflection points are not found); SH = step height; RSH = relative step height; ZL = zone length.

Ba6	Noise Level= 3			Diff. Limit= 25	Min.Zone Dis.= 5	
	Zone	Type	I/Z	SH	RSH	ZL
Leading		3	-	224	0.0000	-
	1	1	I	327	0.0295	3.40
	2	1	I	382	0.0453	2.38
	3	4	I	619	0.1132	1.22
	4	3	I	692	0.1342	16.27
	5	1	I	770	0.1565	6.95
	6	3	I	880	0.1881	2.25
	7	3	I	1044	0.2351	4.52
	8	3	Z	1141	0.2629	12.30
	9	1	Z	1350	0.3228	70.70
	10	3	I	1363	0.4134	14.00
Terminator		2	-	3712	1.0000	-

Testing of the program

Fig. 4 shows the isotachopherogram of a standard mixture. In Table III the results are shown of the zone length measurements for five analysis of the sample with an injection amount of 4 μl, which corresponds to an absolute injection amount of 1 nmol for each component. To test the program for small zones, we also analysed 1 μl. These results are also shown in Table III. In Table IV the results are shown for the relative step-height measurements for the same injection amounts.

It can be seen that the automatic measurements for both the zone lengths and step heights agree with the manual measurements. The coefficients of variation of the zone-length measurements vary from 0.5 to 3% for both methods. With the manual measurement, however, a recorder speed of 30 cm/min was used, whereas the normal speed is 6 cm/min. If the latter speed had been used a larger error (30 cm/min ± 0.1 s,

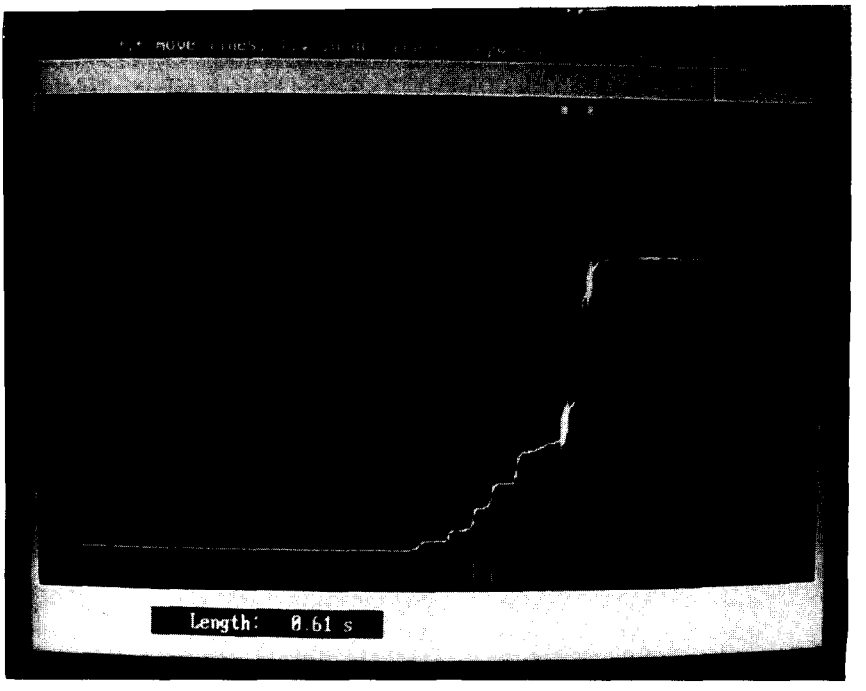

Fig. 3. Screen display of an isotachopherogram.

