Program in BASIC to combine the data from two channels of an integrator, and its use in the calculation of residues per 1000 residues for amino-acid analyses

K. John Cronin
Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, UK,

Paul G. Clarke
Trivector Systems Ltd, Sunderland Road, Sandy, Bedfordshire SG19 1RB, UK,

and John J. Harding
Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, UK

Introduction

Single-channel integrators have been used with gas-liquid chromatography (g.l.c.) equipment and amino-acid analysers for some years and are adequate for many g.l.c. applications. During amino-acid analysis with the conventional ninhydrin system most amino-acids give a blue product but others, notably proline and hydroxyproline, give a yellow product. These products are measured separately, so for manual calculation a two-pen, or three-pen, recorder has always been provided. Attempts to use a single-channel integrator for amino-acid analysis are inevitably unsuccessful. Measuring just the blue channel at 570 nm leads to gross inaccuracies for the small peaks of proline and hydroxyproline; combining the two colorimeter outputs leads to base-line problems, as does the use of an intermediate wavelength.

There are now integrators available that enable the output from two or more channels to be integrated, but it is not always possible to combine the results from two channels. Amino-acid analysis results for proteins have been presented as residues per 1000 residues (or per 100 residues)—this method was first introduced as an aid to comparative studies and for this it is necessary to take data for proline and hydroxyproline if present, from the 440 nm channel and combine it with data for all the other amino-acids from the 570 nm channel.

This paper presents a program that allows the stored data from two channels of a Trilab 3 integrator to be combined to give a full amino-acid analysis expressed in residues/1000 residues. The operator may elect to omit earlier blocks of data to reach an appropriate standard, and may also reassign peaks that were wrongly assigned in the automatic mode.

With minimal modification the program could be adapted to combine data from two g.l.c. runs on a single sample and express results in any convenient form.

Materials and methods

The outputs from the 570 nm and 440 nm colorimeters of an amino-acid analyser (the LKB 4400, produced by LKB Ltd, Milton Road, Cambridge, UK) are taken via head amplifiers into two of the four channels of a Trilab 3 integrator (Trivector Systems Ltd, Sunderland Road, Sandy, Bedfordshire, UK).

The manufacturer's operating system and chromatography programs are used for data collection, immediate print-out and storage on mini-cassettes (Philips Data Systems Ltd).

After data collection the manufacturer's basic compiler (Version 5.9) was loaded, followed by the program listed in figure 1.

The program

The REM statements make the program almost self-explanatory (figure 1). In line 40 the string 'B' represents the three letter codes for the expected amino-acids in order of elution; individual codes can then be extracted using the substring function (for example, line 120). Line 45 sets up the arrays for the two channels with the first array also serving for the assembly of the print-out. Lines 50 to 90 feed in the amount of each amino-acid in the standard analysis: 5 nmol for all amino-acids except hydroxyproline (10 nmol). The program moves on to line 200 and the operator is asked to load the DATA tape, and then the tape is opened (subroutine 11000 to 11020). The operator next has the opportunity to bypass any early runs (lines 231–235 and subroutine 17000 in figure 1; see the print-out in figure 2). The operator is then asked to enter the total number of runs before the standard data is read from the tape (subroutine 12000). Subroutine 11130 is also used at this stage—it converts strings that have been stored as numbers back to strings. The computer checks the channel before printing-out the number of the sample used as a standard. Lines 264 and 266 transfer the data for hydroxyproline and proline from the B channel array into the appropriate positions in the A channel. Lines 267–275, with subroutine 2000, allow new areas to be fed in for selected peaks where the automatic assignment has been incorrect.

Faulty assignment is usually caused by drifting retention times while the analyser is running overnight. Lines 278 to 330 then provide calculation of response factors for the standard and subroutine 100 allows them to be printed-out if required. From line 340 the program allows calculation of each run using subroutines 14000, 18200 and 11130 to read the data, the header and to change stored numbers to strings (line 14035 converts the retention-time units from seconds to minutes). Both channels are accessed before going to subroutine 17000 which bypasses the data for unnamed peaks. The areas for hydroxyproline and
proline, peaks 1 and 2 on the B channel, are transferred into the main channel array to be the first and seventh peaks respectively (lines 364 and 365). Next comes the reassignment routine (lines 367 to 376 including subroutine 2000), which allows new areas to be assigned to any of the amino-acids and sets the retention times of reassigned peaks to zero as a reminder. Then the concentrations are calculated (lines 379-400), and converted to residues/1000 residues (lines 430 to 730) before the report is printed (subroutine 16000) the array cleared (subroutine 18000) and the tape closed (line 999).

