Notes

CHROM. 4000

Computer program for calculation of gradients for column chromatographic elution

Linear elution gradients are useful in many of the applications of column chromatography[1] but more complex patterns of salt or pH alteration during elution are sometimes helpful in achieving specific separations. Reliable devices have been described for the formation of such gradients[2] and it is a simple matter to calculate the gradient that will be produced by such a device when specified concentrations

```
      PROGRAM GRACAL C (INPUT, OUTPUT, TAPE5 = INPUT, TAPE6 = OUTPUT)
C     READ DESIRED GRADIENT AT 100 PERCENTAGE POINTS (COUT)
      DIMENSION COUT(100), CIN(9), CONT(9,100), C(100), DEV(100),
     1DSQ(100),   FRAC(9,100), AVCOR(9), PER(100), A(100,3)
      DATA SDSQ1/0.0/
      DECR = 1000.
  100 READ (5,200) COUT
  200 FORMAT (10(F8.3))
      GO TO 250
  225 DECR=0.5*DECR
  250 CONTINUE
C     MAKE INITIAL ESTIMATES OF CHAMBER CONCENTRATIONS, CIN
      CIN(1)=COUT(1)
      CIN(2)=COUT(12)
      CIN(3)=COUT(24)
      CIN(4)=COUT(37)
      CIN(5)=COUT(50)
      CIN(6)=COUT(61)
      CIN(7)=COUT(74)
      CIN(8)=COUT(86)
      CIN(9)=COUT(100)
C       COMPUTE LATEST GRADIENT, C
      I=0
  300 DO 400 J=1,100
      A=(FLOAT(J))/100.
      CONT(1,J)=CIN(1)*((1.-A)**8)
      CONT(2,J)=CIN(2)*8.*((1.-A)**7)*(A)
      CONT(3,J)=CIN(3)*28.*((1.-A)**6)*((A)**2)
      CONT(4,J)=CIN(4)*56.*((1.-A)**5)*((A)**3)
      CONT(5,J)=CIN(5)*70.*((1.-A)**4)*((A)**4)
      CONT(6,J)=CIN(6)*56.*((1.-A)**3)*((A)**5)
      CONT(7,J)=CIN(7)*28.*((1.-A)**2)*((A)**6)
      CONT(8,J)=CIN(8)*8.*(1.-A)*((A)**7)
      CONT(9,J)=CIN(9)*((A)**8)
  400 CONTINUE
      DO 500 J=1,100
      C(J)=0.
  500 CONTINUE
      DO 550 J=1,100
      DO 550 K=1,9
      C(J) =C(J)+CONT(K,J)
  550 CONTINUE
C     COMPUTE DIFFERENCES AND TEST THEM
      DO 600 J=1,100
      DEV(J)=C(J)-COUT(J)
```

Fig. 1. Part one of the gradient calculation program.

of eluant are placed in the mixer, using either general formulas[2] or nomograms designed for the purpose[3]. It is rather more tedious to calculate the concentrations of eluant that must be placed in the mixer to give a specified gradient, and a process of successive approximations from tables of calculated partial values has been suggested as satisfactory for most purposes[2]. This paper gives a program for the calculation of mixer concentrations which will give a good fit to a specified gradient under prescribed experimental conditions. The program can be used with the computer facilities that are now readily available to most investigators.

