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Effect of hydrostatic flow on the efficiency in capillary electrophoresis

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ABSTRACT

An expression is derived for the plate height in capillary electrophoresis for cases where hydrostatic flow (HF) is present. HF will occur whenever the buffer levels at both ends of the capillary are not at the same height. Whereas the plate height equation for an HF-free system has only a molecular diffusion term, the plate equation in the presence of HF has an additional term which is a resistance to mass transfer term. The second term is a function of capillary radius, the hydrostatic velocity, the solute diffusion coefficient and the electrophoretic velocity. Unlike in chromatography, the mass transfer term usually does not increase with increasing solute velocity. Nonetheless, the contribution of this additional term to the total plate height can be substantial for wide capillaries and large solute molecules. We calculated maximum allowable buffer height differences, Δh_{max}, for a given loss in plate height. It was found that for large molecules and wide capillaries, Δh_{max} can be less than 1 mm, making severe demands on the instrumental design. With small solutes and narrow capillaries, the requirement for extract buffer levelling at both ends of the capillary is less acute.

INTRODUCTION

The use of capillary electrophoresis, because of its high efficiency and separation power, is growing continuously. In particular, capillary electrophoresis holds the promise of becoming the separation method of choice for biomacromolecules such as proteins and nucleic acids. Frequently, however, the theoretical efficiencies cannot be attained in practice and peaks of retained solutes are much broader than expected.

There are several possible contributors to the excess, or extra-column, zone broadening. Perhaps the most studied contribution is that due to the Joule heating effect. Knox and Grant [1,2] and Grushka *et al.* [3] discussed in detail the effects of temperature gradients on the efficiency in capillary electrophoresis. In general, it is felt that temperature effects are of minor importance provided that the capillary radius is small and that the ionic strength of the running buffer is not too high.

Other contributions to zone broadening have been investigated. For example, Martin and co-workers [4,5] studied the effect of wall distortion of the plug flow in capillary electrophoresis. Lukacs and Jorgenson [6], although they did not consider extra-column effects, examined the dependence of the efficiency on several experimental parameters. Sepaniak and co-workers [7,8] investigated the effects of sample

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injection, applied voltage, buffer concentration and column dimensions on the efficiency of micellar electrokinetic capillary chromatography systems. Jones *et al.* [9] discussed several other contributions to zone broadening.

This paper examines whether the presence of a hydrostatic flow component is a possible source of additional zone broadening. When the buffer levels in the reservoirs at the capillary ends are not equal, pressure-induced flow, known as hydrostatic flow (HF), results. This flow will be superimposed on the electrophoretic flow (EF). HF can be either in the same direction as, or opposite to, EF. The velocity profile associated with HF, as in any pressure-induced flow, is parabolic in nature. Therefore, HF can contribute to zone broadening, making the experimental efficiency less than the theoretical prediction.

THEORY

The velocity profile of HF is given by

$$u_{\mathrm{Hs}}(r) = 2u_{\mathrm{s}}\left(1 - \frac{r^2}{a^2}\right) \tag{1}$$

where u_{Hs} is hydrostatic velocity, r is the radial position, a is capillary radius and u_{s} is the cross-sectional average of the hydrostatic velocity, which is given by

$$u_{\mathrm{s}} = \frac{\Delta h \rho g a^2}{8\eta L} \tag{2}$$

Δh is the height difference between the buffer levels, ρ is the buffer density, g is the gravitational acceleration, η is the buffer viscosity and L is the capillary length (see Appendix for units). The overall velocity of the solute is

$$u(r) = u_{\mathrm{ef}} \pm 2u_{\mathrm{s}}\left(1 - \frac{r^2}{a^2}\right) \tag{3}$$

where u_{ef} is the electrophoretic velocity.

Eqn. 3 can be used, in conjunction with the following mass balance equation, in order to obtain the plate-height equation:

$$\frac{\partial C}{\partial t} = D\left[\frac{1}{r}\cdot\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right)\right] + D\cdot\frac{\partial^2 C}{\partial x^2} - u(r)\cdot\frac{\partial C}{\partial x} \tag{4}$$

where C is the solute concentration, D is the solute's diffusion coefficient, t is analysis time and x is longitudinal direction. Suitable boundary and initial values, needed to solve eqn. 4, are

$$C(t,\infty,r) = 0$$

$$C(0,0,r) = C_0$$

$$C(0,x,r) = 0$$

$$\partial C(t,x,a)/\partial r = 0$$

$$\partial C(t,x,0)/\partial r = 0$$

$$u(a) = u_{ef}$$

Eqn. 4 was solved in a manner similar to that described previously [3], using the method of Gill (*e.g.*, ref. 10). The solution yields a concentration dispersion expression which, in turn, allows the derivation of the following plate-height (H) equation (Appendix A in ref. 3 gives the details of the derivation):

$$H = \frac{2D}{u_{ef} \pm u_s} + \frac{a^2 u_s^2}{24D(u_{ef} \pm u_s)} \tag{5}$$

This paper assumes that, in addition to hydrostatic flow, molecular diffusion is the major broadening mechanism. Other contributions, such as temperature gradients, wall adsorption or extra-column effects, are not taken into account here. These additional contributions can be added, via their variances, to give a total H expression.