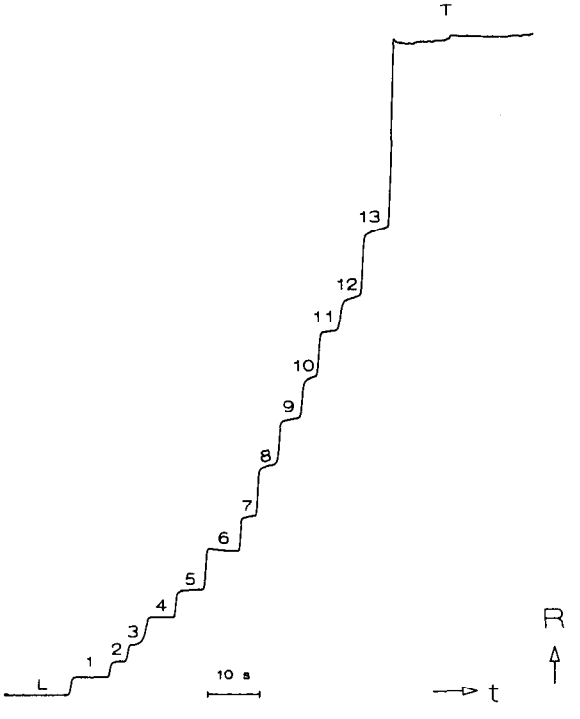

Fig. 4. Isotachopherogram of a test mixture analysed using the electrolyte system in Table I. L = Leading; 1 = sulphate; 2 = chlorate; 3 = chromate; 4 = malonate; 5 = pyrazole-3,5-dicarboxylate; 6 = adipate; 7 = acetate; 8 = β-chloropropionate; 9 = benzoate; 10 = naphthalene-2-monosulphonate; 11 = glutamate; 12 = enanthate; 13 = benzylaspartate; T = terminator. t = time; R = resistance.

TABLE III

MEAN AND STANDARD DEVIATION (S.D.) FOR THE MANUAL (USING A LINE FEED RECORDER) AND AUTOMATIC (USING THE COMPUTER) ZONE-LENGTH MEASUREMENTS ON THE TEST MIXTURE (n = 5)

The numbers correspond to the zones indicated in Fig. 4.

Zone No.	*Injection amount*							
	1 nmol				*0.25 nmol*			
	Manual		*Computer*		*Manual*		*Computer*	
	Mean (s)	*S.D.*	*Mean (s)*	*S.D.*	*Mean (s)*	*S.D.*	*Mean (s)*	*S.D.*
1	7.39	0.42	7.32	0.48	2.16	0.10	2.16	0.08
2	3.25	0.00	3.24	0.04	1.21	0.02	1.20	0.05
3	3.35	0.06	3.38	0.06	0.79	0.02	0.79	0.03
4	5.42	0.06	5.43	0.08	1.56	0.02	1.54	0.02
5	5.31	0.02	5.32	0.04	1.50	0.03	1.50	0.02
6	6.09	0.04	6.09	0.07	2.38	0.07	2.37	0.06
7	3.15	0.06	3.12	0.07	1.03	0.03	1.03	0.03
8	3.78	0.06	3.76	0.05	1.18	0.02	1.19	0.04
9	4.02	0.04	4.03	0.05	1.23	0.01	1.24	0.01
10	2.83	0.06	2.84	0.06	0.86	0.02	0.86	0.01
11	3.77	0.07	3.81	0.07	1.06	0.01	1.07	0.01
12	3.71	0.08	3.73	0.09	1.02	0.02	1.02	0.03
13	4.99	0.16	5.02	0.18	1.43	0.03	1.41	0.02

TABLE IV

MEAN AND STANDARD DEVIATION (S.D.) FOR THE MANUAL (USING A LINE FEED RECORDER) AND AUTOMATIC (USING THE COMPUTER) RELATIVE STEP-HEIGHT MEASUREMENTS ON THE TEST MIXTURE (n = 5)

The numbers correspond to the zones indicated in Fig. 4.

Zone No.	*Injection amount*							
	1 nmol				*0.25 nmol*			
	Manual		*Computer*		*Manual*		*Computer*	
	Mean	*S.D.*	*Mean*	*S.D.*	*Mean*	*S.D.*	*Mean*	*S.D.*
1	0.029	0.0007	0.029	0.0005	0.029	0.0009	0.030	0.0006
2	0.051	0.0000	0.051	0.0007	0.051	0.0006	0.053	0.0006
3	0.078	0.0005	0.079	0.0012	0.081	0.0009	0.084	0.0010
4	0.119	0.0005	0.119	0.0008	0.116	0.0009	0.116	0.0007
5	0.161	0.0005	0.161	0.0007	0.160	0.0020	0.160	0.0008
6	0.219	0.0005	0.223	0.0011	0.222	0.0027	0.221	0.0011
7	0.269	0.0007	0.269	0.0007	0.275	0.0010	0.273	0.0011
8	0.348	0.0018	0.347	0.0008	0.353	0.0040	0.350	0.0017
9	0.419	0.0019	0.418	0.0015	0.424	0.0026	0.419	0.0018
10	0.483	0.0009	0.483	0.0015	0.487	0.0044	0.480	0.0022
11	0.552	0.0018	0.552	0.0018	0.555	0.0032	0.550	0.0017
12	0.605	0.0019	0.604	0.0021	0.603	0.0041	0.598	0.0023
13	0.709	0.002	0.705	0.0006	0.704	0.0047	0.699	0.0030

