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A COMPUTER PROGRAM IN BASIC FOR CALCULATING GRADIENTS USED IN CHROMATOGRAPHY, ELECTROPHORESIS AND CENTRIFUGATION

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Abstract—Concentration gradients are often used in separations based on chromatographic, electrophoretic and centrifugal methods. In this report, a BASIC computer program for calculating and graphically representing gradients is described. This GRADIENT program is intended to be run on IBM-compatible computers.

Centrifugation Chromatography Concentration gradients Density gradients Electrophoresis

INTRODUCTION

Gradients are widely used in a variety of methods for separation of complex chemical mixtures. These methods include various types of chromatography, electrophoresis and centrifugation.

The apparatus used to prepare these gradients commonly comprises two chambers, a supply chamber and a mixing chamber, containing solutions of different compositions. The solution from the supply chamber is gradually fed into, and mixed with, the contents of the mixing chamber while continuously removing solution from the mixing chamber. In this way the outflow starts off with a composition identical to that of the mixing chamber and gradually changes towards that of the supply chamber.

Depending on the relative flow rates into and out of the mixing chamber, the resulting gradients can be linear, convex (including exponential) or concave. These flow rates may be independently controlled using suitable pumps, or interdependent if one or both of these flows are controlled by hydrostatic pressure. In this latter case, providing both chambers are cylindrical, the relative flow rates are easily determined by knowing the cross-sectional areas.

The concentration (C) in the outflow at any time (t) may be calculated using expressions adapted from Lakshmanan and Lieberman [1].

$$C = C_m + f(C_s - C_m) \tag{1}$$

$$f = 1 - [1 - t(R_m - R_s)/V_m]\,[R_s/(R_m - R_s)] \tag{2}$$

where C_s and R_s represent the solute concentration in, and the flow rate from, the supply chamber while C_m and R_m are the corresponding parameters for the mixing chamber; V_m is the volume of the mixing chamber. In the special case in which R_s is equal to R_m, a different expression for f is used.

$$f = 1 - \exp(-tR_m/V_m). \tag{3}$$

These exponential gradients may be obtained by equalizing the pump rates or, in a gravity-fed apparatus, by using a closed vessel for the mixing chamber so that the volume V_m remains constant.

In this report I describe a BASIC computer program for calculating gradients according to these equations. This GRADIENT program is intended for IBM-compatible computers and requires some form of graphics display.