The print-out

A print-out demonstrating the options available is shown in figure 2. After the invitation to load a DATA tape the operator was asked if he wished to skip some blocks. He chose to do so as the first two chromatographic separations were poor. The print-out gave the sample numbers for the bypassed data and then after being told the total number of runs proceeded with the calibration. The operator chose to reassign three peaks in the basic region (HYL, HIS and LYS) which had been incorrectly assigned; and chose to have the standard concentrations, response factors and peak numbers printed. Moving on to the analysis of a lens protein hydrolysate again, the operator was invited to reassign peaks and did so choosing to set hydroxy-

Discussion

Changes to line 700 would enable the results to be presented in a different form, for example residues/100 residues and with a few extra lines residues/peptide or residues/g could be calculated. Exchange of the routines used to read data from tape would permit the use of this program with other microcomputer systems programmable in BASIC that are used as integrators. This program should have wide applicability to systems where data from two channels must be merged into a single analysis, whether the two channels represent two features of a single run, or two g.l.c., high-performance liquid chromatography or other chromatographic separations.

Acknowledgements

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Figure 1. BASIC program combining data from two channels and calculating amino-acid analysis results as residues/1000 residues.

Continued on pages 27–29
200 REM MASTER SEGMENT
210 ?"Please load DATA tape and press (RETURN)"
220 INPUT X$
230 GOSUB 11000:REM OPEN TAPE
231 INPUT("DO YOU WANT TO SKIP THE FIRST n BLOCKS? (YES/NO) ")Y$
232 IF Y$="NO" GOTO 240
233 INPUT("ENTER No. OF BLOCKS TO BE BYPASSED ")N
234 FOR K=1 TO N
235 GOSUB 17000
236 NEXT K
240 INPUT("How many pairs of runs including STD? ")R
245 GOSUB 12000:REM READ STD A(B)
246 GOSUB 12000:REM READ STD B(A)
247 REM STD PEAK REASSIGNMENT
249 INPUT("Do you want to reassign STD peaks or areas? (Y/N) ")Z$
250 IF Z$="N" GOTO 270
252 GOSUB 20000:REM REASSIGN
253 A(1,1)=A
255 GOTO 269
270 REM CALCULATE RESPONSE FACTORS
275 FOR I=1 TO R-1
278 A(1,0)=A(1,1)/A(1,2)
280 NEXT I
285 REM RESPONSE FACTOR= AREA/CONC.
286 GOSUB 100
287 REM CALCULATE EACH RUN IN TURN
289 FOR K=1 TO R-1
290 A(1,0)=A(1,1)/A(1,2)
295 NEXT K
296 REM SAMPLE PEAK ASSIGNMENT
299 INPUT("Do you want to reassign SAMPLE peaks or areas? (Y/N) ")Z$
300 IF Z$="N" GOTO 379
302 GOSUB 20000:REM REASSIGN
303 A(1,E,3)=A
305 A(1,E,2)=0.0
306 GOTO 369
309 REM CALCULATE CONCNs
310 FOR I=1 TO R-1
312 IF A(1,I,0)=0 THEN 400
313 A(1,I,4)=A(1,I,3)/A(1,I,0)
315 NEXT I
318 REM CONC=AREA/RESPONSE FACTOR
320 F=0
325 REM CALCULATE TOTAL CONCN
329 FOR I=1 TO R-1
330 F=F+A(1,I,4)
335 NEXT I
336 IF F=0 THEN F=1000
340 REM CALCULATE CONC/TOTAL*1000 FOR EACH PEAK
345 T=0
350 FOR I=1 TO R-1
355 A(1,I,5)=A(1,I,4)*1000/F
360 T=T+A(1,I,5)
365 NEXT I
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```
730 NEXT I
740 REM PRINT REPORT
750 GOSUB 16000
755 ?
756 ? "TOTAL RESIDUES = ";T[6.3]
757 ?
758 GOSUB 18000:REM CLEAR
760 NEXT K
999 CLOSE #DATA
1000 END
2000 REM REASSIGNMENT
2010 INPUT("Type NUMBER of peak to be reassigned ")E
2024 P=3*E-2
2025 ?SUBSTR$(B$P,3);?
2030 INPUT(" Type its AREA ")A
2040 RETURN
1100 REM OPEN TAPE
1101 OPEN IN#,DATA,TAPE,RA,"GLC RESULTS"