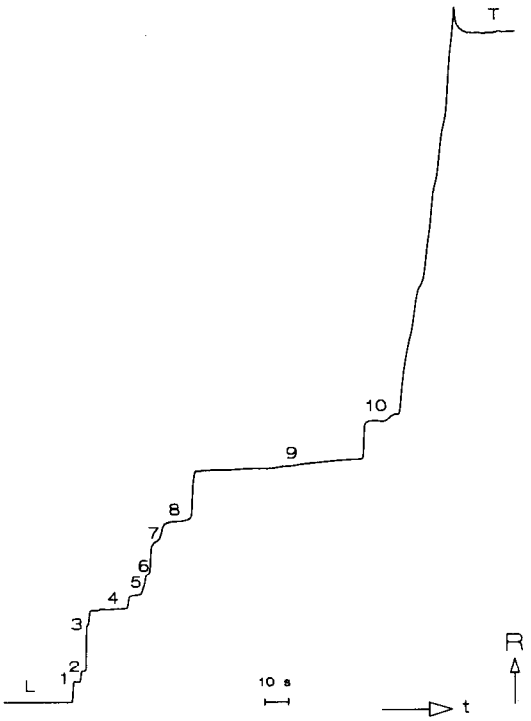

Fig. 5. Isotachopherogram of a beer sample analysed using the electrolyte system in Table I. L = Leading; 1 = sulphate; 3 = formate; 4 = malate; 5 = citrate; 10 = phosphate; 2,6,7,8,9 = not identified; T = terminator. t = time; R = resistance.

TABLE V

MEAN AND STANDARD DEVIATION (S.D.) FOR THE MANUAL (USING A LINE FEED RECORDER) AND AUTOMATIC (USING THE COMPUTER) ZONE-LENGTH AND RELATIVE STEP-HEIGHT MEASUREMENTS ON THE BEER SAMPLE (n = 10)

The numbers corresponds with the zones indicated in Fig. 5. The numbers in parenthesis denote the number of zones which are measured "manually" using the graphics screen plot.

Zone No.	*Zone length*				*Step height*			
	Manual		*Computer*		*Manual*		*Computer*	
	Mean	*S.D.*	*Mean*	*S.D.*	*Mean*	*S.D.*	*Mean*	*S.D.*
1	3.27	0.17	3.30	0.19	0.029	0.0003	0.030	0.0004
2	2.31	0.09	2.34	0.07	0.044	0.0004	0.045	0.0003
3	1.21	0.01	1.22	0.02	0.111	0.0010	0.113	0.0010
4	16.20	0.10	16.18	0.12	0.136	0.0015	0.134	0.0013
5	6.93	0.05	6.96	0.07(1)	0.158	0.0012	0.157	0.0010
6	2.19	0.02	2.22	0.04	0.185	0.0020	0.188	0.0015
7	4.57	0.07	4.55	0.09(1)	0.236	0.0015	0.234	0.0019
8	12.60	0.35	12.55	0.58(3)	0.264	0.0025	0.262	0.0023
9	69.45	1.20	69.53	1.30	0.325	0.0039	0.327	0.0042
10	14.40	0.37	14.37	0.41(1)	0.410	0.0018	0.412	0.0024

60 cm/min ± 0.5 s), due to the thickness of the recorder pen, could be expected for the manual measurements, especially for small zones.

Finally, the program was tested with a complex sample. A 1-μl volume of beer was injected ten times and Fig. 5 shows the isotachopherogram obtained. The zone lengths and relative step heights of the ten zones were measured by the computer and manually, using the analogue differentiated signal on the recorder. The results are given in Table V. The automatic measurements correspond well with the manual measurements. In Table V those zones which were measured "manually", using the graphics screen plot, when the automatic data processing failed are indicated.

CONCLUSIONS

The use of this computer program for data acquisition in capillary isotachophoresis will expand its applications in routine analysis. The program can measure zone lengths and step heights automatically or "manually" using a graphics screen plot. For most applications the automatic data processing will be sufficiently accurate. For complex samples a manual correction may be necessary for some zones. Tests with the program have shown that the automatic measurements agree with the manual measurements.

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