Method of calculation

The program is written in Fortran IV and has been used on a Control Data Corporation Model 6400 Computer System at the University of Colorado Computer Center. The commercially available gradient mixing devices are customarily nine-chambered and the program is designed specifically for such an apparatus although it could readily be modified to accomodate a larger or smaller number of chambers by altering the appropriate indices in the calculation. The user specifies the desired

```
 600  CONTINUE
      SUMD=0.
      DO 800 J=1,100
      SUMD=SUMD+DEV(J)
 800  CONTINUE
      IF(SUMD.GT.1.E+90) GO TO  225
      DO 801 J=1,100
      DSQ(J)=0.
 801  CONTINUE
      DO 8011 J=1,100
      DSQ(J)=DEV(J)**2
8011  CONTINUE
      SDSQ=0.
      DO 802 J=1,100
      SDSQ=SDSQ+DSQ(J)
 802  CONTINUE
      IF (SDSQ.LT. 1. E-04) GO TO 8980
      I=1
      IF(I.GT. 35.AND.SDSQ.GT.SDSQ1) GO TO 8920
      SDSQ1=SDSQ
      IF(I.GE.999) GO TO 8900
      DO 820 K=1,9
      DO 820 L=1,100
      FRAC(K,L)=0.
 820  CONTINUE
      DO 900 K=1,9
      DO 900 L=1,100
      IF(DEV(L)) 822,822,824
 822  SIGN=1.
      GO TO 826
 824  SIGN=-1.
 826  FRAC(K,L)=CONT(K,L)*SIGN*DSQ(L)/C(L)
 900  CONTINUE
      DO 910 K=1,9
      AVCOR(K)=0.
 910  CONTINUE
      DO 1000 K=1,9
      KK=(12*K)-11
      DO 1000 L=1,KK
      B=FLOAT(KK)
      AVCOR(K)=(AVCOR(K)+FRAC(K,L))/B
      CIN(K)=CIN(K)+DECR*AVCOR(K)
```

Fig. 2. Part two of the gradient calculation program.

shape of the gradient in terms of molar concentrations at 100 equally spaced points along the gradient. The program then calculates by successive approximations the concentrations of eluant to be placed in the mixing chamber to give the desired gradient. The resulting input concentrations and the calculated mixer output are tabulated numerically, along with the percentage deviation of each calculated point from the corresponding input datum. In order to estimate quickly the adequacy of the correspondence between the calculated and the desired gradient, a standard printer plot subroutine is then instructed to display the results of the calculation[4].

In the programming no attempt was made to condense maximally the computer instructions with respect either to machine space or computing time, because the extended version will probably be more readily modified by future users to meet special conditions. The approximation method used is one of successive bisection of the error at each iteration of the calculation and was selected in order to avoid difficulties with conditional convergences[5]. The relatively slow convergence of such a method is no disadvantage with modern high speed computing machines. The program is arranged to stop when the agreement between the desired and calculated gradients becomes better than a specified error (which may readily be altered by the user), or to stop if the approximation process ceases to converge, or to stop after a specified number of iterations (999 as written but simply adjustable by the user). The final result of the calculation is tabulated and plotted graphically for inspection in all cases.

```
1000 CONTINUE
     GO TO 300
8900 WRITE (6,8910) I
8910 FORMAT(1H1,34HSTANDARD TOLERANCE EXCEEDED AFTER ,I3, 1X,10HITERAT
    2IONS)
8920 WRITE (6,8930) I
8930 FORMAT (1H1,37HAPPROXIMATION PROCESS DIVERGES AFTER ,I3,1X, 10HITE
    2RATIONS)
     GO TO 9000
8980 WRITE (6,8990) I
8990 FORMAT (1H1,37HFITTED WITHIN STANDARD TOLERANCE IN ,I3,1X,10HITERA
    2TIONS)
9000 DO 9100 J=1,100
     PER(J)=(DEV(J)/COUT(J))*100.
9100 CONTINUE
     WRITE (6,9200)
9200 FORMAT(1H0, 32H                CHAMBER    MOLARITY//)
     WRITE (6,9300) (K, CIN(K),K=1,9)
9300 FORMAT (15X,I3,8X,F6.4)
     WRITE (6,9400)
9400 FORMAT(1H0,66H     FRACTION     SPECIFIED     CALCULATED     PER
    2CENT DEVIATION//)
     WRITE (6,9500) (L,COUT(L),C(L),PER(L),L=1,100)
9500 FORMAT(8X,I3,9X,F6.4, 6X,F6.4,16X,F6.2)
     DO 9600 L=1,100
     A(L,1)=L
     A(L,2)=COUT(L)
     A(L,3)=C(L)
9600 CONTINUE
     N=100
     M=3
     NLL=100
     CALL PLOT (A,N,M,NLL)
     STOP
     END
```