RESULTS AND DISCUSSION

A listing of the program is shown in Fig. 1 while the result from a typical run is illustrated by Fig. 2. The manner in which the program is used will be described with reference to the appearance of the display screen as shown by Fig. 2.

```
1000 '       P R O G R A M   G R A D I E N T
1010 '
1020 'A program to calculate gradients for chromatography.
1030 '
1040 'Written by R.G. Duggleby, Department of Biochemistry, University of
1050 'Queensland, St.Lucia, QLD  4067, Australia.
1060 '
1070 'The experimental setup consists of two chambers referred to as the
1080 '"Supply Vessel" and the "Mixing Vessel". The input variables are:
1090 '
1100 '    CS and CM - The concentrations in the supply and mixing vessels
1110 '    FS and FM - The flow rates out of the supply and mixing vessels
1120 '    VS and VM - The volumes of the supply and mixing vessels
1130 '    VT        - The total volume of the gradient
1140 '
1150 'The program calculates:
1160 '
1170 '    CT and TT - The solute concentration at time TT
1180 '
1190 'Set graphics screen parameters from a data statement. The values are:
1200 'Screen, Pixels Vertical and Pixels Horizontal
1210 'Typical combinations are
1220 'DATA  2,  200, 640:'CGA card
1230 'DATA  9,  350, 640:'EGA card
1240 '
1250 DATA  9,  350, 640: 'EGA card
1260 READ SM%, PV%, PH%: TR% = 25: TC% = 80: 'Always use 25 rows, 80 columns
1270 'Read default input values
1280 DATA  1,  1,  1,  2,  0,  2,  1
1290 READ CS, FS, VS, VT, CM, FM, VM
1300 '
2000 GOSUB 3000: 'Draw the experimental set-up
2010 GOSUB 4000: 'Input the variables
2020 GOSUB 5000: 'Draw the gradient
2030 GOSUB 6000: 'Tracking pointer mode
2040 GOSUB 7000: 'End
2050 GOTO 2000
3000 'Draw the experimental set-up
3010 KEY OFF : CLS : SCREEN SM%: IF SM% = 9 THEN PALETTE 1, 63
3020 LOCATE 8, 6: PRINT "Supply concentration   "; CS
3030 LOCATE 9, 6: PRINT "Supply flow rate       "; FS
3040 LOCATE 10, 6: PRINT "Supply vessel volume   "; VS
3050 LOCATE 11, 6: PRINT "Total gradient volume  "; VT
3060 LOCATE 8, 46: PRINT "Mixing concentration   "; CM
3070 LOCATE 9, 46: PRINT "Mixing flow rate       "; FM
3080 LOCATE 10, 46: PRINT "Mixing vessel volume   "; VM
3090 LOCATE 11, 44: ET = VT / FM: PRINT "[ Note total time is     "; ET; "]";
3100 FOR R% = 1 TO 4: LOCATE R%, 6: PRINT CHR$(179); SPACE$(20); CHR$(179)
3110 LOCATE R%, 46: PRINT CHR$(179); SPACE$(20); CHR$(179); : NEXT
3120 LOCATE 5, 6: PRINT CHR$(179); SPACE$(20); CHR$(198); STRING$(18, 205);
3130 LOCATE , 46: PRINT CHR$(181); SPACE$(20); CHR$(198); CHR$(205);
3140 LOCATE 6, 6: PRINT CHR$(192); STRING$(20, 196); CHR$(217);
3150 LOCATE , 46: PRINT CHR$(192); STRING$(20, 196); CHR$(217);
3160 LOCATE 1, 10: PRINT "Supply vessel";
3170 LOCATE 1, 50: PRINT "Mixing vessel";
3180 TV% = PV% / TR%: GY1% = PV% - 2.5 * TV%: GY2% = (12.5) * TV%
3190 TH% = PH% / TC%: GX1% = 5.5 * TH%: GX2% = PH% - 4.5 * TH%
3200 I% = (GX2% - GX1%) / 40: X1% = GX1% + I%: X2% = GX2% - I%
3210 I% = (GY2% - GY1%) / 20: Y1% = GY1% + I%: Y2% = GY2% - I%:
3220 LINE (GX1%, GY1%)-(GX2%, GY2%), 1, B: FOR I% = 1 TO 5
3230 X% = (GX2% - GX1%) * I% / 5 + GX1%: Y% = (GY2% - GY1%) * I% / 5 + GY1%
3240 LINE (GX1%, Y%)-(X1%, Y%), 1: LINE (GX2%, Y%)-(X2%, Y%), 1
3250 LINE (X%, GY1%)-(X%, Y1%), 1: LINE (X%, GY2%)-(X%, Y2%), 1
3260 NEXT: RETURN
4000 'Input the variables
4010 IV(1) = CS: IV(2) = CM: IV(3) = FS: IV(4) = FM
4020 IV(5) = VS: IV(6) = VM: IV(7) = VT: I% = 1: SB% = 6: SC% = 2
4030 LOCATE TR%, 6: PRINT "Set gradient conditions, then press <escape>";
4040 CC% = TC% * ((I% + 1) MOD 2) / 2 + 28
4050 CR% = 7 + INT((I% + 1) / 2): LOCATE CR%, CC%: PRINT CHR$(26);
4060 IK$ = INKEY$: IF IK$ = "" THEN GOSUB 4510: GOTO 4060
4070 IF IK$ = CHR$(27) THEN 4250
4080 IF LEN(IK$) = 2 THEN 4190
4090 C% = INSTR(1, ".0123456789", IK$): IF C% = 0 THEN 4060
4100 ST$ = IK$: LOCATE , CC% + 2
4110 PRINT IK$; CHR$(254); SPACE$(9); : LOCATE , CC% + 4
4120 IK$ = INKEY$: IF LEN(IK$) <> 1 THEN GOSUB 4510: GOTO 4120
```