1102 RETURN
1110 H1(1)=H1(1)+64
1120 H1(0)=1
1130 CHANGE H1 TO H1$
1131 H2(0)=3
1140 CHANGE H2 TO H2$
1150 H3(0)=25
1160 CHANGE H3 TO H3$
1170 H4(0)=2
1180 CHANGE H4 TO H4$
1190 H5(0)=1
1200 CHANGE H5 TO H5$
1210 H6(0)=5
1220 CHANGE H6 TO H6$
1230 H7(0)=10
1240 CHANGE H7 TO H7$
1250 RETURN
1260 REM READ STD FROM A & B
1270 GOSUB 18200:REM READ HEADER
1280 IF G=1 THEN 999
1290 GOSUB 11130:REM CHANGE NO TO $
1291 IF H1$="B" THEN 13030
1292 ?"CALIBRATION OF CHAN A WITH ",& H2$ & H3$
1300 FOR I= 1 TO H8
1310 INPUT#DATA,N1,N2,N3,A1(I,1),N5
1320 NEXT I
1330 GOTO 13075
1340 ?"CALIBRATION OF CHAN B WITH ",& H2$ & H3$
1350 FOR I= 1 TO H8
1360 INPUT#DATA,N1,N2,N3,B1(I,1),N5
1370 NEXT I
1380 GOSUB 18200:REM SKIP
1390 RETURN
14000 REM READ SAMPLE A & B
1410 GOSUB 18200:REM READ HEADER
1420 IF G=1 THEN 999
1430 GOSUB 11130:REM CHANGE NO. TO $
1440 IF H1$="B" THEN 15015
1450 S1$=H2$
1460 FOR I= 1 TO H8
1470 INPUT#DATA,A1(I,1),N2,A1(I,2),A1(I,3),N5
1480 A1(I,2)=A1(I,2)/&0
```

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```basic
14040 NEXT I
14045 GOTO 15045
15015 S2* = H2*
15020 FOR I = 1 TO H8
15030 INPUT DATA, B1(I,1), N2, B1(I,2), B1(I,3), N5
15035 B1(I,2) = B1(I,2) / 60
15040 NEXT I
15045 GOSUB 17000: REM SKIP
15050 RETURN
16000 REM PRINT REPORT
16020 ? "SAMPLES " S1$ " & " S2$
16030 ? " RET TIME NAME AREA CONCN RESIDUES/1000"
16040 FOR I = 1 TO A2
16050 J = 3 * I - 2
16060 A$ = SUBSTR$(B$, J, 3)
16070 «TA(I,2)[6.3],
16080 TA$, 16089 TA(I,3)[6.3], A1(I,4)[6.3], A1(I,5)[6.3]
16100 NEXT I
16110 RETURN
17000 REM IGNORE NEXT SAMPLE ON TAPE
17100 GOSUB 18200: REM READ HEADER
17110 IF G = 1 THEN 999
17140 GOSUB 11130: REM CHANGE NO. TO $ 17150 IF HS* <> "$E" THEN "Skipping "$2$&H3$
17160 FOR I = 1 TO H8
17170 INPUT DATA, N1, N2, N3, N4, N5
17180 NEXT I
17190 RETURN
18000 REM CLEAR TABLE
18010 FOR I = 1 TO 23
18020 FOR J = 1 TO 5
18030 A1(I, J) = 0
18040 NEXT J
18050 NEXT I
18060 IF I = 1 TO 5
18070 FOR J = 1 TO 5
18080 B1(I, J) = 0
18090 NEXT J
18100 NEXT I
18110 RETURN
18200 INPUT #DATA, H1(1)
18210 IF H1(1) >= 1 THEN IF H1(1) <= 4 THEN 18240
18220 G = 1
18230 RETURN
18240 INPUT #DATA, H2(1), H2(2), H2(3)
18250 FOR I = 1 TO 25
18260 INPUT #DATA, H3(I)
18270 NEXT I
18280 INPUT #DATA, H4(1), H4(2)
18290 INPUT #DATA, H5(I)
18300 INPUT #DATA, H6(1), H6(2), H6(3), H6(4), H6(5)
18310 FOR I = 1 TO 10
18320 INPUT #DATA, H7(I)
18330 NEXT I
18340 INPUT #DATA, H8
18350 RETURN

End of figure 1.
```
Amino acid analysis res/1000 3.0 *kjc/pgc/jjh 1981
Please load DATA tape and press (RETURN)?
DO YOU WANT TO SKIP THE FIRST n BLOCKS? (YES/NO) YES
ENTER No. OF BLOCKS TO BE BYPASSED 8
Skipping B07
Skipping A07
Skipping B08
Skipping A08
How many pairs of runs including STD? 7
CALIBRATION OF CHAN B WITH B07
CALIBRATION OF CHAN A WITH A09
Do you want to reassign STD peaks or areas? (Y/N) Y
Type NUMBER of peak to be reassigned 17
HYL Type its AREA 682
Do you want to reassign STD peaks or areas? (Y/N) Y
Type NUMBER of peak to be reassigned 18
HIS Type its AREA 703
Do you want to reassign STD peaks or areas? (Y/N) Y
Type NUMBER of peak to be reassigned 19
LYS Type its AREA 775
Do you want to reassign STD peaks or areas? (Y/N) N
Do you wish to see STD concentrations (Y/N)? Y
Channel A:
|    |   |
|----|---|
| ASP | 5 |
| THR | 5 |
| SER | 5 |
| HIS | 5 |
| GLU | 5 |
| PRO | 5 |
| GLY | 5 |
| VAL | 5 |
| MET | 5 |
| ILE | 5 |
| LEU | 5 |
| TYR | 5 |
| PHE | 5 |
| HYL | 5 |
| HIS | 5 |
| LYS | 5 |
| ARG | 5 |

