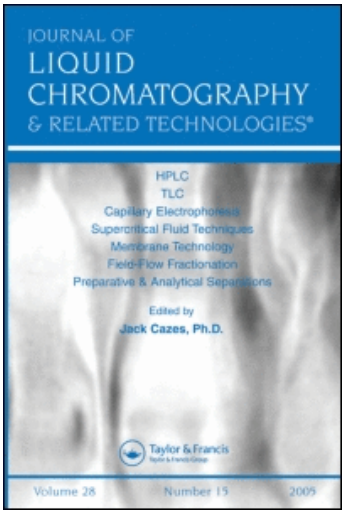

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PHYSICOCHEMICAL MEASUREMENTS IN LIQUIDS BY SIMPLE HPLC INSTRUMENTATION

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ABSTRACT

A method is described for measuring physicochemical quantities in liquid and liquid/solid systems by means of HPLC instrumentation. It has been applied with benzene as analyte, chloroform or acetonitrile as liquid solvents, and silica gel as solid phase stuck on the internal wall of a cylindrical tube.

The relevant theoretical analysis has been found for determining diffusion coefficients into liquid systems, and kinetic parameters pertaining to the adsorption, desorption, and surface reaction of the analyte with the solid stuck on the wall. The method has been tested for various lengths of time keeping the system stagnant before analysing it by HPLC.

INTRODUCTION

A considerable number of papers dealing with physicochemical measurements by gas chromatography have been published in recent years, and various reviews on this subject have been appeared. As examples, one may cite one in

1998[1] and one in 1999[2]. Much less work has been published on physicochemical measurements by liquid chromatography, although physicochemical quantities in liquids, like diffusion coefficients, are measured with difficulty by non-chromatographic techniques.

One possible reason for that is the difficulty, or rather impossibility to employ in liquid chromatography, modern methods like flow perturbation gas chromatography,[3] thus avoiding effects due to longitudinal diffusion along the chromatographic column and other effects due to the carrier gas flow. Analogous effects due to the flow of the chromatographic liquid are faced in HPLC, but they cannot possibly be overcome by disturbing the flow, since the drive force of this is based on a high pressure gradient along the column.

An obvious possibility, though, is to set aside temporarily the chromatographic process, conduct the physicochemical experiments for a definite time in a separate column filled with a stagnant solvent, and then analyze the contents of the latter by connecting it directly to the entrance of the HPLC, running with the same solvent as that of the stagnant column.

What about the stationary phase of the columns? The analytical column of HPLC can be a conventional one, chosen to separate the analytes used in the physicochemical column. The latter may not contain any stationary solid phase, like that used in the first publication of this kind,[4] or it may have its internal wall covered with an adsorbent material or a supported liquid, like that of analogous gas chromatographic methods,[5-7] or lastly it may be filled with a solid adsorbent or a supported liquid.

The present work refers to the second possibility above, the relevant theoretical analysis and some results being obtained with silica gel as stationary phase stuck on the internal wall, chloroform or acetonitrile as solvent, and benzene as analyte.

THEORY

This can be developed with reference to Figure 1. The important point is obviously the fact that, instead of injecting the analyte(s) under study into port 6 of the six-port valve, as usual, it is introduced either at the top ($z = L$) or at the bottom ($z = 0$) of the diffusion column, filled with pure solvent. Then it is left to diffuse freely along this column (downwards or upwards) for several hours (16-48 h), without operating the chromatograph during this time period. After that, small sections (5mm high) from the entire length of the diffusion column are analyzed by leaving them enter the valve loop, and then the chromatographic column operated at appropriate conditions, by turning the valve to the broken lines position. This is repeated for all sections of the diffusion col-