Fig. 1. (*continued overleaf*)

```
4130 IF IK$ = CHR$(13) THEN 4160
4140 C% = INSTR(1, ".0123456789", IK$): IF C% = 0 THEN 4120
4150 ST$ = ST$ + IK$: LOCATE , POS(0) - 1: PRINT IK$; CHR$(254); : GOTO 4120
4160 IV(I%) = VAL(ST$): LOCATE , POS(0) - 1: PRINT " ";
4170 LOCATE 11, 44: PRINT SPACE$(36); : LOCATE 11, 44: ET = IV(7) / IV(4)
4180 PRINT "[ Note total time is     "; ET; "]"; : GOTO 4040
4190 C% = ASC(RIGHT$(IK$, 1)): LOCATE CR%, CC%: PRINT " ";
4200 IF C% = 72 THEN I% = I% - 2: GOTO 4240
4210 IF C% = 75 THEN I% = I% - 1: GOTO 4240
4220 IF C% = 77 THEN I% = I% + 1: GOTO 4240
4230 IF C% = 80 THEN I% = I% + 2: GOTO 4240
4240 I% = ((I% + 6) MOD 7) + 1: GOTO 4040
4250 'Check the values are valid
4260 CS = IV(1): CM = IV(2): FS = IV(3): FM = IV(4)
4270 VS = IV(5): VM = IV(6): VT = IV(7)
4280 IF FM <= FS THEN 4310
4290 IF ET <= VM / (FM - FS) THEN 4310
4300 LOCATE 18, 20: PRINT "Mixing chamber will run dry"; : GOTO 4350
4310 IF ET <= VS / FS THEN 4330
4320 LOCATE 18, 20: PRINT "Supply chamber will run dry"; : GOTO 4350
4330 IF CM <> CS THEN 4390
4340 LOCATE 18, 20: PRINT "Concentrations must differ";
4350 LOCATE 19, 20: PRINT "Press any key": BEEP
4360 IK$ = INKEY$: IF IK$ = "" THEN GOSUB 4510: GOTO 4360
4370 LOCATE 18, 20: PRINT SPACE$(50); : LOCATE 19, 20: PRINT SPACE$(50);
4380 GOTO 4040
4390 SW% = 0: IF CM > CS THEN SWAP CS, CM: SWAP GY1%, GY2%: SW% = 1
4400 FY$ = "#####": CT = CS
4410 IF CT < 999.5 THEN FY$ = "###.#": IF CT < 99.95 THEN FY$ = "##.##"
4420 IF CT < 9.995 THEN FY$ = "#.###": IF CT < .9995 THEN FY$ = ".####"
4430 FOR I% = 0 TO 5: C = CM + (CS - CM) * I% / 5
4440 LOCATE 23 - 2 * I%, 1: PRINT USING FY$; C; : NEXT
4450 LOCATE 14, 1: PRINT "Conc."; : FX$ = "#####"
4460 IF ET < 999.5 THEN FX$ = "###.#": IF ET < 99.95 THEN FX$ = "##.##"
4470 IF ET < 9.995 THEN FX$ = "#.###": IF ET < .9995 THEN FX$ = ".####"
4480 FOR I% = 0 TO 5: T = ET * I% / 5
4490 LOCATE 24, 14 * I% + 3: PRINT USING FX$; T; : NEXT
4500 LOCATE 24, 67: PRINT "Time"; : RETURN
4510 SS% = 10 - SB% / 2: IF SB% = 2 OR SB% = 12 THEN SC% = -SC%
4520 CL% = CSRLIN: CP% = POS(0): LOCATE 5, 47
4530 PRINT SPACE$(SS%); STRING$(SB%, 220); SPACE$(SS%);
4540 SB% = SB% + SC%: LOCATE CL%, CP%: RETURN
5000 'Draw the gradient
5010 PSET (GX1%, GY1%), 1: IF FM = FS THEN 5060
5020 EX = FS / (FM - FS): RR = (FM - FS) / VM
5030 FOR I% = 1 TO 100: TT = I% * ET / 100
5040 FC = (1 - (1 - TT * RR) ^ EX): CT = CM + (CS - CM) * FC
5050 GOSUB 5090: NEXT: RETURN
5060 RR = FM / VM: FOR I% = 1 TO 100: TT = I% * ET / 100
5070 FC = (1 - EXP(-TT * RR)): CT = CM + (CS - CM) * FC
5080 GOSUB 5090: NEXT: RETURN
5090 GOSUB 5100: LINE -(X%, Y%), 1: RETURN
5100 X% = GX1% + (GX2% - GX1%) * (I% / 100)
5110 Y% = GY1% + (GY2% - GY1%) * FC: RETURN
5120 GOSUB 5100: FOR J% = Y% - 5 TO Y% + 5
5130 PSET (X%, J%), 1 - SGN(POINT(X%, J%)): NEXT: RETURN
6000 'Tracking pointer mode
6010 LOCATE TR%, 6: PRINT SPACE$(TC% - 6); : I% = 50
6020 LOCATE TR%, 6: PRINT "Move pointer or <escape>"; : FV$ = "#####"
6030 IF VT < 999.5 THEN FV$ = "###.#": IF VT < 99.95 THEN FV$ = "##.##"
6040 IF VT < 9.995 THEN FV$ = "#.###": IF VT < .9995 THEN FV$ = ".####"
6050 F$ = "Time = " + FX$ + ";" + " Vol. = " + FV$ + ";" + " Conc. = " + FY$
6060 TT = I% * ET / 100:  IF FM <> FS THEN 6080
6070 FC = (1 - EXP(-TT * RR)): GOTO 6090
6080 FC = (1 - (1 - TT * RR) ^ EX)
6090 CT = CM + (CS - CM) * FC: IF SW% = 1 THEN CT = CS + (CM - CS) * FC
6100 LOCATE TR%, 33: PRINT USING F$; TT, VT * I% / 100, CT; : GOSUB 5120
6110 IK$ = INKEY$: IF IK$ = CHR$(27) THEN RETURN
6120 IF LEN(IK$) <> 2 THEN GOSUB 4510: GOTO 6110
6130 C% = ASC(RIGHT$(IK$, 1)): IF ABS(76 - C%) <> 1 THEN 6110
6140 GOSUB 5120: I% = ((I% + C% + 25) MOD 101): GOTO 6060
7000 'Check whether to end
7010 LOCATE TR%, 6: PRINT SPACE$(TC% - 6);
7020 LOCATE TR%, 6: PRINT "Calculate another gradient (Y/N) ";
7030 YN$ = INKEY$: IF YN$ = "" THEN GOSUB 4510: GOTO 7030
7040 YN% = INSTR(1, "YNNyn", YN$): IF YN% = 0 THEN 7030
7050 YN% = YN% MOD 3: PRINT MID$("YN", YN%, 1); : IF YN% = 2 THEN END
7060 IF SW% = 1 THEN SWAP CS, CM: SWAP GY1%, GY2%
7070 RETURN
```