DISCUSSION

Contribution of the hydrostatic flow effect to plate height

In eqn. 5, the hydrostatic velocity is added to or subtracted from the electrophoretic velocity. In the following discussion we shall assume that the hydrostatic velocity is in the same direction as the electrophoretic velocity. The extension of the treatment to cases where the two velocity components are in opposite directions is fairly straightforward.

Eqn. 5 shows that the existence of HF results in an additional term in the plate-height equation which resembles the resistance to mass transfer in the mobile phase term in chromatography. The equivalent term in chromatography is a linear function of the average mobile phase velocity; the contribution of the resistance to mass transfer in the mobile phase to the plate height increases linearly with the average velocity of the mobile phase. In the present case, the velocity dependence is more complicated and it is a function of both u_{ef} and u_s. Assuming that the buffer height difference is constant (*i.e.*, it is equipment dependent), then the HF term in eqn. 5 is an inverse function of the electrophoretic velocity as in the molecular diffusion term. Thus, provided that Δh is constant, the relative importance of the HF term to the plate height is roughly a constant at all EF velocities.

Eqn. 5 has another very important implication. The existence of the HF component introduces an aliasing effect on the H behaviour. The additional flow component changes not only H but also the experiment migration velocity. As a result, the H *vs.* velocity curve can change in some unexpected ways. As will be discussed in a later section, this change in the H curve is particularly troublesome with small solute molecules.

Fig. 1 shows three H plots for a large solute ($D = 1 \cdot 10^{-10}$ m^2/s). The solid line

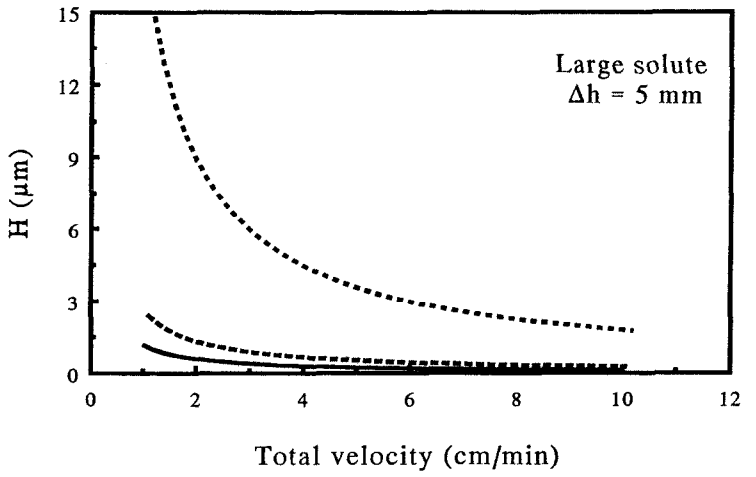

Fig. 1. Effect of capillary radius on H plots for a large solute ($D = 1 \cdot 10^{-10}$ m^2/s) when $\Delta h = 5$ mm. The solid line depicts the theoretical behaviour; the dashed line is for a 50-μm radius capillary; the dotted line is for a 75-μm capillary. The values of other parameters are given in the Appendix.

is the theoretical H behaviour ($H = 2D/u_{ef}$). Each line refers to a different capillary radius. Fig. 1 shows that as the capillary radius decreases, the effect of hydrostatic flow diminishes owing to a decrease in u_s. The velocity of the hydrostatic flow component is 0.207 and 0.0919 for the 75- and 50-μm capillaries, respectively (the relevant data for the calculation of u_s can be found in the caption of Fig. 1 and in the Appendix). It should be pointed out that for a 25-μm radius capillary, the plate-height line is identical with the theoretical line, within 1–2%.