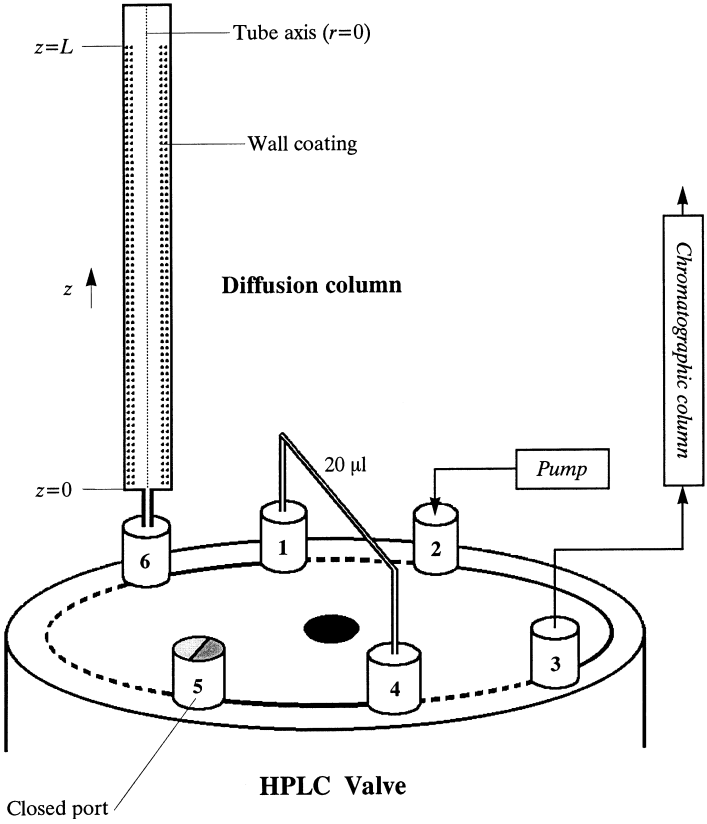

Figure 1. Schematic representation of the experimental set-up for the determination of physicochemical quantities by HPLC.

umn, thus obtaining a series of sharp peaks giving the concentration of the analyte as a function of coordinate z along the diffusion column. An example is given in Figure 2.

The question naturally arising is: which mathematical equation describes the experimental appearance of Figure 2, and what physicochemical measurements can that be theoretically based on? The following theoretical analysis answers this question. It starts with the mass balance equation of the analyte A in the diffusion column covered with a thin solid layer at the internal wall:

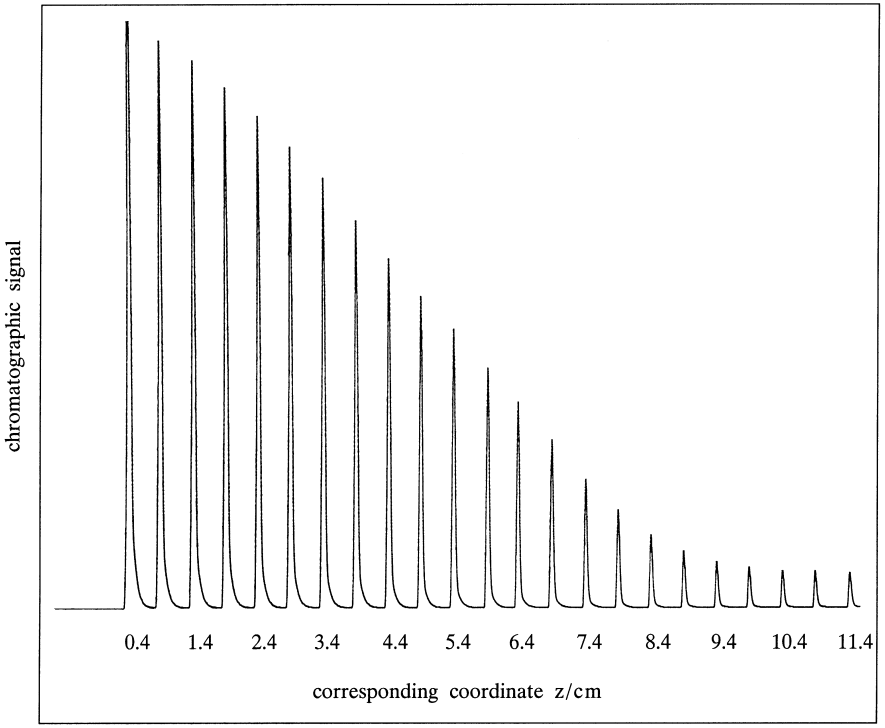

Figure 2. Signal of the UV detector (Lambda-Max, Model 481, LC Spectrophotometer, Waters, MILLIPORE®, recorded by Waters 745 Data Module, MILLIPORE®) of HPLC as a function of *z*, obtained at 18°C with 50 µL of benzene, injected onto the diffusion column 15.9 cm long x 3 mm I.D., at the end $z = 0$. The column was filled with acetonitrile as solvent, and the chromatographic sampling started 48 h after the injection.

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial z^2} - D\left(\frac{\partial^2 c}{\partial r^2} + \frac{1}{r}\frac{\partial c}{\partial r}\right) \tag{1}$$

where $c = c(t, z, r)$ is the concentration of A in the stagnant solvent as a function of t, z, and r (mol/cm^3); t = time measured from the injection moment of A into the diffusion column (s); z = length coordinate along the diffusion column (cm); r = distance from the tube axis (cm); D = diffusion coefficient of A into the liquid solvent (cm^2/s).