Fig. 1. Listing of the GRADIENT computer program.

The upper part of the screen shows a simplified diagram of the apparatus. The section below this gives values for the five variables of equations (1)–(3) which define the apparatus, plus three other quantities: the supply vessel volume, the total gradient volume and the total time. With the exception of the total time, all other values may be changed at will. Selecting the variable to be changed is achieved by using the keyboard cursor keys to move the arrow which is shown in Fig. 2 adjacent to the "Mixing flow rate" variable. If undesirable combinations are selected (e,g, if one of the chambers will run dry) a warning is issued.

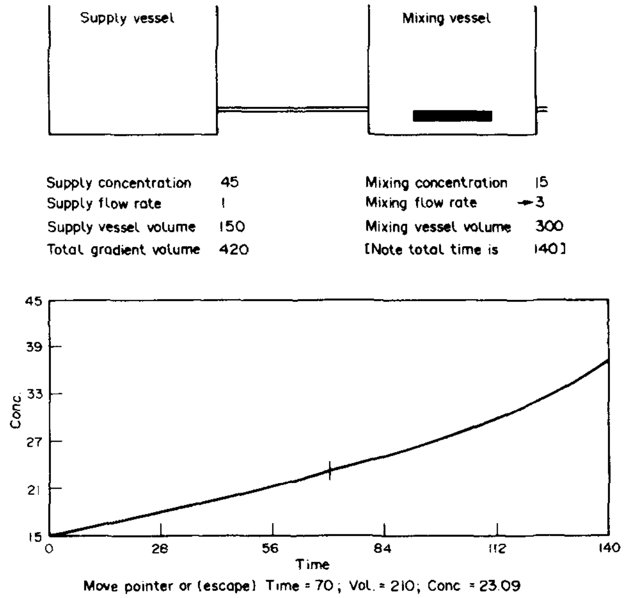

Fig. 2. Results from a typical run of the GRADIENT computer program.

The lower part of the screen shows the gradient which will result, in the form of a graph of concentration vs time. Below this are given values of time, volume and concentration at a particular place in the gradient. In Fig. 2 this is half way along the curve, as indicated by a pointer consisting of a small vertical bar. By using the keyboard cursor keys, this pointer may be moved across the entire curve to determine the exact concentration at any place in the gradient.

The program is available on 5.25" 360 kbyte disk from the author upon request; a small fee is charged to cover costs.

SUMMARY

The GRADIENT computer program simplifies the calculations needed to construct concentration and density gradients using a two-chamber mixing apparatus. The program is written in BASIC for IBM-compatible computers and gives both numerical and graphical representations the information. These linear and curved gradients are widely used in separations based on chromatographic, electrophoretic and centrifugal methods.

REFERENCE

1. T. K. Lakshmanan and S. Lieberman, *Arch. Biochem. Biophys.* **53**, 258–281 (1954).

About the Author—RON DUGGLEBY was born in London, England in 1945. He received a B.Sc. degree in biochemistry from the University of Hull, England in 1966 and M.Sc. (1970) and Ph.D. (1972) degrees in biology from Queen's University at Kingston, Canada. After postdoctoral fellowships at the University of California (Loss Angeles) and the University of Ottawa, he was appointed research fellow in biochemistry at the Australian National University where he remained until 1979. He is now reader in biochemistry at the University of Queensland. His current research interests are centred around the analysis and simulation of biochemical data, particularly in the area of enzyme kinetics.