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ON THE EFFICIENCY OF GEL ELECTROPHORESIS

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SUMMARY

A mathematical expression for the plate height in polyacrylamide gel electrophoresis is derived on the basis of the conservation of mass.

Comparison of the theoretical with the experimental values justifies the assumption that diffusion and the velocity profile due to the electrical heat production are the main causes of peak broadening in polyacrylamide gel electrophoresis.

Some applications of the theory to the optimization of gel electrophoresis experiments and to the estimation of the homogeneity of protein preparations are described.

INTRODUCTION

As in chromatography[1], resolution in gel electrophoresis can be defined as $R_s = \Delta Z/4\sigma$, where ΔZ is the distance between the centres of gravity of two neighbouring zones and σ is the standard deviation of a zone. If L is the distance from the centre of gravity of the zone to the origin, m the mobility of one of the two compounds and Δm the mobility difference, then

$$R_s = \frac{L}{4\sigma} \cdot \frac{\Delta m}{m} \qquad (1)$$

The expression $\Delta m/m$ can be regarded as the selectivity of this separation method for the two compounds and can be influenced by changing the gel concentration[2] or pH (ref. 3). To achieve optimal resolution at a fixed selectivity and column length, σ has to be minimal.

LUNNEY *et al.*[4] have made an empirical approach to this problem. RICHARDS AND LECANIDOU[5] have developed a partial theory to explain the effect of various factors on the band width of RNA zones in polyacrylamide gel electrophoresis.

As we used an electrophoresis system consisting of a column with an elution chamber and a UV detector, the standard deviation of the concentration–time curve on the recorder must be considered due to the combined effects of the dispersion in the column and in the detection system. For gas chromatography, the effect of the detector on the retention time and zone dispersion was calculated by JOHNSON AND STROSS[6]. Their formulae can also be applied to the combination of an elution chamber

and a detector cell. We can therefore concentrate our attention on the zone dispersion in the column.

THEORY

In gel electrophoresis, convection can be ignored as a cause of zone dispersion. If the column is homogeneous and the load is chosen to be low enough, the dispersion will be the result only of diffusion and the electrical heat production in the column.

The differential equation based on the conservation of mass for "ideal electrophoresis"[7] is:

$$\frac{\partial C}{\partial t} = D\Delta C - U(\rho)\frac{\partial C}{\partial x} \tag{2}$$

The axis of the electrophoresis tube coincides with the x-axis of the cylindrical coordinate system x, ρ, θ.

From the temperature gradient in the column, which was given by PORATH[8], HJERTÉN[9] has derived the velocity profile. This profile is represented by:

$$U(\rho) = U\{1 + q(1 - \rho^2)\} \tag{3}$$

where

$$q = \frac{BI^2}{4\pi^2 a^2 \varkappa \lambda T^2}$$

and

$$U = mE = \frac{mI}{\pi a^2 \varkappa}$$

is the velocity at the wall.

Substitution of eqn. 3 into eqn. 2 gives the differential equation:

$$\frac{1}{D}\cdot\frac{\partial C}{\partial t} = \Delta C - \frac{U}{D}[1 + q(1 - \rho^2)]\frac{\partial C}{\partial x} \tag{4}$$

where D is assumed to be constant.

ARIS[10] and TAYLOR[11] have dealt with the effect of a parabolic velocity profile on the dispersion. We have followed essentially the mathematical procedure described by ARIS.

If the coordinates are transformed to a dimensionless form and an origin is chosen that moves with the speed of the centre of gravity of the zone, eqn. 4 becomes:

$$\frac{\partial C}{\partial \tau} = \frac{\partial^2 C}{\partial \xi^2} + \frac{1}{\rho}\cdot\frac{\partial}{\partial \rho}\left(\rho\frac{\partial C}{\partial \rho}\right) - \frac{\mu q}{2}(1 - 2\rho^2)\frac{\partial C}{\partial \xi} \tag{5}$$