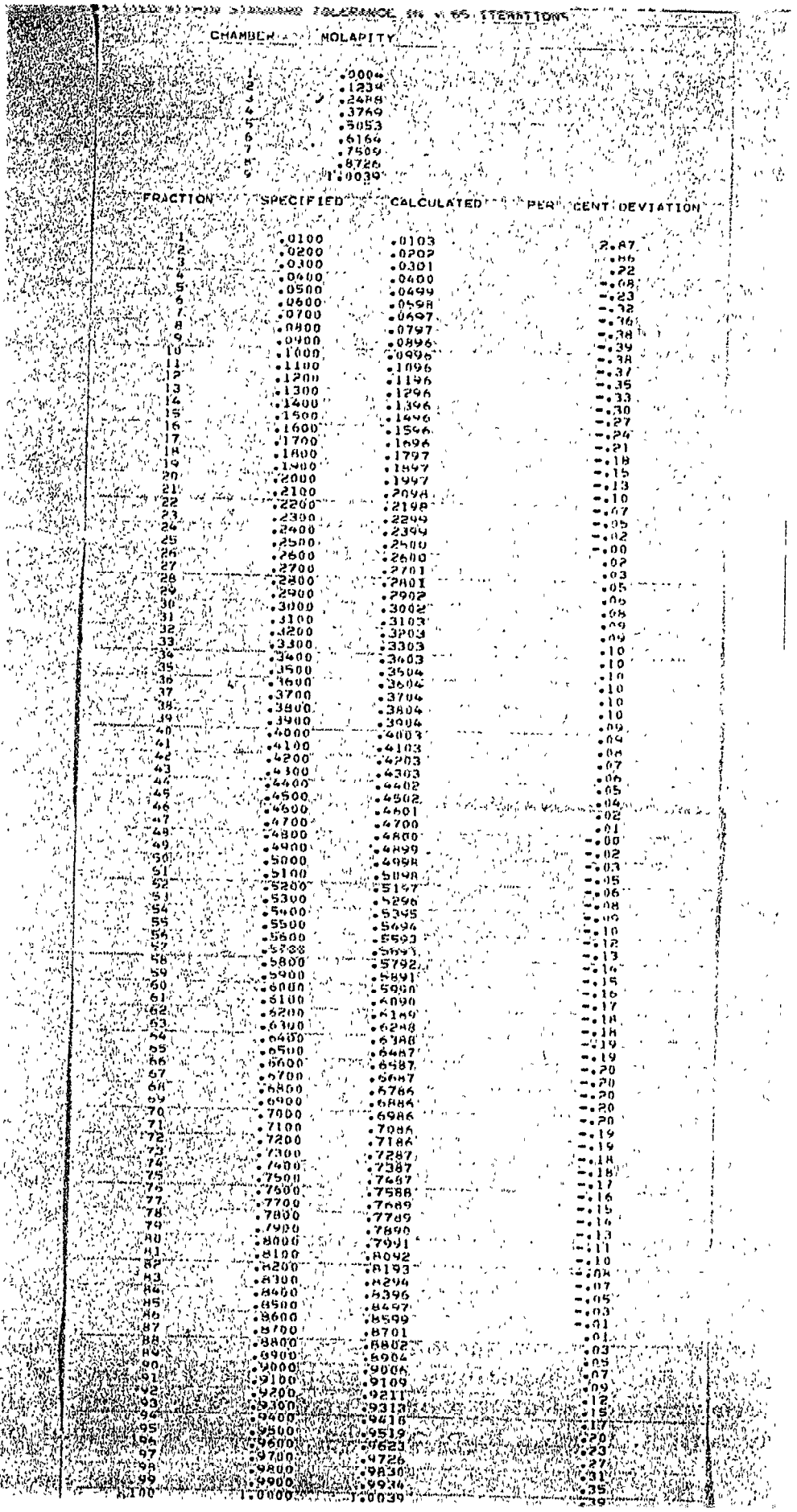
Fig. 3. Part three of the gradient calculation program.

```
      SUBROUTINE PLOT (A,N,M,NLL)
      DIMENSION OUT(101),YPR(11),ANG(9),A(100,3)
    1 FORMAT (1H1,50X26HDESIRED = 1 CALCULATED = 2//)
    2 FORMAT (1X,F11.4,5X,101A1)
    3 FORMAT (2X)
    5 FORMAT (10A1)
    7 FORMAT (    16X,101H.
     1    .         .         .         .         .         .         .)
      MY=5
      MX=6
    8 FORMAT (//,9X,11F10.4)
   20 WRITE(MX,1)
      READ(MY,5) BLANK,(ANG(I),I=1,9)
      XSCAL=(A(N)-A(1))/(FLOAT(NLL-1))
      M1=N+1
      M2=M*N
      YMIN=A(M1)
      YMAX=YMIN
      DO 40 J=M1,M2
      IF (A(J)-YMIN) 28,26,26
   26 IF (A(J)-YMAX) 40,40,30
   28 YMIN=A(J)
      GO TO 40
   30 YMAX=A(J)
   40 CONTINUE
      YSCAL=(YMAX-YMIN)/100.
      XB=A(1)
      L=1
      MYX=M-1
      I=1
   45 F=I-1
      XPR=XB+F*XSCAL
      IF(A(L)-XPR) 50,50,70
   50 DO55 IX=1,101
   55 OUT(IX)=BLANK
      DO 60 J=1,MYX
      LL=L+J*N
      JP=((A(LL)-YMIN)/YSCAL)+1.
      OUT(JP)=ANG(J)
   60 CONTINUE
      WRITE(MX,2)XPR,(OUT(IZ),IZ=1,101)
      L=L+1
      GO TO 80
   70 WRITE(MX,3)
   80 I=I+1
      IF(I-NLL)45,84,86
   84 XPR=A(N)
      GO TO 50
   86 WRITE(MX,7)
      YPR(1)=YMIN
      DO 90 KN=1,9
   90 YPR(KN+1)=YPR(KN)+YSCAL*10.
      YPR(11)=YMAX
      WRITE(MX,8)(YPR(IP),IP=1,11)
      RETURN
      END
```