The initial condition can be described by Dirac's delta function $\delta(z\text{-}L)$, for an instantaneous injection at $z = L$ of an amount m(mol)of A:

$$c(0,z,r) = \frac{m}{a_z}\delta(z-L) \tag{2}$$

where a_z is the cross sectional area (cm^2) of the tube part containing the solvent.

The solution of Eq. (1) has been based[5] on taking the double laplace transforms of each term with respect to t and z, resulting in a second-order differential equation of the independent variable r. This is easily solved with respect to r, giving a series solution which is approximated by the first two terms of a modified Bessel function of zero order:

$$\bar{C} = -\frac{b}{a^2} + \Lambda_0\left(1+\frac{a^2r^2}{4}\right) \tag{3}$$

where $\bar{C}$ is the double Laplace transform of c with respect to t and z (transform parameters p and s, respectively), Λ_0 a constant to be determined and a and b being given by the relations

$$a^2 = s^2 - q^2,\ q^2 = p/D \tag{4}$$

$$b = \frac{m}{a_z D}\exp(-Ls) - sC(0) - C'(0) \tag{5}$$

where $C(0)$ = t transform of c at $z = 0$, and $C'(0) = (dC/dz)_{z=0}$.

There are two radial boundary conditions, one at the tube axis ($r = 0$), and the other at the tube wall ($r = R$). The first is $(\partial c/\partial r)_{r=0} = 0$ or its double Laplace transform $\left(\partial \bar{C}/\partial r\right)_{r=0} = 0$, and this is satisfied by Eq. (3). The second boundary condition is $-D\left(\partial \bar{C}/\partial r\right)_{r=R} = 0$ when there is no coating on the wall, since in this case there is neither a flux through the tube walls nor an adsorption at the wall $r = R$. From Eq. (3) one then obtains $-D(\Lambda_0 a^2 R/2)=0$, i.e., $\Lambda_0 = 0$, when Eq. (3) becomes

$$\bar{C} = -\frac{m}{a_z D}\frac{\exp(-Ls)}{s^2 - q^2} + \frac{sC(0)}{s^2 - q^2} + \frac{C'(0)}{s^2 - q^2} \tag{6}$$

If there is a solid coating on the wall, the boundary condition at $r = R$ is not zero as before, but

$$-D\left(\frac{\partial \bar{C}}{\partial r}\right)_{r=R} = \frac{k_{-1}}{S}\left(\bar{C}_s^* - \bar{C}_s\right) \tag{7}$$

where $k_{-1}(s^{-1})$ is the rate constant for desorption of the analyte from the solid coating, $S(cm^2/g)$ is the specific surface area of the solid and $\bar{C}_s^*, \bar{C}_s$ the double Laplace transforms with respect to t and z of the adsorbed analyte concentration at equilibrium and far from it, respectively. By employing a linear adsorption isotherm of the analyte on the solid support at the wall

$$K = \frac{c_s^*}{c} \tag{8}$$

Eq. (6) is again obtained, with the only difference that, instead of q^2, f^2 appears, where

$$f^2 = q^2 - \lambda^2 = \frac{1}{D}\left(p - \lambda^2 D\right) \tag{9}$$

and

$$\lambda^2 = \frac{4k_1k_2}{2D\left(k_{-1} + k_2\right) + R^2 k_1 k_2} \tag{10}$$

the k_1 (s^{-1}) being adsorption rate constant of analyte A on the wall coating, and k_2 (s^{-1}) the rate constant of a possible first-order or pseudo first-order surface reaction of the adsorbed A. The details for the derivation of Eqs.(9) and (10) can be found elsewhere,[5] Eq. (10) being applied after a long enough time for steady-state conditions to be applied.

Taking now the inverse Laplace transformation of Eq. (6) with respect to s, one finds

$$C = -\frac{m}{a_z Dq}\sinh q(z-L)\cdot u(z-L) + C(0)\ \cosh\ qz + \frac{C'(0)}{q}\sinh\ qz \quad (11)$$

Since C'(0) = $(dC/dz)_{z=0}$ = 0, the last term of Eq. (11) is omitted, and because at $z = L$ it is also $(dC/dz)_{z=L} = 0$, differentiating Eq. (11) with respect to z at $z = L$ and setting it equal to zero, one finds the value of $C(0)$:

$$C(0) = \frac{m}{a_z Dq}\cdot\frac{1}{\sinh\ qL} \quad (12)$$

This can be substituted back into Eq. (11), giving

$$C = \frac{m}{a_z Dq}\left[\frac{\cosh qz}{\sinh qL} - \sinh q(z-L)\cdot u(z-L)\right] \quad (13)$$