Fig. 2 shows H values for a large solute in a 50-μm radius capillary at three

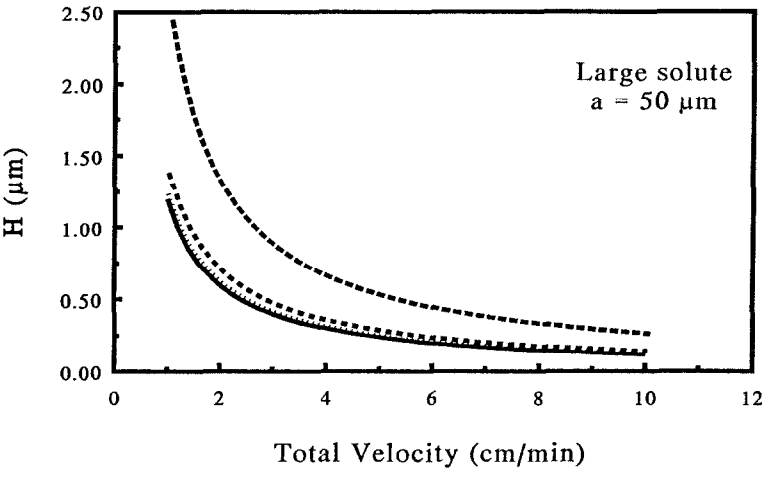

Fig. 2. Effect of Δh on the plate height for a large solute ($D = 1 \cdot 10^{-10}$ m^2/s) in a 50-μm radius capillary. The solid line is for $\Delta h = 0$ (theoretical behaviour); the dotted line is for $\Delta h = 1$ mm; the short dashed line is for $\Delta h = 2$ mm; the dashed line is for $\Delta h = 5$ mm. The values of other parameters are indicated in the Appendix.

different Δh values, 1, 2 and 5 mm. Also shown is the theoretical H behaviour. As expected, the larger is Δh, the greater is the contribution of hydrostatic flow to the plate height.

Figs. 1 and 2 indicate two main points. (a) Assuming that Δh is constant, the hydrostatic flow contribution does not cause the H curve to give a hyperbolic shape as in chromatography. As mentioned previously, the relative effect of HF is independent of the EF velocities. (b) For large solutes, the contribution of HF can be significant. For example, in the case of the 50-μm radius capillary, a 5-mm Δh can double the H values. Even a 2-mm height difference can cause a 15–20% increase in H. With the 75-μm radius capillary the situation is much worse; a 2-mm height difference triples H, whereas a 5-mm height difference increases H by a factor of 10. The HF velocity in this last example is 0.207 cm/min. Thus, at the low u_{ef} values, u_s is about 20% of the total velocity and the H curve at the low velocity is "aliased" toward slightly higher velocities than the theoretical curve. At high velocities, the contribution of u_s to the total velocity is only about 2% and the aliasing is not too noticeable.

With small solute molecules the HF effect is much less pronounced. Fig. 3 shows the H behaviour for a solute whose diffusion coefficient is $1 \cdot 10^{-9}$ m^2/s in a 75-μm radius capillary when Δh is 5 mm. The solid line is the theoretical behaviour and the dashed line is the H behaviour for $\Delta h = 5$ mm. Fig. 3 demonstrates very well the aliasing effect mentioned above: it looks as if the theoretical curve is shifted towards faster velocities and higher plate values. The dashed line is the "experimentally" observed H behaviour in the presence of HF. As the dashed line lies slightly above the theoretical line, the experimental conclusion is that there is a slight loss in efficiency. From a purely formalistic point of view, this conclusion is correct. However, at low u_{ef} velocities, the presence of the HF component actually improves slightly the efficiency *vis-à-vis* the expected value at the given EF velocity. For example, the plate height at $u_{ef} = 1$ cm/min is higher than the plate height at the total velocity of 1.2 cm/min ($u_{ef} =$

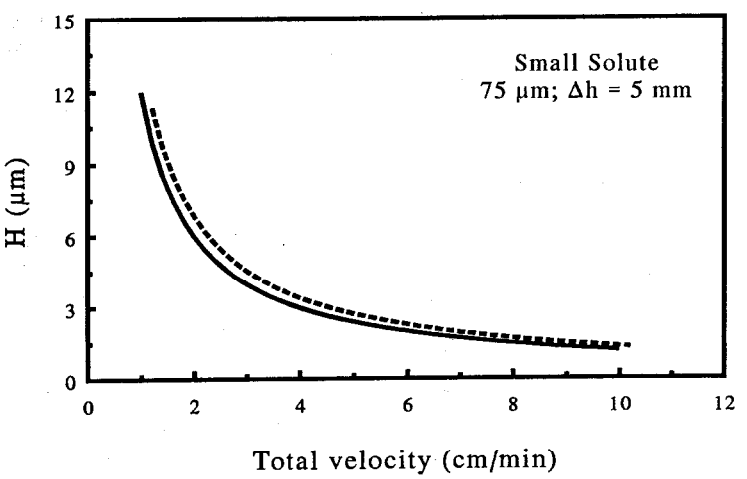

Fig. 3. Plate-height behaviour for small solute ($D = 1 \cdot 10^{-9}$ m^2/s) when $\Delta h = 5$ mm. The solid line is the theoretical curve; the dashed line is for a 75-μm radius capillary. The values of other parameters are indicated in the Appendix.