Fig. 4. Program for graphic representation of the results of the calculation.

Results and discussion

A complete listing of the program and subroutines is given in Figs. 1–4. Fig. 5 shows the printed output and Fig. 6 demonstrates the graph that is obtained when the input consists of instructions to calculate a linear gradient. The results of the calculation are obviously correct. When a more complex gradient is specified the



CHAMBER	MOLARITY
1	.0004
2	.1234
3	.2488
4	.3749
5	.5053
6	.6164
7	.7509
8	.8726
9	1.0039

FRACTION	SPECIFIED	CALCULATED	PER CENT DEVIATION
1	.0100	.0103	2.87
2	.0200	.0202	.86
3	.0300	.0301	.22
4	.0400	.0400	.08
5	.0500	.0499	-.23
6	.0600	.0598	-.32
7	.0700	.0697	-.36
8	.0800	.0797	-.38
9	.0900	.0896	-.39
10	.1000	.0996	-.38
11	.1100	.1096	-.37
12	.1200	.1196	-.35
13	.1300	.1296	-.33
14	.1400	.1396	-.30
15	.1500	.1496	-.27
16	.1600	.1596	-.24
17	.1700	.1696	-.21
18	.1800	.1797	-.18
19	.1900	.1897	-.15
20	.2000	.1997	-.13
21	.2100	.2098	-.10
22	.2200	.2198	-.07
23	.2300	.2299	-.05
24	.2400	.2399	-.02
25	.2500	.2500	.00
26	.2600	.2600	.02
27	.2700	.2701	.03
28	.2800	.2801	.05
29	.2900	.2902	.06
30	.3000	.3002	.06
31	.3100	.3103	.08
32	.3200	.3203	.09
33	.3300	.3303	.09
34	.3400	.3403	.10
35	.3500	.3504	.10
36	.3600	.3604	.10
37	.3700	.3704	.10
38	.3800	.3804	.10
39	.3900	.3904	.10
40	.4000	.4003	.09
41	.4100	.4103	.09
42	.4200	.4203	.08
43	.4300	.4303	.07
44	.4400	.4402	.06
45	.4500	.4502	.05
46	.4600	.4601	.04
47	.4700	.4700	.02
48	.4800	.4800	.01
49	.4900	.4899	.00
50	.5000	.4998	-.02
51	.5100	.5098	-.03
52	.5200	.5197	-.05
53	.5300	.5296	-.06
54	.5400	.5395	-.08
55	.5500	.5494	-.09
56	.5600	.5593	-.10
57	.5700	.5693	-.12
58	.5800	.5792	-.13
59	.5900	.5891	-.14
60	.6000	.5990	-.15
61	.6100	.6090	-.16
62	.6200	.6189	-.17
63	.6300	.6288	-.18
64	.6400	.6388	-.19
65	.6500	.6487	-.19
66	.6600	.6587	-.19
67	.6700	.6687	-.20
68	.6800	.6786	-.20
69	.6900	.6886	-.20
70	.7000	.6986	-.20
71	.7100	.7086	-.20
72	.7200	.7186	-.19
73	.7300	.7287	-.19
74	.7400	.7387	-.18
75	.7500	.7487	-.18
76	.7600	.7588	-.17
77	.7700	.7689	-.16
78	.7800	.7789	-.15
79	.7900	.7890	-.14
80	.8000	.7991	-.13
81	.8100	.8092	-.11
82	.8200	.8193	-.10
83	.8300	.8294	-.08
84	.8400	.8396	-.07
85	.8500	.8497	-.05
86	.8600	.8599	-.03
87	.8700	.8701	-.01
88	.8800	.8802	.01
89	.8900	.8904	.03
90	.9000	.9006	.05
91	.9100	.9109	.07
92	.9200	.9211	.09
93	.9300	.9313	.12
94	.9400	.9416	.15
95	.9500	.9519	.17
96	.9600	.9623	.20
97	.9700	.9726	.23
98	.9800	.9830	.27
99	.9900	.9934	.31
100	1.0000	1.0039	.35

Fig. 5. Numerical output for a linear gradient input specification.