The term containing θ vanishes because of the cylinder symmetry. The transformed coordinates are given by:

$$\xi = x - U(1 + \tfrac{1}{2}q)t/a$$

$$\rho = r/a$$

$$\tau = Dt/a^2$$

$$\mu = Ua/D$$

Eqn. 5 has the same form as the equation ARIS obtained for the dispersion of a solute by viscous flow of a liquid through a tube of circular cross-section. His results can therefore be used for the calculation of the zone profile and zone dispersion. The zone profile (C_1) of an initially flat zone of constant concentration as a function of ρ and τ is given by eqn. 30 in ref. 10. It is necessary to replace μ in that equation by $\mu q/2$ to obtain the zone profile in the electrophoresis tube:

$$C_1 = \frac{\mu q}{8}(\tfrac{1}{3} - \rho^2 + \tfrac{1}{2}\rho^4) + 4\mu q \sum_{n=1}^{\infty} \alpha_n^{-4} \cdot \frac{J_0(\alpha_n \rho)}{J_0(\alpha_n)} \cdot \exp(-\alpha_n^2 \tau) \qquad (6)^*$$

where α_n is the n-th zero of the Bessel function of the first order.

The zone dispersion (m_2) as a function of time was also given by ARIS and becomes in our case:

$$m_2 = m_{20} + 2\left(1 + \frac{\mu^2 q^2}{192}\right)\tau - 32\mu^2 q^2 \sum_{n=1}^{\infty} \alpha_n^{-8}[1 - \exp(-\alpha_n^2 \tau)] \qquad (7)^{**}$$

If these equations are written in the coordinates ρ and t, one obtains:

$$C_1 = \frac{\mu q}{8}(\tfrac{1}{3} - \rho^2 + \tfrac{1}{2}\rho^4) + 4\mu q \sum_{n=1}^{\infty} \alpha_n^{-4} \cdot \frac{J_0(\alpha_n \rho)}{J_0(\alpha_n)} \cdot \exp\left(-\frac{\alpha_n^2 Dt}{a^2}\right) \qquad (8)$$

and

$$m_2 = m_{20} + 2\left(1 + \frac{\mu^2 q^2}{192}\right)\frac{Dt}{a^2} - 32\mu^2 q^2 \sum_{n=1}^{\infty} \alpha_n^{-8}\left[1 - \exp\left(-\frac{\alpha_n^2 Dt}{a^2}\right)\right] \qquad (9)$$

To obtain the band-profile P and the variance σ^2, C_1 and m_2 must be multiplied by a and a^2 respectively: $P = aC_1$; $\sigma^2 = a^2 m_2$.

As $H = \sigma^2/L$ and $t = \pi a^2 L \varkappa / mI$, the plate height is completely expressed in experimental quantities.

MATERIALS AND METHODS

The experiments were carried out on an LKB Uniphor 7900 column electrophoresis system[12] except for the elution stopper. In order to be able to use a single gel column over a long period—up to 8 days—it was necessary to modify the elution chamber of the LKB apparatus. The details of the modified elution stopper are shown in Fig. 1. The principle of elution was described by HJERTÉN[13].

The buffer system used has been described elsewhere[14]. The gel ($T = 5\%$, $C = 3\%$[15]) was prepared as described by DAVIS[16].

* The sign for the second term in this expression has to be + in ARIS' formula.
** The sign for the third term has to be −, as a consequence of the preceding footnote.

Bromophenol blue and penicillinase from *Bacillus licheniformis* were used as samples in the electrophoresis experiments. The penicillinase was the extracellular enzyme isolated from *B. licheniformis* 749/C cultures according to the method described by POLLOCK[17].

Plate heights at different current strengths were obtained from the concentration–time recordings.