1 cm/min; u_s = 0.2 cm/min for a 75-μm radius capillary and Δh = 5 mm). The lowering of the plate height can best be demonstrated by plotting both curves in Fig. 3 against the electrophoretic velocity, that is, eliminating the hydrostatic velocity from the dashed line. In such a plot, the dashed line, at u_{ef} velocities of up to about 2 cm/min, would lie below the theoretical line. At high electrophoretic velocities, the dashed line would lie above the solid line.

Figs. 1–3 lead to the following important conclusion: the larger the solute molecule and the wider the capillary, the more critical is the need to balance the buffer reservoirs at the ends of the tubing. Narrow capillaries are advantageous not only because of their excellent heat transfer characteristics, but also owing to the much smaller HF effect.

The existence of an optimum Δh

As discussed above, provided that u_{ef} and u_s have the same sign, the presence of an HF component can yield better H values. The improvement in efficiency occurs because the HF component causes the solute molecules to elute faster, thus lowering the contribution of molecular diffusion. For each u_{ef} there is a unique Δh value which will give the best possible H for the total migration velocity $u_{ef} + u_s$. The optimum Δh can be found as follows: the ratio of eqn. 5 to the theoretical H equation is

$$\frac{H_s}{H} = \frac{u_{ef}}{u_{ef} + u_s} + \frac{a^2 u_s^2 u_{ef}}{48D^2(u_{ef} + u_s)} \tag{6}$$

H_s is the expression in eqn. 5. Eqn. 6 is differentiated with respect to u_s, set equal to zero and solved for $u_{s_{opt}}$:

$$u_{s_{opt}} = -u_{ef} + \frac{1}{a}\sqrt{a^2 u_{ef}^2 + 48D^2} \tag{7}$$

Eqn. 7, together with eqn. 2, allows the calculation of an optimum Δh value. Fig. 4 plots the optimum Δh values *vs.* u_{ef} for large molecules with three capillary radii. As the radius becomes larger, the optimum height difference becomes smaller. Also, the optimum Δh values decrease with increasing electrophoretic velocities. Moreover, with large molecules the optimum Δh values are very small, usually significantly less than 1 mm. As a consequence, for large solutes, the optimum u_s values are very small and the improvement in H is usually negligible.

Fig. 5 plots optimun Δh values *vs.* u_{ef} for small molecules with three capillary radii. As expected, Δh is much higher for small molecules. In fact, at slow EF velocities, the height difference leading to an improved H can be tens of centimetres, especially with the 25-μm radius capillary. Here, the improvement in H can be noticeable. Table I gives some H values with and without the HF component. At very low EF velocities, the improvements in H and the migration time can be substantial. However, owing to the very long migration times at such low EF velocities, the benefit of HF is questionable, except where very low voltages are desired. At more reasonable u_{ef} values, such as 3.5 cm/min, the improvement in H is not as impressive; *e.g.*, with a 25-μm capillary H improves from 3.43 to 3.25, a 5% decrease. With larger capillaries the improvement is even smaller.

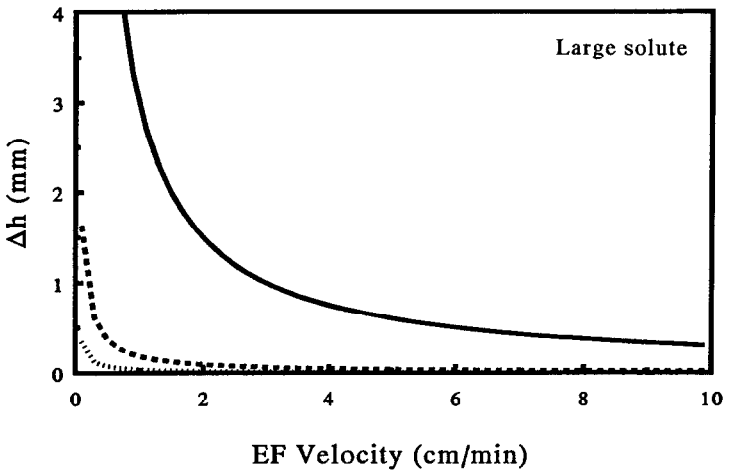

Fig. 4. Height difference which will give the optimum H value at a given u_{ef}. The curves are for large solute ($D = 1 \cdot 10^{-10}$ m^2/s). The solid line is for a 25-μm radius capillary; the dashed line is for a 50-μm capillary; the dotted line is for a 75-μm capillary. The values of other parameters are indicated in the Appendix.