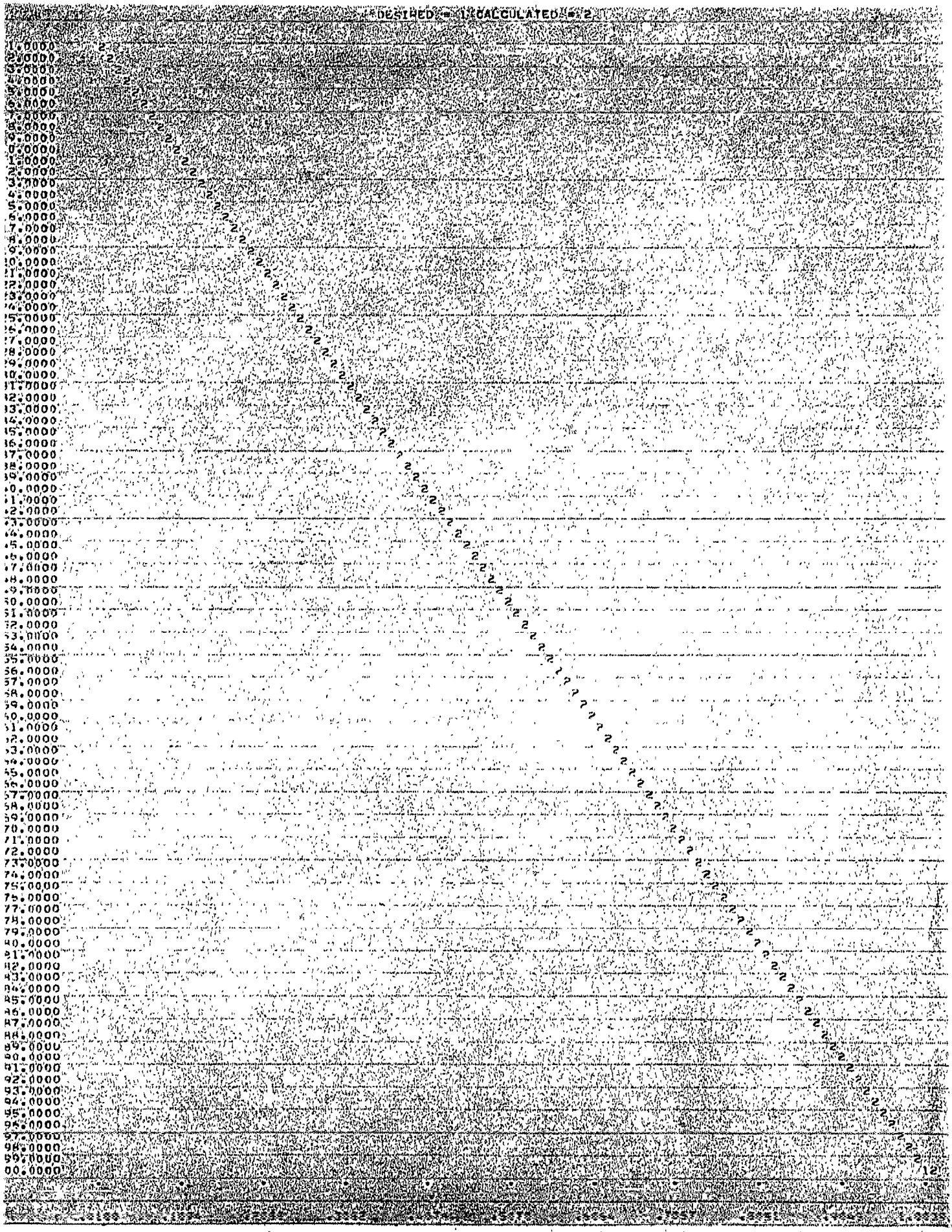