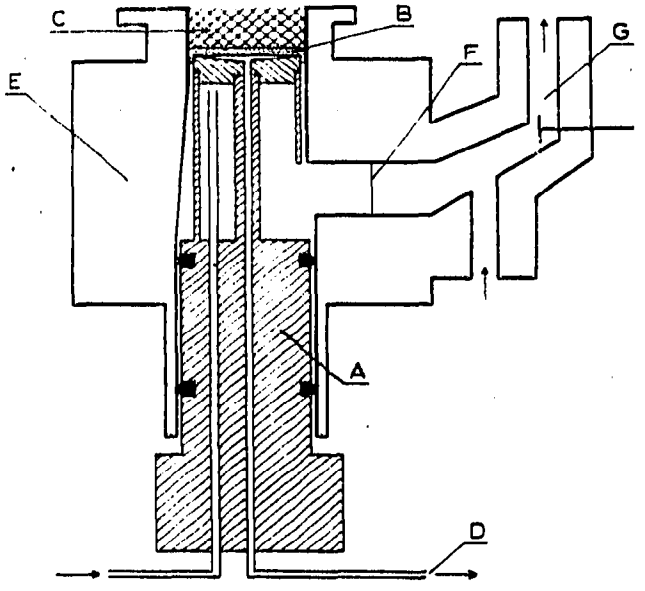

Fig. 1. Modified elution-stopper for the LKB-7900 Column Electrophoresis system. A = Perspex body of the elution stopper; B = porous polyethylene disk; C = polyacrylamide gel column; D = elution buffer; E = Perspex circulation coupling; F = Visking dialysis membrane; G = electrode buffer.

RESULTS AND DISCUSSION

For the experimental verification of eqns. 8 and 9, the parameters $\varkappa$, D, m, T, λ, B, L, and I have to be determined.

RICHARDS AND LECANIDOU[5] have shown that for a gel of less than 10% polyacrylamide the specific conductance of the aqueous buffer solution may be used. As our gel consisted of 5% polyacrylamide we have chosen for B and λ the values for water, *viz.*, $B = 2400°$ K; $\lambda = 5.73 \times 10^{-3}$ J s^{-1}cm^{-1}deg^{-1}. At higher gel concentrations, the values of $\varkappa$, B and λ have to be determined in separate experiments. As

$$\frac{m(T_1)}{m(T_2)} = \frac{\eta(T_2)}{\eta(T_1)} = \exp\left[B\left(\frac{1}{T_2} - \frac{1}{T_1}\right)\right] \qquad (10)$$

this can be achieved for B by measuring the mobility at different temperatures.

The diffusion coefficient and the mobility can be obtained from the electrophoresis experiments. The mobility is given by $m = \pi a^2 L\varkappa/tI$.

The diffusion coefficient can be calculated from the slope of the linear part of the H–I^{-1} curve (Fig. 2), which is $2\pi a^2\varkappa/m \cdot D$, as can be seen from eqn. 9 at low

current strengths. From our data between 2 and 6 mA, we calculated with the least-squares method a value for D of 1.4×10^{-6} cm^2s^{-1} at 10° in the 5 % polyacrylamide gel. Simultaneously, the constant term in the plate heights was found to be 1.1×10^{-3} cm. This constant term is due to the dispersion in the detector and to the term m_{20} in eqn. 9. The experimental data are not sufficiently accurate to separate the dispersion in the detector from the dispersion caused by irregularities in the