The equivalent increase in efficiency and decrease in migration time can be accomplished by eliminating HF and increasing the applied electric field. If the electric field is increased so that the migration time is equal to that in the system with HF, the efficiency improvement will be better than indicated above. Therefore, from an efficiency point of view, it is always better to increase the electrophoretic velocity. However, when an increase in the applied field is detrimental to the system, then intentionally unlevelled buffer reservoirs may be beneficial.

The above discussion about a decrease in migration time and an increase in efficiency as a result of HF assumes that the added flow component is in the direction

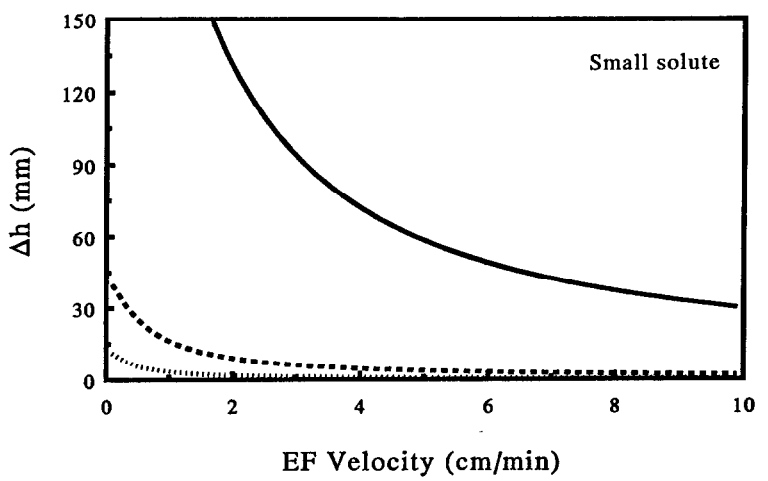

Fig. 5. Height difference which will give the optimum H value at a given u_{ef}. The curves are for small solute ($D = 1 \cdot 10^{-9}$ m^2/s). The solid line is for a 25-μm radius capillary; the dashed line is for a 50-μm capillary; the dotted line is for a 75-μm capillary. The values of other parameters are indicated in the Appendix.

TABLE I

IMPROVEMENT IN EFFICIENCY AS A RESULT OF HYDROSTATIC FLOW

H_s represents H with HF; t_{Rs} represents migration time with HF.

u_{ef} (cm/min)	a (μm)	u_s (cm/min)	Δh (mm)	H (μm)	H_s (μm)	t_R (s)	t_{Rs} (s)
0.1	25	1.56	340	120	13.6	36 000	2156
	50	0.737	40	120	25.6	36 000	4301
	75	0.463	11	120	36.2	36 000	6394
1.1	25	0.811	180	10.9	7.77	3237	1884
	50	0.243	13	10.9	9.7	3237	2680
	75	0.113	2.7	10.9	10.3	3237	2968
3.5	25	0.375	82	3.43	3.25	1029	929
	50	0.097	5.3	3.43	3.38	1029	1001
	75	0.044	1.1	3.43	3.41	1029	1016

of the electrophoretic flow. If HF is in the opposite direction, then the migration times will increase and the efficiency will decrease.

Maximum allowed height difference (Δh_{max}) for a given loss in plate height

It is of practical importance to be able to calculate Δh values for a given loss in H. However, because of the aliasing effect, the calculation of the maximum allowable Δh is not straightforward. An equation for Δh_{max} can be derived in one of two ways. The first approach assumes a given electrophoretic velocity, with its associated theoretical H value, and then proceeds to calculate Δh_{max} based on the assumed u_{ef}. In this approach, the derivation of Δh_{max} is done as follows: if we can tolerate a fraction x loss in H, then

$$\frac{H_s}{H} = 1 + x \tag{8}$$

Eqns. 8, 5 and 2 yield an expression for the Δh which is responsible of the above loss in efficiency:

$$\Delta h_{max} = \frac{(8\eta L)[48D^2 y \pm \sqrt{(48D^2 y)^2 - 192a^2 D^2 u_{ef}^2 (1 - y)}]}{2a^4 u_{ef} \rho g} \tag{9}$$

where $y = 1 + x$. Only the positive root of eqn. 9 is physically significant. The strong inverse dependence on the capillary radius should be noted. Fig. 6 plots the maximum height difference which will result in a 20% loss ($x = 0.2$) in H for large molecules as a function of electrophoretic velocity. Fig. 6 shows that Δh_{max}, which will cause 20% decrease in H, is relatively insensitive to electrophoretic velocity, especially with wide capillaries. It also shows the strong dependence of Δh_{max} on the radius; the narrower the capillary, the larger is the maximum height difference. For example, for a 25-μm radius capillary, the height difference between the buffer levels can be as large as 1.8