This is the equation of the analyte concentration c (transformed with respect to t) as a function of the length coordinate z. Under our experimental conditions (cf. Figure 1),

$z \le L$, and this permits omission of the second term in brackets, since for $z<L$, $u(z-L)=0$ and $\sinh q(z-L)=-\sinh q(L-z)<0$, whereas for $z=L$, $u(z-L)=1$ and $\sinh q(z-L)=\sinh q\cdot 0=0$.

The remaining term gives c by inverse Laplace transformation with respect to the time parameter p, which is related to q by Eq. (4), i.e., $q=\sqrt{p/D}$. Thus

$$c = L_p^{-1}\frac{m\sqrt{D}}{a_z D}\cdot\frac{1}{\sqrt{p}}\cdot\frac{\cosh\left(\frac{z}{\sqrt{D}}\sqrt{p}\right)}{\sinh\left(\frac{L}{\sqrt{D}}\sqrt{p}\right)} \quad (14)$$

According to Oberhettinger and Badii[8], the right-hand size of Eq. (14) is given by

$$\frac{m}{a_z L}\theta_4\left(\frac{z}{2L}\,\middle|\, t\frac{D}{L^2}\right)$$

where θ_4 is an elliptic theta function of variables, given analytically on p. 422 of Ref. 8. Using this, one finds

$$c = \frac{m}{a_z\sqrt{\pi Dt}} \sum_{n=-\infty}^{\infty} \exp\left[-\left(\frac{z}{2L} + n + \frac{1}{2} \right)^2 L^2 \Big/ Dt \right] \tag{15}$$

For n = -2, -1, 0, 1 the exponential variable in brackets becomes $(3L-z)^2/4Dt$, $(L-z)^2/4Dt$, $(L+z)^2/4Dt$, $(3L+z)^2/4Dt$, respectively. Taking the absolutely smallest exponential variable for the same z and t values, i.e., $(L-z)^2/4Dt$, one may approximate Eq. (15) as

$$c = \frac{m}{a_z\sqrt{\pi Dt}} \exp\left[-\frac{(L-z)^2}{4Dt} \right] \tag{16}$$

the rest of the terms becoming negligible under the experimental conditions employed, i.e., the values of z, L and t, given the order of magnitude of D from the literature.

If the initial condition refers to an instantaneous injection of the analyte, not at $z = L$ (cf. Fig 1) but at $z = 0$, i.e., at the bottom of the diffusion column, this is described by

$$c(0,z,r) = \frac{m}{a_z}\delta(z) \tag{17}$$

instead of Eq. (2). The solution of Eq. (1) then follows a route analogous to that described before, ending with the expression

$$c = \frac{m}{a_z\sqrt{\pi Dt}} \sum_{n=-\infty}^{\infty} \exp\left[-\left(\frac{L-z}{2L} + n + \frac{1}{2} \right)^2 L^2 \Big/ Dt \right] \tag{18}$$

instead of Eq. (15). This is expected, since it is equivalent to changing the variable z by $z' = L - z$ in the marking of the ends of the diffusion column.

For n = -2, -1, 0, 1 as before, the exponential variable in the brackets of Eq. (18) becomes $(2L+z)^2/4Dt$, $z^2/4Dt$, $(2L-z)^2/4Dt$, $(4L-z)^2/4Dt$, respectively. Taking the absolutely smaller exponential variable, as before, i.e., $z^2/4Dt$, Eq. (18) reduces to

$$c = \frac{m}{a_z\sqrt{\pi Dt}} \exp\left(-\frac{z^2}{4Dt} \right) \tag{19}$$

which is equivalent to Eq. (16).

In the presence of a solid coating on the wall, q in Eq. (12) is substituted by f of Eq. (9) and this has the final effect of multiplying Eqs.(16) and (19) by $\exp(\lambda^2 Dt)$, λ^2 being given in terms of the rate constants k_1 (adsorption), k_{-1} (desorption) and k_2 (surface reaction), according to Eq. (10).

EXPERIMENTAL

Materials and Procedure

The benzene analyte was the product Uvasol®; acetonitrile and chloroform used as chromatographic solvents were the products LiChrosolv®, all obtained from Merck. Silica gel was the product Kieselgel 60G from Merck.