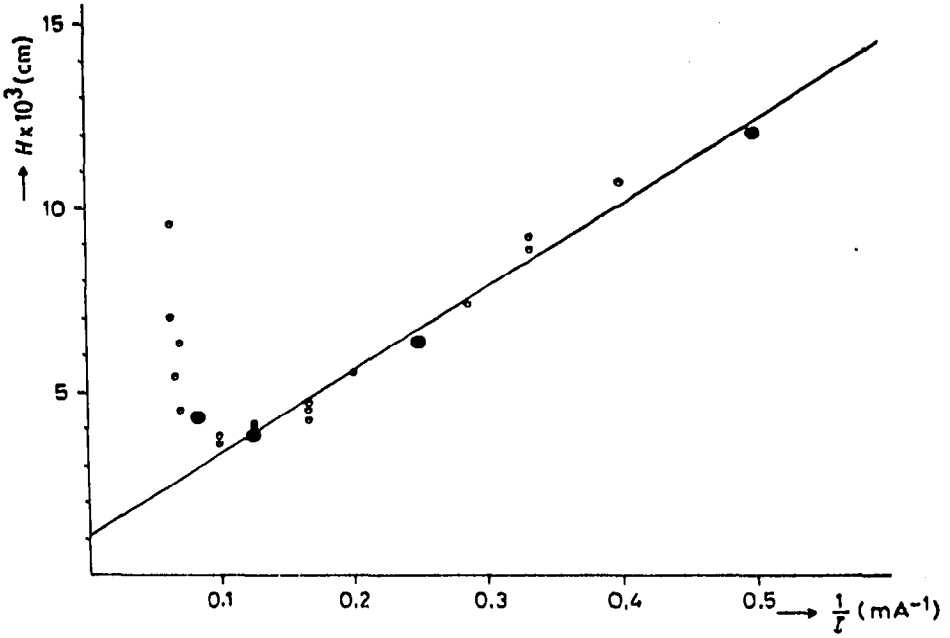

Fig. 2. Graph of H against $1/I$. ○ = measured values of H, ● = two coinciding values for bromophenol blue. The straight line is the best line (least-squares method) through the points corresponding to $1/I$ values of 0.167 and higher. $\varkappa = 2.23 \times 10^{-4}$ $\Omega^{-1}cm^{-1}$; $T = 283.0°$ K; $B = 2400°$ K; $\lambda = 5.73 \times 10^{-3}$ J s^{-1} cm^{-1} deg^{-1}; $m = 13.5 \times 10^{-5}$ cm^2 s^{-1} V^{-1}; $D = 1.4 \times 10^{-6}$ cm^2s^{-1}.

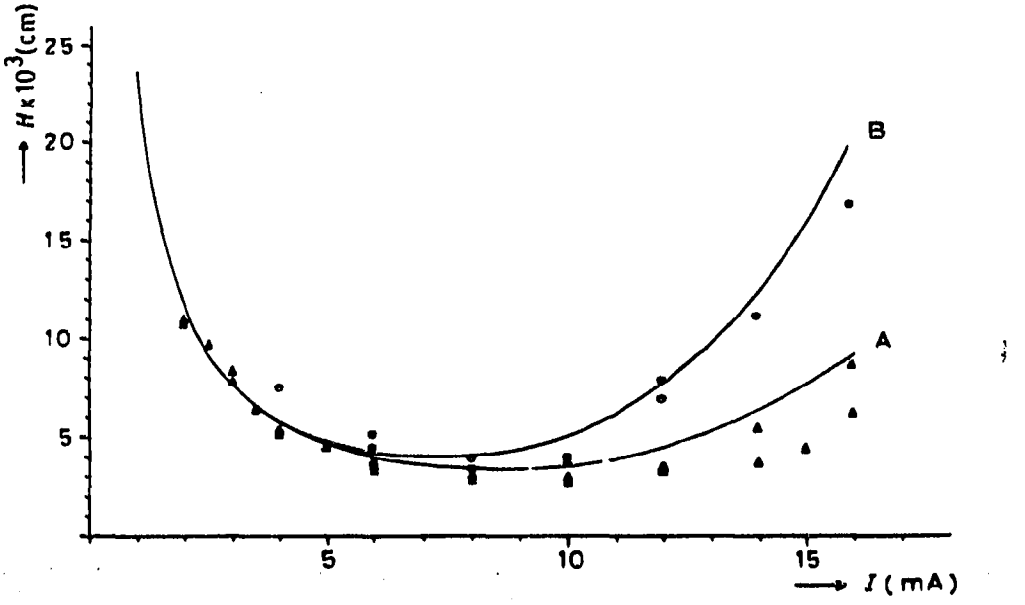

Fig. 3. Graphs of H against I for bromophenol blue. A, calculated curve on 10-cm column; B, calculated curve on 25-cm column. ▲ = Observed values on 10-cm column; ○ = observed values on 25-cm column. The parameters used are the same as in Fig. 2.

column, which lead to m_{20}. The factors that affect the regularity of the gel polymerization were discussed by CRAMBACH AND RODBARD[18]. A homogeneous gel matrix will be especially important for the electrophoresis of proteins.