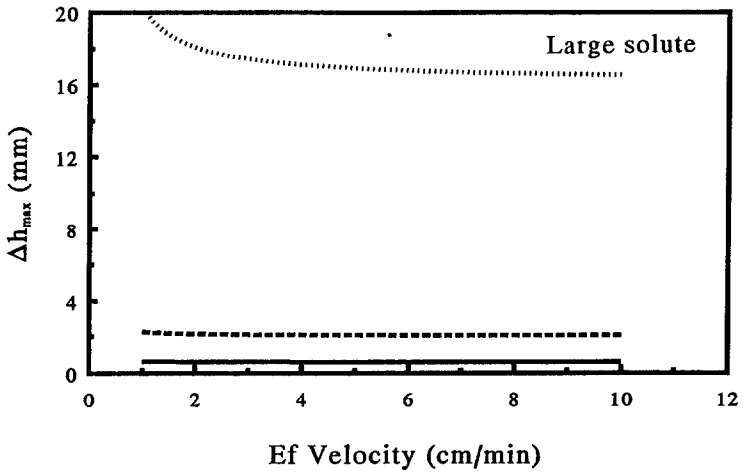

Fig. 6. Maximum allowed height differences which will cause a 20% decrease in plate height. Large solute ($D = 1 \cdot 10^{-10}$ m^2/s). The solid line is for a 25-μm radius capillary; the dashed line is for a 50-μm capillary; the dotted line is for a 75-μm capillary. The values of other parameters are indicated in the Appendix.

cm. In practice, actual height differences between the buffer levels can be easily maintained below that Δh_{max}. However, with 75-μm radius capillary, Δh should be less than about 0.61 mm to ensure a loss in H of less than 20%. Such a Δh is much more difficult to attain in practice. Even if we allow 40% loss in H, Δh_{max} for the 75-μm capillary, as calculated from eqn. 9, should be less than 0.85 mm, which still may be difficult to realize experimentally.

With large molecules, as in the examples in the previous section, Δh_{max} and the associated hydrostatic velocities are small. Therefore, the velocity shift in the H curve is barely noticeable. However, with small molecules, Δh_{max} and u_s can be large. For example, Fig. 7 plots the dependence of Δh_{max} that will cause a 20% loss in H on electrophoretic velocity for a solute with $D = 1 \cdot 10^{-9}$ m^2/s. Some of the characteristics of Fig. 7 are similar to those of Fig. 6, namely, a strong dependence of the allowed height difference on capillary radius and relative independence of Δh_{max} on u_{ef} in wide capillaries. Fig. 7 shows that for a 75-μm capillary, the allowed height difference, for a loss of 20% in H, is about 6.8 mm. However, with narrow capillaries, Δh_{max} can be fairly high, *e.g.*, with a 25-μm radius the allowed Δh is above 200 mm even at high velocities. Therefore, we might conclude that with narrow capillaries and small solutes, exact levelling of the two reservoirs is not essential, and almost any reasonable arrangement of the buffer solutions should yield close to theoretical H values. That conclusion is erroneous because, if Δh is high, the associated u_s is also very high, possibly higher than u_{ef}, causing a severe aliasing effect. The H curve will be very distorted, and the loss in efficiency, at the total velocity of $u_{ef} + u_s$, will be much higher than expected based on the electrophoretic velocity for which the calculations of Δh_{max} were made. Fig. 8 plots the H values associated with Δh_{max} curves in Fig. 7. Particularly noteworthy is the H behaviour for the 25-μm radius capillary. Owing to the very high hydrostatic velocity, the H curve cannot be measured at total velocities below about 4 cm/min. The highest H value for the 25-μm capillary is 14.4 μm. This value was