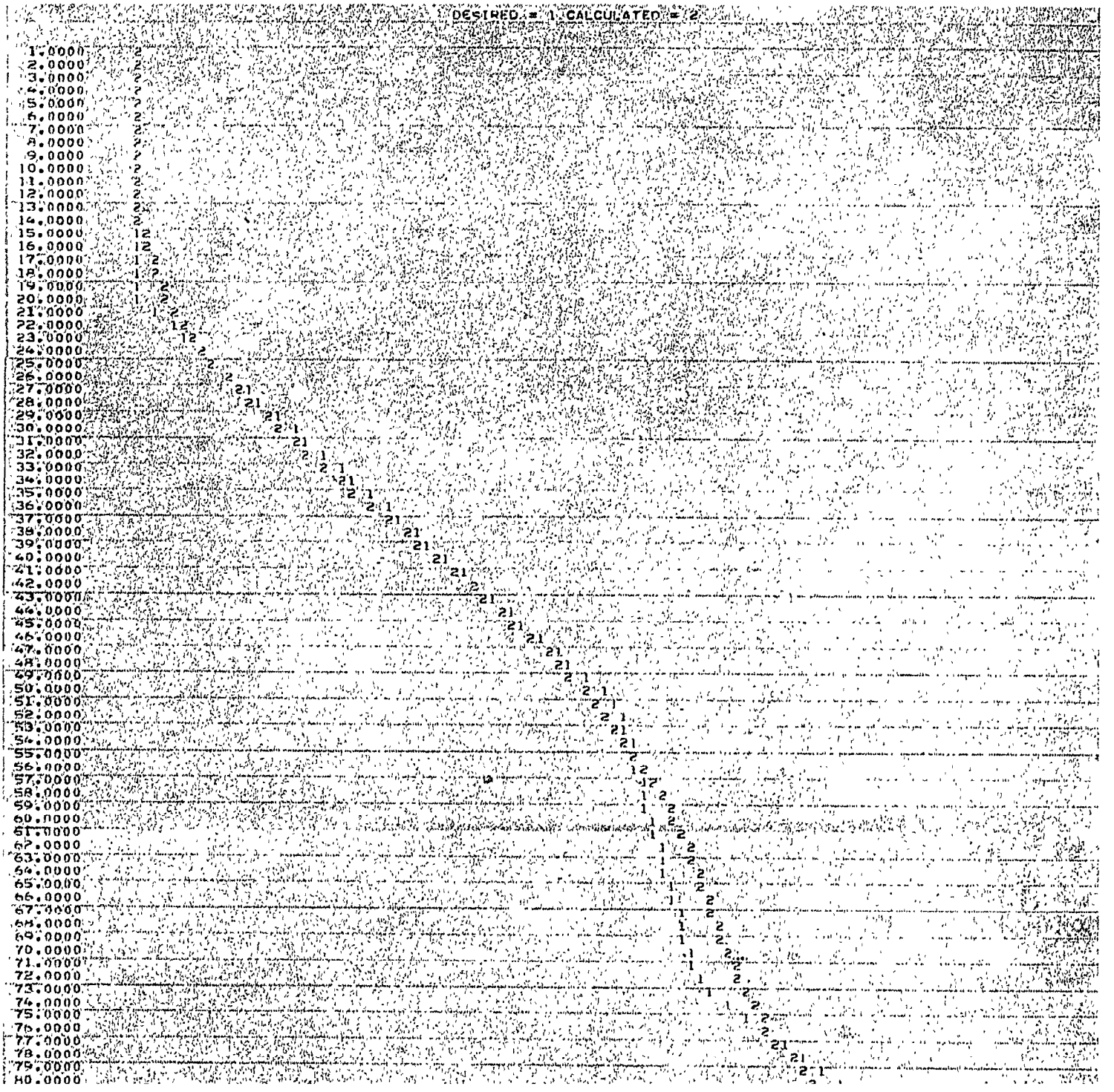