The calculated graphs of H against I are given in Fig. 3, together with the experimental values for column lengths of 10 and 25 cm.

It can be seen from Fig. 3 that there is good agreement between the calculated and experimental values of H. This is an indication of the correctness of the physical model on which the theory is based.

A second method of testing our theory was found in the comparison of the calculated and experimental zone profiles (P) at various stages of the electrophoresis experiments. Fig. 4 shows the agreement of the experiments with the calculated curves, which also gives strong support for the correctness of the underlying physical model.

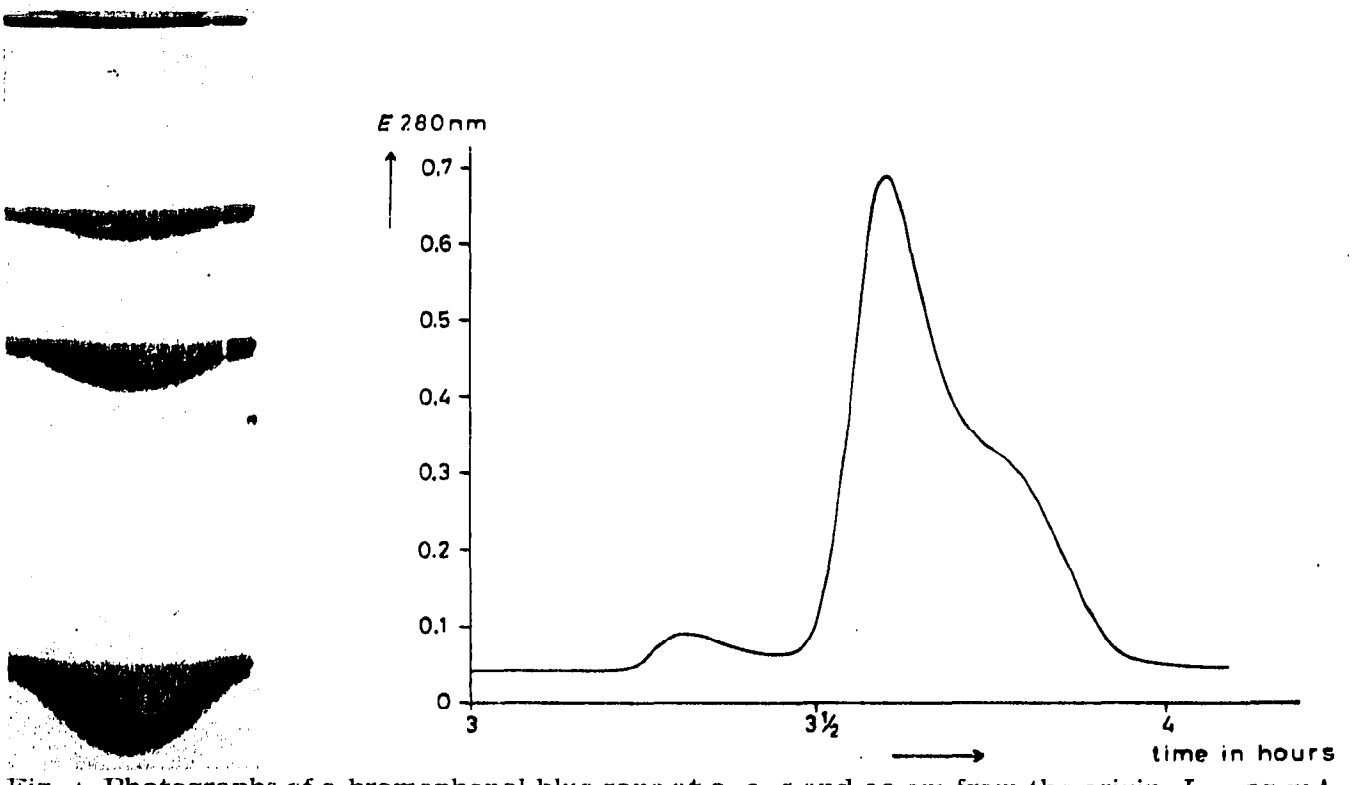

Fig. 4. Photographs of a bromophenol blue zone at 0, 3, 5 and 10 cm from the origin. I = 17 mA. The other parameters are the same as for Fig. 2. The lines in the last three zones are the calculated zone profiles (P).