After injecting a small volume (50 μL) of the liquid analyte onto the top or the bottom of the diffusion column, i.e., at $z = L$ or $z = 0$, respectively (cf. Figure 1), and having left it to diffuse freely along the liquid column of length L = 14.9 - 26 cm for a time t = 16-48 h, the concentration c of the analyte was measured as a function of z by simply opening for 10 s port 5 of the valve and letting the volume of a small height (5 mm) of the diffusion column to enter the valve loop. The port is closed again and the valve is turned to the other position, shown by the broken lines, so that the loop contents are introduced and analysed into the chromatographic column (μPorasil™ Packings, Normal Phase Chromatography, Waters, Millipore®). They give a sharp peak of height H.

The above procedure is repeated many sequential times (around 30), injecting once per min, with the result to obtain a series of peaks of the analyte corresponding to different heights of the liquid in the diffusion column, i.e., different values of z (cf. Figure 2).

Calculations

According to Eqs.(16) and (19), the height H (cm) of the chromatographic peaks is proportional to c (mol cm^{-3}), and therefore a plot of ln$H$ versus $(L\text{-}z)^2$ or z^2, according to the relations

$$\ln H = \ln\left(\frac{gm}{a_z\sqrt{\pi Dt}}\right) - \frac{(L-z)^2}{4Dt} \qquad (20)$$

$$\ln H = \ln\left(\frac{gm}{a_z\sqrt{\pi Dt}}\right) - \frac{z^2}{4Dt} \tag{21}$$

where g is a calibration factor in cm/mol cm^{-3}, is expected to be linear with slope $-1/4Dt$ and intercept $\ln\left(gm/a_z\sqrt{\pi Dt}\right)$. Knowing t (time elapsing between solute injection and starting of the chromatographic analysis), the diffusion coefficient D of the solute into the solvent used can easily be calculated from the slope. Then, g can be found from the intercept of the plot, since m, a_z, π, D and t are known quantities.

In the presence of a solid coating on the wall of the diffusion column, the intercept will be $\ln\left[\left(gm/a_z\sqrt{\pi Dt}\right)\exp\left(\lambda^2 Dt\right)\right]$, from which λ^2 of Eq. (10) can be obtained using the $\left(gm/a_z\sqrt{\pi Dt}\right)$ value calculated from an experiment without coating.

Equations (16) and (19) are of the form $H=A\exp[B(L-z)^2]$ and $H=A\exp(Bz^2)$, respectively. They can be improved by adding to each of them a second exponential function of the same form:

$$H = A_1\exp\left[B_1(L-z)^2\right] + A_2\exp\left[B_2(L-z)^2\right] \tag{22}$$

$$H = A_1\exp\left(B_1 z^2\right) + A_2\exp\left(B_2 z^2\right) \tag{23}$$

to correct for possible perturbations due to other unknown diffusive phenomena. Then, all coefficients A_1, B_1, A_2, B_2 can be calculated by non-linear regression analysis published elsewhere.[9] Two PC programs in GW-BASIC are given in the Appendices 1 and 2 here for all calculations. They are based on the program mentioned before,[9] modified according to Eq. (22) for injection of the analyte at $z = L$, or Eq. (23) for the injection at $z = 0$.

RESULTS AND DISCUSSION

The methodology described here is exemplified by using liquid benzene diffusing into chloroform and acetonitrile for various time periods, after injection of 50 μL of benzene either at $z = L$ or at $z = 0$ (cf. Figure 1). Experiments were conducted in the absence of any internal wall coating and also in the presence of a thin wall coating of silica gel. The diffusion coefficients D calculated

Table 1

Diffusion Coefficients (D), Calibration Factors of HPLC (g), Adsorption Parameters (λ^2), and Squared Correlation Coefficients (r^2) for Benzene as Solute, Left for Various Time Periods (t), at Ambient Temperature (18°C)

Solvent	Injection Point	t (h)	Wall Coating	D (cm^2s^{-1})	g ($cm/mol\ cm^{-3}$)	λ^2 (cm^{-2})	r^2 r^2
Acetonitrile	$z = 0$	16	None	4.60×10^{-5}	6727982	---	0.997
Acetonitrile	$z = 0$	29	None	3.15×10^{-5}	6567330	---	0.993
Acetonitrile	$z = 0$	48	None	2.67×10^{-5}	7743744	---	0.994
Acetonitrile	$z = 0$	18.5	SiO_2	4.29×10^{-5}	---	1.22×10^{-2}	0.997
Chloroform	$z = L$	48	None	7.89×10^{-6}	4.609×10^{7}	---	0.938
Chloroform	$z = L$	48	SiO_2	1.13×10^{-5}	---	-0.536	0.746
Acetonitrile	$z = 0$	29	SiO_2	3.91×10^{-5}	---	0.665	0.980
Acetonitrile	$z = 0$	48	SiO_2	5.55×10^{-5}	---	0.542	0.986

as described in the previous Section 4, together with the respective calibration factor g of the HPLC system, the values of λ^2, and the squared correlation coefficients of the non-linear regression of the experimental data by the PC programs of the Appendices, are collected in Table 1.