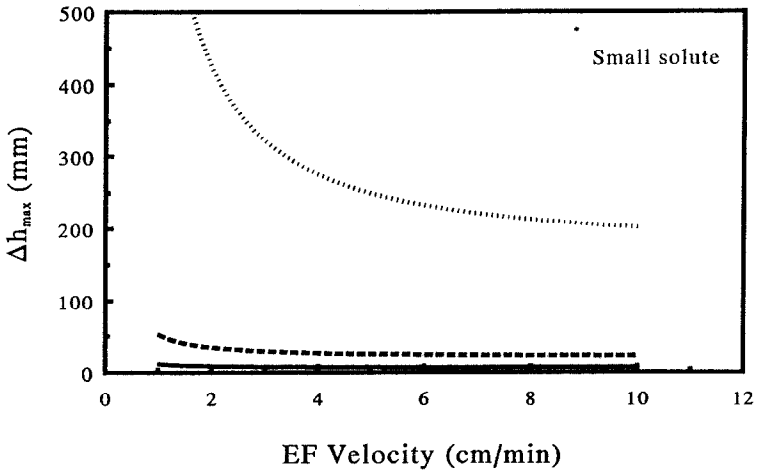

Fig. 7. Maximum allowed height differences which will cause a 20% decrease in plate height. Small solute ($D = 1 \cdot 10^{-9}$ m^2/s). Solid line is for a 25-μm radius capillary; dashed line is for a 50-μm capillary; dotted line for a 75-μm capillary. The values of other parameters are indicated in the Appendix.

calculated using $u_{ef} = 1$ cm/min as the reference electrophoretic velocity. The theoretical H value, at that u_{ef}, is 12 μm. Therefore, the value of 14.4 μm does represent a 20% loss, as was stipulated in the calculation. However, the apparent H curve is at much higher velocities and the *apparent* loss in the efficiency is much greater.

The "hook" shape of the curves for the 25-μm capillary in Fig. 8 is due to the very high u_s at low u_{ef} values. However, it should be stressed that the above discussion centred on the *maximum* allowed Δh for a given loss in H. In practice, it is expected that the actual Δh values will be, at most, a few millimetres. Therefore, the experimental H curves can be higher than expected, but not as high as depicted in Fig. 8.

The second approach to the calculation of Δh_{max} assumes an electrophoretic velocity, which we will indicate by u_{ef1}, and its associated theoretical H value. The next step is to equate a fractional increase in that H to the plate expression in eqn. 5, coupled with the condition that the total velocity, in the modified H expression, equals u_{ef1}. This last conditions ensures that we compare two H values, one theoretical and the other as a result of a buffer height difference, at the same migration velocity. As we have here two unknowns; namely, u_s and a new electrophoretic velocity, u_{ef2}, we need to solve two equations:

$$u_{ef1} = u_{ef2} + u_s \tag{10}$$

and

$$(1 + x)\frac{2D}{u_{ef1}} = \frac{2D}{u_{ef2} + u_s} + \frac{a^2 u_s^2}{24D(u_{ef2} + u_s)} \tag{11}$$

where x is the fractional loss in H. The solution for u_s is

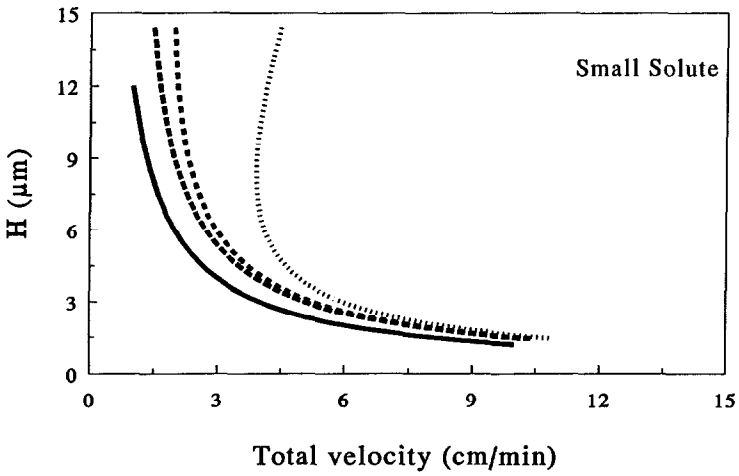

Fig. 8. Plate heights, in the presence of Δh_{max} which will cause a 20% loss in efficiency, as a function of total velocity for a small solute. Eqn. 9 is used to calculate Δh_{max}. The solid line represents theoretical H behaviour; the dashed line is for a 75-μm radius capillary; the short dashed line is for a 50-μm capillary; the dotted line is for a 25-μm capillary. The values of other parameters are indicated in the Appendix.

$$u_s = \frac{\sqrt{48xD}}{a} \tag{12}$$

Eqn. 12, together with eqn. 2, allows us to write an expression for Δh_{max}:

$$\Delta h_{max} = \frac{8\sqrt{48x\eta LD}}{\rho g a^3} \tag{13}$$

Eqn. 13 is much simpler than eqn. 9 although some similarities exist, *e.g.*, the strong inverse dependence of Δh_{max} on the radius of the capillary (see Fig. 9). In the limit of small diffusion coefficients, high u_{ef} and wide capillaries, eqn. 9 reduces to eqn. 13. In the present case, and for a given buffer system, u_s and Δh_{max} depend only on the diffusion coefficient of the solute and on the capillary radius. Hence the H curve for the uneven buffers will be shifted upward, from the theoretical curve, by the fractional efficiency loss, x, but it will not be distorted in the velocity direction; that is, at each velocity, the upper curve will be a fraction x higher than the theoretical curve.