Fig. 5. Recorded peak for bromophenol blue at I = 16 mA. The small peak at 3.3 h is considered to be an impurity in the bromophenol blue sample.

At high current strengths—in our case above 14 mA—the recorded peaks become not only relatively broad, but also asymmetric (Fig. 5). For these peaks, it is not possible to make an accurate determination of the variance. Probably this is a reason for the smaller reproducibility in H at these current strengths (Fig. 3).

In order to check whether it is also possible to predict the plate height for a

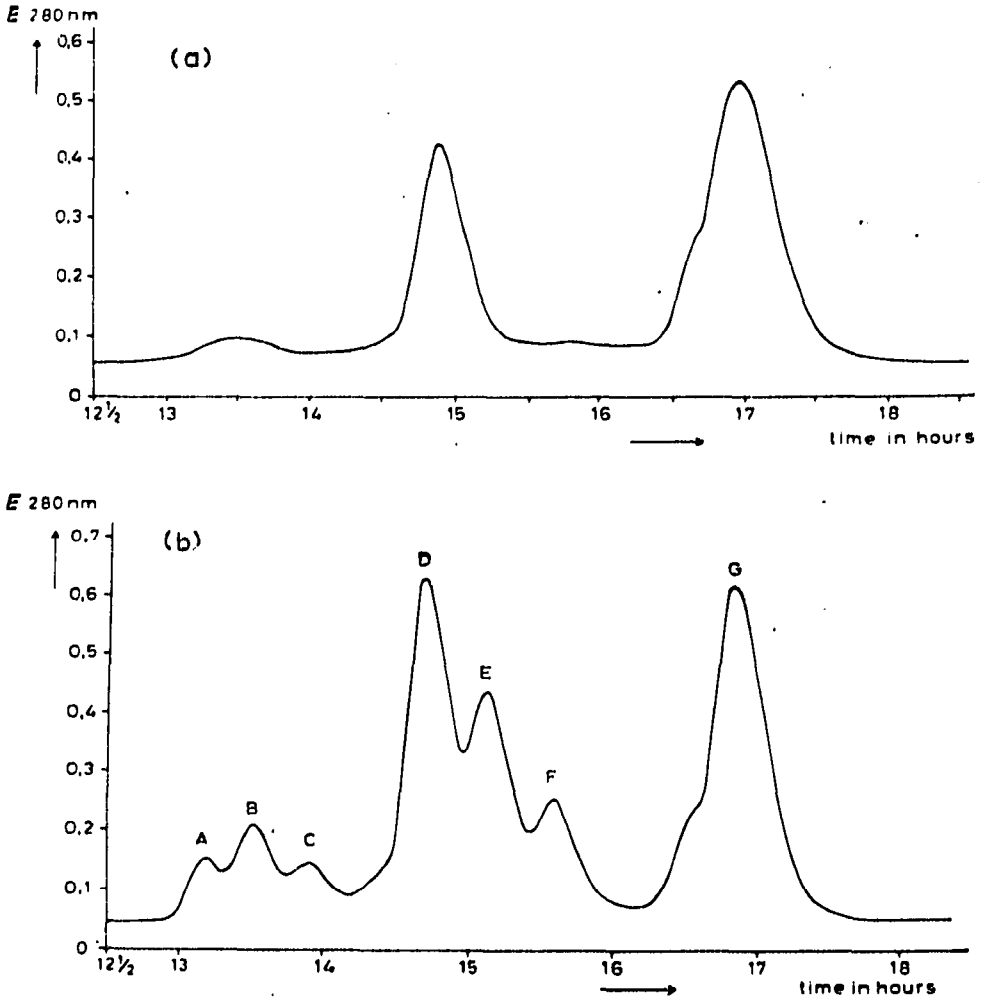

Fig. 6. Electropherograms of samples of penicillinase. (a) Freshly prepared sample; (b) aged sample. I = 10 mA.