From the r^2 values, one can judge that Eq. (23), pertaining to the injection of the solute at the bottom of the diffusion column, describes the experimental findings in a better way than Eq. (22) referring to injection at the top. This assumption is validated by the values of the diffusion coefficients D found by the two alternatives, and the positive values of λ^2 when the injection is made at $z = 0$. The values of D are of the correct order of magnitude known from the literature for the diffusion in liquids at ordinary ambient temperatures. Moreover, they seem independent of the time t used for equilibration of the diffusion column. This is not the case, however, for the adsorption parameter λ^2, which was found 1.22×10^{-2} for $t = 18.5$ h and 0.665 or 0.542 for 29 or 48 h, respectively.

Obviously, more experiments are required to confirm the various hypotheses used in the calculations.

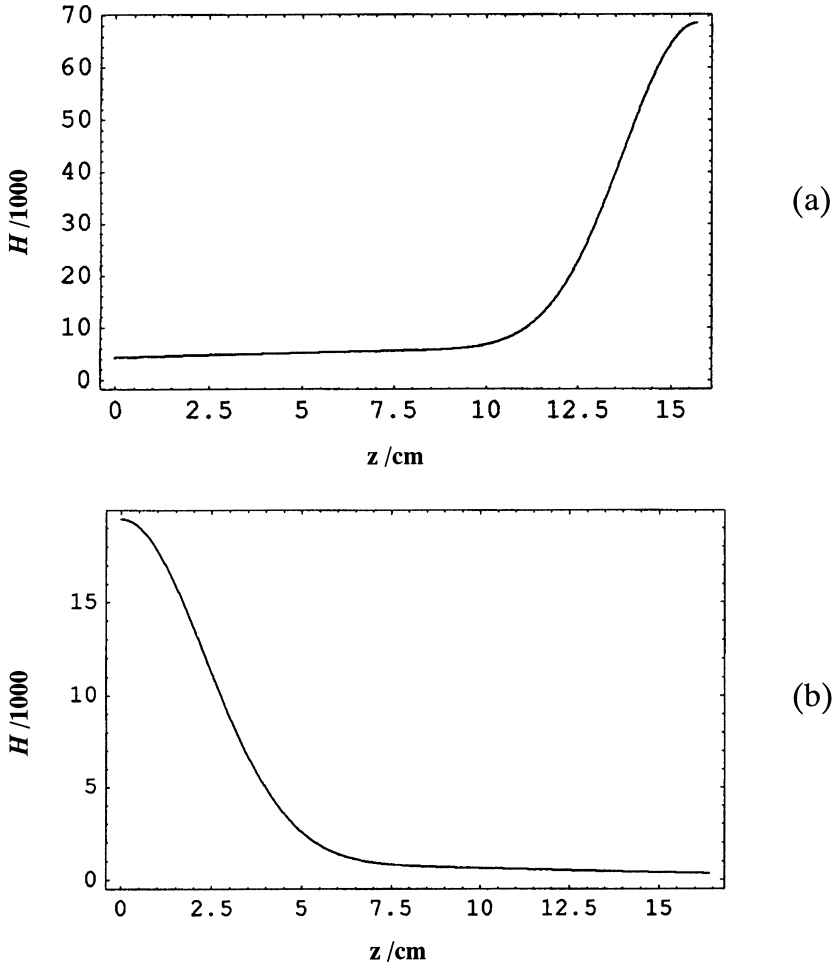

Figure 3. Plots of Eq. 22 (a) and Eq. 23 (b), using the parameters A_1, A_2, B_1 and B_2, as calculated by the PC programs of the Appendices from the experimental measurements.

If one plots H versus z according to Eqs. (22) and (23), using the values of A_1, A_2, B_1, B_2 calculated by the PC programs of the Appendix, and the known value of L, graphs like those shown in Figure 3 are obtained. It is seen that Figure 3 (b) resembles in shape Figure 2, where experimental measurements of H and z are used.