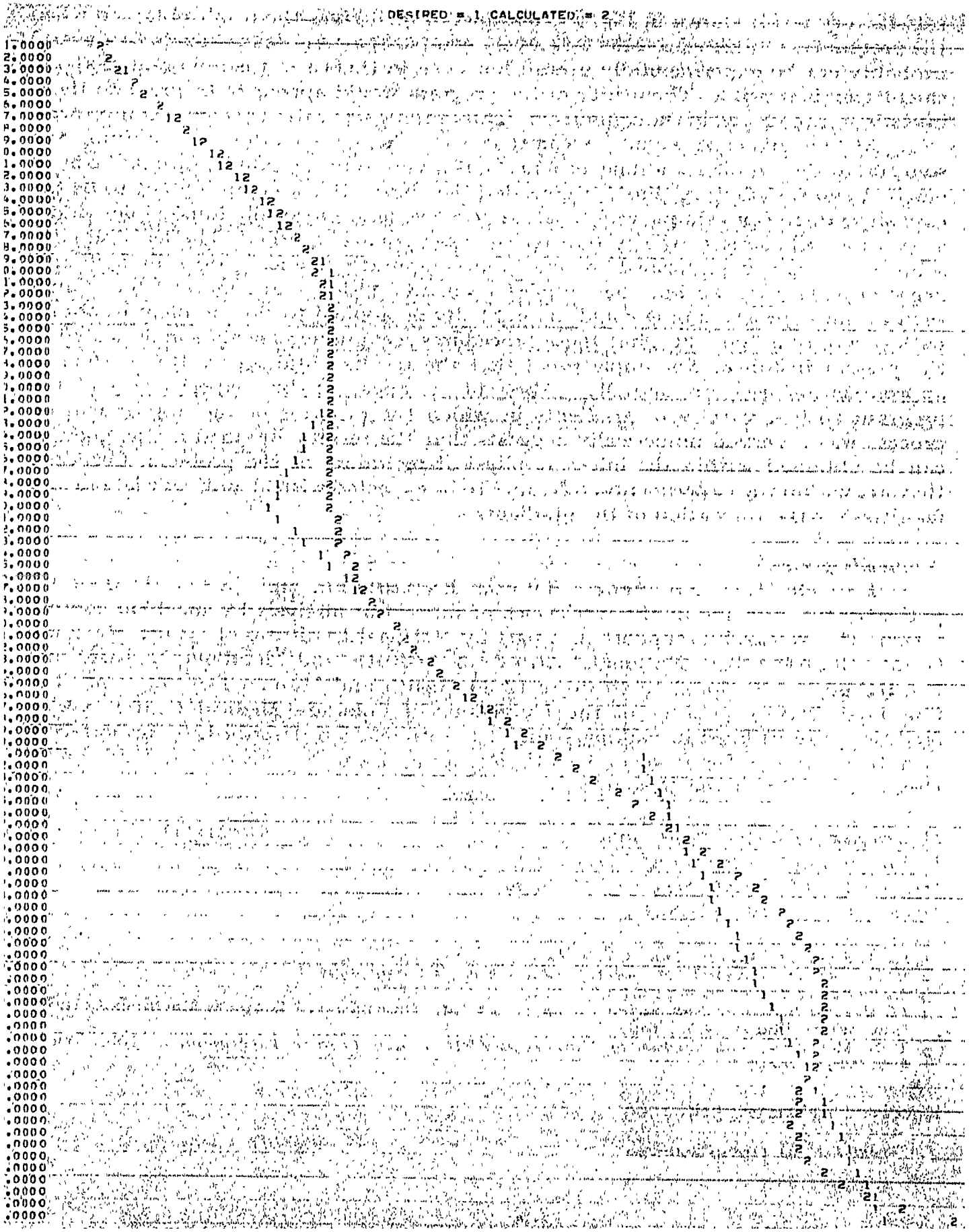