Channel B:

|    |   |
|----|---|
| HYP | 19 |
| PRO | 5 |

Figure 2. Print-out for the standard.
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Do you want to reassign SAMPLE peaks or areas? (Y/N) Y
Type NUMBER of peak to be reassigned 1
HYP Type its AREA 0
Do you want to reassign SAMPLE peaks or areas? (Y/N) Y
Type NUMBER of peak to be reassigned 18
HIS Type its AREA 3075
Do you want to reassign SAMPLE peaks or areas? (Y/N) Y
Type NUMBER of peak to be reassigned 19
LYS Type its AREA 5078
Do you want to reassign SAMPLE peaks or areas? (Y/N) N

| RET TIME | NAME | AREA    | CONCN  | RESIDUES/1000 |
|----------|------|---------|--------|---------------|
| 14.450   | ASP  | 8820.239| 79.189 | 115.108       |
| 16.550   | THR  | 3516.942| 28.793 | 41.852        |
| 17.650   | SER  | 7243.011| 56.008 | 81.412        |
| 21.150   | GLU  | 11767.428| 92.503 | 134.461       |
| 23.150   | PRO  | 1940.265| 34.498 | 50.145        |
| 26.000   | GLY  | 7315.697| 49.223 | 71.549        |
| 28.100   | ALA  | 4479.188| 30.682 | 44.599        |
| 33.200   | VAL  | 4927.038| 36.453 | 52.988        |
| 36.800   | MET  | 2201.389| 14.644 | 21.286        |
| 40.000   | ILE  | 4502.904| 29.704 | 43.178        |
| 41.450   | LEU  | 7914.270| 48.813 | 70.954        |
| 46.500   | TYR  | 4979.409| 35.735 | 51.944        |
| 48.200   | PHE  | 5970.130| 39.193 | 56.971        |
| 48.800   | HYD  | .000    | .000   | .000          |
| 49.000   | HIS  | 3075.000| 21.871 | 31.791        |
| 50.000   | LYS  | 5078.000| 32.761 | 47.621        |
| 75.000   | ARG  | 7122.040| 57.885 | 84.141        |

TOTAL RESIDUES = 1000.000

Figure 3. Print-out for lens protein.