APPENDIX 1

PC Program in GW-BASIC for Calculations Based on Eq. (20) (Injection of Solute at $z = L$).

```
10 REM   Least Square Fit of Function H=A1*exp(B1*(L1-Z)
^2)+A2*EXP(B2*(L1-Z) ^2)
20 REM  Calculation of D and g
30 INPUT"Total number of pairs H, z=";N
40 INPUT"Total time/s=";T
50 INPUT"Total length L/cm=";L1
60 INPUT"Total volume of liquid in cm^3=";V
70 INPUT"Amount in mol injected=";MOL
80 DIM  H(N), T(N), Y(N), U(N), D(N), Z(N)
90 FOR I=l  TO  N
100     READ  H(I), Z(I)
110 NEXT  I
140 N2=INT(N/5+.5)
150 MAX=-10:OPT=0
160 FOR J=N2  TO  N-3
170    K=N-J+1:L=N
180    FOR I=K  TO  L
190        Y(I)=LOG(H(I))
200        T(I)=(L1-Z(I)) ^2
210    NEXT  I
220 GOSUB 2040
230 A1=EXP(A)
240 BI=B
250 SA1=SA
260 SB1=SB
270    K=l  :L=N-J
280    FOR I=K  TO  L
290        U(I)=H(I)-A1*EXP(B1*(L1-Z(I) ) ^2)
300    Y(I)=LOG(ABS(U(I)))
310    NEXT  I
320 FOR I=K  TO  L
330 T(I)=(L1-Z(I)) ^2
340 NEXT  I
350 GOSUB 2040
360 A2=EXP(A)
370 B2=B
380 SA2=SA
390 SB2=SB
400 IF  OPT=1  THEN   570
410 Cl=0
420 C2=0
```

```
430 C3=0
440 FOR I=l  TO  N
450    D(I)=H(I)-A1*EXP(B1*(Ll-Z(I) ) ^2)-A2*EXP(B2*(Ll-Z(I) ) ^2)
460 C1=C1+D(I) ^2
470 C2=C2+H(I) ^2
480 C3=C3+H(I)
490 NEXT  I
500 R=1-C1/(C2-C3^2/N)
510 IF R>MAX  THEN  MAX=R : JM=J
520 NEXT  J
550 J=JM : OPT=1
560 GOTO 170
570 LPRINT
580 LPRINT “Intercept Ln(A1) and its Standard error=”;LOG(A1)  “+-”SA1
590 LPRINT “Slope Bl and its Standard error=”;Bl  “+-”SBI
600 LPRINT
610 LPRINT “Intercept Ln(A2) and its Standard error=”;LOG(A2)  “+-”SA2
620 LPRINT “Slope B2 and its Standard error=”;B2  “+-”SB2
630 LPRINT
640 LPRINT “Square of maximum correlation coefficient r^2=”;MAX
650 LPRINT
660 LPRINT “Optimum values of points for 1st and 2nd exponential functions,
          respectively=”; JM”and”N-JM
670 LPRINT
680 D1=-1/(4*B1*T) :D2=-1/(4*B2*T)
690 LPRINT “Diffusion Coefficient D from B1 and B2 in cm^2/s =”;D1”,”D2
700 LPRINT
710  G1=A1*V*SQR(ABS(-3.14159/4/B1)  )/MOL/L1:G2=A2*V*SQR(ABS
(-3.14159/4/B2))/MOL/L1
720 LPRINT
730 LPRINT “Calibration factor of HPLC in cm per mol/cm^3 to divide the
peak-height in cm g1 and g2=”;G1”,”G2
2000 REM Enter DATA in pairs: peak Height, distance z ,starting from the small
values of z and ending with the biggest one
2010 DATA
2020 DATA
2030 END
2040 REM Linear regression of Y(I) = A + B T(I)
2050 S1=0
2060 S2=0
2070 S3=0
2080 S4=0
2090 S5=0
2100   FOR I=K  TO  L
2110   S1=S1+T(I)
```

```
2120   S2=S2+T(I)^2
2130   S3=S3+Y(I)
2140   S4=S4+Y(I)^2
2150   S5=S5+T(I)*Y(I)
2160 NEXT  I
2170 Z=L-K+1
2180 M1=S5-S1*S3/Z
2190 M2=S2-SI^2/Z
2200 M3=S4-S3^2/Z
2210 A=(S3-S1*M1/M2)/Z
2220 B=M1/M2
2230 SYT=SQR(ABS(S4-A*S3-B*S5)/(Z-2) )
2240 SA=SYT*SQR(S2/Z/M2)
2250 SB=SYT/SQR(M2)
2260 RETURN
```