protein zone, we carried out some experiments with penicillinase[17]. At 10 mA on a 25-cm column, we obtained the elution patterns shown in Fig. 6. The diffusion coefficient of penicillinase (mol. wt. = 30000) was estimated in the following way. The diffusion coefficient at 20° of a protein with a molecular weight (M) of 30000 is given by (ref. 19, p. 88):

$$D^{20}_{H_2O} = 3.2 \times 10^{-5} M^{-\frac{1}{2}} = 1.03 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$$

The diffusion coefficient at 10° can be calculated from the relation:

$$D^T = \frac{T}{293} \cdot \frac{1.005}{\eta_T} \cdot D^{20}$$

which results in $D^{10}_{H_2O} = 7.6 \times 10^{-7}$ cm^{2}s^{-1} for penicillinase. ORNSTEIN[20] has given a calculation method for the pore radius of polyacrylamide gels. For a gel of 5% polyacrylamide we obtained a pore radius of 32 Å. The radius of a globular protein with a molecular weight of 30000 is about 20 Å. Therefore, from the relation of Pappenheimer (see ref. 19, p. 740), the diffusion coefficient at 10° in a 5% poly-

acrylamide gel will be 2.8×10^{-8} cm^2s^{-1}. From our electrophoresis experiments the mobility of penicillinase was found to be 4.2×10^{-5} $cm^2s^{-1}V^{-1}$.

From these data we calculated a plate height of 3.3×10^{-3} cm at 10 mA. The experimental value of 3.0×10^{-3} cm for the central peak of the penicillinase pattern in Fig. 6a agrees very well with the calculated value. It can therefore be concluded that it is possible to make a reliable prediction of the standard deviation of a single protein zone.

For the analysis of aged penicillinase preparations, a column of 25 cm length was necessary in order to obtain a satisfactory resolution of the various enzymatically active proteins (Fig. 6b). The peaks D and G in Fig. 6b correspond to a plate height of 3×10^{-3} cm, which indicates that these peaks are single as far as this analysis method is concerned.

The comparison of the theoretically predicted value of the standard deviation of a zone with the experimental value can be used as a criterion[21] of the purity of a sample. For a more accurate determination of the standard deviation of a single peak, a reference compound can be used. If precautions are taken in order to prevent distortion of single peaks by heat production, the moment analysis given by GRUSHKA *et al.*[22] can be used as a method for the discernment of overlapping peaks in polyacrylamide gel electrophoresis.

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SYMBOLS

a = inner radius of the electrophoresis tube
B = constant which indicates the temperature dependence of the viscosity: $\eta(T) = A \exp(B/T)$
C_1 = band profile in the dimensionless coordinates
$C(x,r,\theta,t)$ = concentration at the point x, r, θ and time t
D = diffusion coefficient
E = field strength
H = a^2m_2/L = plate height
I = electrical current
$J_0(x)$ = Bessel function of x of zero order
L = distance moved by a zone
m = electrophoretic mobility
m_2 = variance of a zone in dimensionless coordinates
P = aC_1 = zone profile
R_s = resolution of two zones
r = distance from a point to the axis of the cylinder
s = area of the cross-section of the tube
T = temperature of the wall of the gel column
t = time
$U(r)$ = velocity of a zone along a line (r)
x,r,θ = coordinates of a cylindrical coordinate system.

ΔZ = distance between two zones
α_n = the n-th zero of the Bessel function of the first order
$\varkappa$ = specific conductance of the electrophoresis buffer at the temperature of the wall
λ = thermal conductivity
μ = Ua/D
ξ = $[x-U(1 + \frac{1}{2}q)\, t]/a$
ξ, ρ, τ = dimensionless variables corresponding to x, r and t
ρ = r/a
σ = standard deviation of a zone
τ = Dt/a^2

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