Table II gives typical maximum allowed height differences for several capillary radii, several losses in H and for a large and a small solute. The general trend of the data is similar to those in Figs. 6 and 7. Thus, for large molecules, the requirement for buffer levelling is much greater. The demands for smaller Δh_{max} increase in severity as the capillary radius increases.

For large molecules, Δh_{max} values calculated from eqn. 9 or 13 are nearly identical, especially at high migration velocities. For small solutes, eqn. 13 yields lower allowed Δh values than eqn. 9, for a given loss in H. The results in the Table II indicate again the need to keep the buffer levels at equal heights in order to minimize the effects of hydrostatic flow.

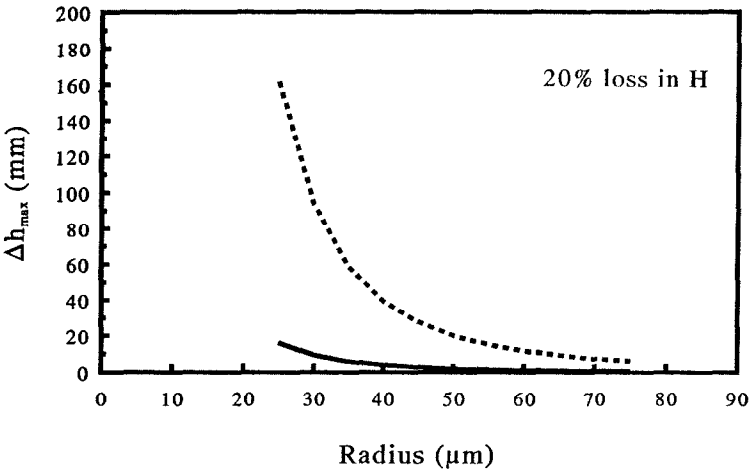

Fig. 9. Δh_{max}, for a 20% loss in H, *versus* capillary radius. Eqn. 13 was used to calculate the height difference. The solid line represents the behaviour for a large solute and the dashed line for a small solute.

CONCLUSIONS

The existence of hydrostatic flow in capillary zone electrophoresis can cause an additional zone broadening, which lowers the expected efficiency of the method. The hydrostatic flow effect is particularly important with large solutes and wide capillaries. In such cases, theory shows that the height levels of the buffer solutions, at both ends of the capillary, should be controlled to better than 1 or 2 mm. The situation is less critical with narrow capillaries, or with small molecules. Thus, narrow capillaries are beneficial not only because they minimize the Joule heating effect, but also because they reduce the influence of hydrostatic flow. Experimental work is now being pursued to validate the theoretical prediction.

TABLE II

MAXIMUM ALLOWED HEIGHT DIFFERENCE FOR SEVERAL CAPILLARY RADII AND SEVERAL ACCEPTABLE LOSSES IN H

Shown are values for large solutes (small diffusion coefficient) and small solutes (larger diffusion coefficient). Values used in the calculations are the same as those in the legend for Fig. 1.

Radius (μm)	D (m^2/s)	Δh_{max} (mm)		
		10% loss in H	20% loss in H	40% loss in H
25	$1 \cdot 10^{-10}$	11.44	16.18	22.88
50		1.43	2.02	2.86
75		0.42	0.60	0.85
25	$1 \cdot 10^{-9}$	114.4	161.8	228.8
50		14.3	20.22	28.6
75		4.24	5.99	8.47

ACKNOWLEDGEMENTS

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TABLE AI

UNITS USED IN CALCULATIONS

Symbol	Meaning	Units	Value used
a	Capillary radius	m	See text
C	Solute concentration	mol/m^3	None
D	Diffusion coefficient	m^2/s	See text
g	Gravitational acceleration	m/s^2	9.807
H	Plate height	m	See text
L	Capillary length	m	1
r	Radial position	n	None
t	Time	s	None
u_{ef}	Electrophoretic velocity	m/s	See text
u_{hs}	Hydrostatic velocity	m/s	None
u_s	Average hydrostatic velocity	m/s	See text
Δh	Height difference	m	See text
η	Buffer viscosity	$kg/m \cdot s$	0.001
ρ	Buffer density	kg/m^3	1000

APPENDIX

The calculations in this paper were done using mks units, and these units are listed in Table AI. In the text we use the more familiar units of cm/min for velocity, μm for capillary radius and plate-height values and mm for Δh.

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