APPENDIX 2

PC Program in GW-BASIC for Calculations Based on Eq. (21) (Injection of Solute at $z = 0$).

```
10 REM   Least Square Fit of Function H=A1*exp(B1*(Z) ^2)+A2*EXP(B2*(Z)
^2)
20 REM  Calculation of D and g
30 INPUT"Total number of pairs H, z=";N
40 INPUT"Total time/s=";T
50 INPUT"Total length L in cm=";L1
60 INPUT"Total Volume of liquid in cm^3=";V
70 INPUT"Amount in mol injected=";MOL
80 DIM  H(N), T(N), Y(N), U(N), D(N), Z(N)
90 FOR I=l  TO  N
100     READ  H(I), Z(I)
110 NEXT  I
140 N2=INT(N/5+.5)
150 MAX=-10:OPT=0
160 FOR J=N2  TO  N-3
170    K=N-J+1:L=N
180    FOR I=K  TO  L
190        Y(I)=LOG(H(I))
200        T(I)=Z(I) ^2
210    NEXT  I
220 GOSUB 2040
230 A1=EXP(A)
240 BI=B
```

```
250 SA1=SA
260 SB1=SB
270    K=l  :L=N-J
280    FOR I=K  TO  L
290        U(I)=H(I)-A1*EXP(B1*Z(I) ^2)
300    Y(I)=LOG(ABS(U(I)))
310    NEXT  I
320 FOR I=K  TO  L
330 T(I)=Z(I) ^2
340 NEXT  I
350 GOSUB 2040
360 A2=EXP(A)
370 B2=B
380 SA2=SA
390 SB2=SB
400 IF  OPT=1  THEN   570
410 Cl=0
420 C2=0
430 C3=0
440 FOR I=l  TO  N
450    D(I)=H(I)-A1*EXP(B1*Z(I)^2)-A2*EXP(B2*Z(I)^2)
460 C1=C1+D(I) ^2
470 C2=C2+H(I) ^2
480 C3=C3+H(I)
490 NEXT  I
500 R=1-C1/(C2-C3^2/N)
510 IF R>MAX  THEN  MAX=R : JM=J
520 NEXT  J
550 J=JM : OPT=1
560 GOTO 170
570 LPRINT
580 LPRINT "Intercept Ln(A1) and its Standard error=";LOG(A1)  "+-"SA1
590 LPRINT "Slope Bl and its Standard error=";Bl  "+-"SBI
600 LPRINT
610 LPRINT "Intercept Ln(A2) and its Standard error=";LOG(A2)  "+-"SA2
620 LPRINT "Slope B2 and its Standard error=";B2  "+-"SB2
630 LPRINT
640 LPRINT "Square of maximum correlation coefficient r^2=";MAX
650 LPRINT
660 LPRINT "Optimum values of points for 1st and 2nd exponential functions,
        respectively="; JM"and"N-JM
670 LPRINT
680 D1=-1/(4*B1*T) :D2=-1/(4*B2*T)
690 LPRINT "Diffusion Coefficient D from B1 and B2 in cm^2/s =";D1","D2
700 LPRINT
```

```
710  G1=A1*V*SQR(ABS(-3.14159/4/B1)  )/MOL/L1:G2=A2*V*SQR(ABS
(-3.14159/4/B2))/MOL/L1
720 LPRINT
730 LPRINT "Calibration factor of HPLC in cm per mol/cm^3 to divide the
peak-height in cm g1 and g2=";G1","G2
2000 REM Enter DATA in pairs: peak Height, distance z ,starting from the small
values of z and ending with the biggest one
2010 DATA
2020 DATA
2030 END
2040 REM Linear regression of Y(I) = A + B T(I)
2050 S1=0
2060 S2=0
2070 S3=0
2080 S4=0
2090 S5=0
2100   FOR I=K  TO  L
2110   S1=S1+T(I)
2120   S2=S2+T(I)^2
2130   S3=S3+Y(I)
2140   S4=S4+Y(I)^2
2150   S5=S5+T(I)*Y(I)
2160 NEXT  I
2170 Z=L-K+1
2180 M1=S5-S1*S3/Z
2190 M2=S2-SI^2/Z
2200 M3=S4-S3^2/Z
2210 A=(S3-S1*M1/M2)/Z
2220 B=M1/M2
2230 SYT=SQR(ABS(S4-A*S3-B*S5)/(Z-2) )
2240 SA=SYT*SQR(S2/Z/M2)
2250 SB=SYT/SQR(M2)
2260 RETURN
```


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