Fig. 6. Graphic representation for a linear gradient input specification.


DESIRED = 1 CALCULATED = 2


DESIRED = 1, CALCULATED = 2

satisfactory result shown in Fig. 7 is obtained. Fig. 8 gives the result obtained when the program is requested to calculate a very complex pattern (one which in fact would probably not be experimentally useful) but even in this case it is apparent that the calculation is possible. The utility of the program would appear to be great to those investigators engaged in the column chromatographic separation of complex mixtures.

At first glance it would appear that a least squares method of obtaining the solution to the simple simultaneous equations involved in the problem would be most efficient and satisfactory. Further consideration shows this assumption not to be the case since there is no simple way to avoid negative (and physically impossible) values as possible solutions to the system by the least squares method. The least squares solution is readily performed by machine computation, and it does in fact give negative values for all but the simplest gradients. Other mathematical techniques such as linear programming could undoubtedly be applied to this problem to obtain with certainty a "best fit", but these procedures seem unnecessarily sophisticated in the present instance. No simple proof that the results obtained by the method of approximation used are actually a "best fit" is apparent, but experience with the program with a variety of gradients in which the progress of the approximation process was observed numerically suggests that the results obtained are as good as can be obtained within the inherent physical restraints of the problem. Certainly they are within the experimental tolerance to be expected with all ordinary laboratory facilities for the formation of the gradients.

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Department of Biochemistry and
Department of Obstetrics and Gynecology,
University of Colorado School of Medicine,
Denver, Colo. 80220 (U.S.A.)

DWAIN D. HAGERMAN

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