Strongly nonlinear waves in capillary electrophoresis

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In capillary electrophoresis (CE), separation of charged molecular species is accomplished by exploiting the differential migration of ions in a narrow channel (10–100 μm) in which a strong electric field (∼100 V/m) is applied in the axial direction [1, 2]. The sample ions exist in solution in an electrolytic buffer which is referred to as the background electrolyte (BGE). Separation is accompanied by the competing process of diffusive spreading in the axial direction which causes peak dispersion. Dispersion reduces resolution of the separation and may lower the peak concentration to below the detection threshold. It is therefore detrimental. Any effect that tends to increase axial spreading over the minimum imposed purely by molecular diffusion in the axial direction is referred to as “anomalous dispersion” [3]. The transport problem of ions in the capillary is of considerable interest as it determines the amount of dispersion of the sample peak.

In this paper we are concerned with an effect known as “electromigration dispersion” (EMD) that causes significant anomalous dispersion when the ratio of sample to background ion concentration becomes large enough. For this reason it is also known as the “sample overloading effect”. In CE, it is desirable to have the sample concentration at the inlet as high as possible (to ensure that even trace components are within detectable limits) and buffer conductivity as low as possible (to minimize Joule heat), so that the limitation imposed by EMD quickly becomes significant [4].

The physical mechanism of EMD may be explained roughly in the following way: when the concentration of sample ions is sufficiently high in comparison to that of the background electrolyte, the local electrical conductivity of the solution is altered in the region around the sample peak. However, charge conservation requires the electric current to be the same at all points along the axis of the capillary. If diffusion currents due to concentration inhomogeneities are ignored for the moment, it follows, that the electric field must change axially. This is because Ohm’s law, taken together with current conservation, implies that the product of the conductivity and electric field must remain constant along the capillary. The axially varying electric field then alters the effective migration speed of the sample ions, which in turn alters its concentration distribution. Thus, in the continuum limit, the concentration of sample ions is described by a nonlinear transport equation. As expected, the CE signal exhibits features reminiscent of nonlinear waves familiar from other physical contexts [5, 6].

A one dimensional nonlinear hyperbolic equation for the sample ion concentration may be derived using simplifications that arise from assuming local electroneutrality and from neglecting the diffusivity of ions [7]. The restriction to zero ionic diffusivities was recently removed by Ghosal and Chen [8]. They considered the minimal model of a three ion system – the sample ion, a co-ion and counter-ion. The diffusivities of the three ionic species were assumed equal, though not necessarily zero. The sample ion concentration was then shown to obey a one dimensional nonlinear advection-diffusion equation which reduced to Burgers’ equation if the sample concentration was not too high relative to that of the background ions.

In this paper, we focus on the minimal three ion system considered by Ghosal and Chen [8] but we do not assume that the concentration of sample ions is small. Local electro-neutrality is however an excellent approximation in CE systems, since characteristic length scales are much larger than the Debye length which is on the order of nanometers. We therefore exploit it to reduce the numerical stiffness of the coupled ion transport equations. We identify a small number of parameters that

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I. INTRODUCTION

In capillary electrophoresis (CE), separation of charged molecular species is accomplished by exploiting the differential migration of ions in a narrow channel (10–100 μm) in which a strong electric field (∼100 V/m) is applied in the axial direction [1, 2]. The sample ions exist in solution in an electrolytic buffer which is referred to as the background electrolyte (BGE). Separation is accompanied by the competing process of diffusive spreading in the axial direction which causes peak dispersion. Dispersion reduces resolution of the separation and may lower the peak concentration to below the detection threshold. It is therefore detrimental. Any effect that tends to increase axial spreading over the minimum imposed purely by molecular diffusion in the axial direction is referred to as “anomalous dispersion” [3]. The transport problem of ions in the capillary is of considerable interest as it determines the amount of dispersion of the sample peak.

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In this paper, we focus on the minimal three ion system considered by Ghosal and Chen [8] but we do not assume that the concentration of sample ions is small. Local electro-neutrality is however an excellent approximation in CE systems, since characteristic length scales are much larger than the Debye length which is on the order of nanometers. We therefore exploit it to reduce the numerical stiffness of the coupled ion transport equations. We identify a small number of parameters that

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primarily determine the system evolution and study the
dynamics for a representative range of these parameters.
We show that at low concentrations the peak evolves in
accordance with the weakly nonlinear theory \[5\], but at
high enough concentrations, the dynamics of peak evolu-
tion is qualitatively different as the system is dominated
by the nonlinearity. Surprisingly, in the strongly nonlinear
regime, the peak breaks up into two zones marked by
a critical concentration \((\phi = \phi_c)\) and separated by a dif-
usive boundary. The high concentration zone \((\phi > \phi_c)\)
remains quasi-stationary whereas the low concentration
zone propagates forward forming a “surge front” super-
icially resembling nonlinear wave phenomena familiar
in the context of water waves, such as a river bore \[8\]. The
critical concentration \((\phi_c)\) can be predicted by a simple
argument based on flux conservation. At late times, dis-
ersion ensures that concentrations throughout the do-
main get smaller and the peak once again may be de-
scribed by Burgers’ equation. The complex nonlinear
behavior is a consequence of the nonlinearity inherent
in the Nast-Planck equations of ion transport, just as
the behavior of large amplitude water waves arise from
the nonlinear nature of the Navier-Stoke’s equations of
hydrodynamics.

II. NUMERICAL SIMULATIONS

We set up and solve numerically an idealized problem
in which a sample peak migrates in a background elec-
trolyte. The channel walls are assumed charge neutral,
so that electro-osmotic flow is absent \[18\]. Further, local
electro-neutrality is invoked which enables us to express
the electric field in terms of the instantaneous concen-
tration distributions rather than solve the Poisson’s equa-
tion for the electric potential. This considerably simpli-
fies the numerical work as the Poisson’s equation is stiff
on account of the smallness of the Debye length. Thus,
the problem is reduced to solving a set of one dimen-
sional coupled partial differential equations for the ion
concentration fields.

A. Model System

We will consider a three ion system consisting of sam-
ple ions, co-ions and counter-ions. Results will be ex-
pressed in terms of dimensionless variables: all lengths
are in units of a characteristic length \(w_0\) determined by
the initial peak width, time is in units of \(w_0/v\), where
\(v\) is the migration velocity of an isolated sample ion in
the applied field \((E^\infty)\) and the electric potential is in units
of \(E^\infty w_0\). All concentrations are in units of \(c_i^\infty\), where \(c_i^\infty\) is
the concentration of negative ions in the background elec-
trolyte. In order to define a minimal problem with the
fewest possible parameters, we assume that the mobility
\((u)\) is the same for all the species, and therefore, so is the
diffusivity \((D)\), in accordance with the Einstein relation
\((D_i/u_i = D/u = k_BT\) where \(k_B\) is Boltzmann’s constant
and \(T\) is the absolute temperature). Note however, since
the valence \(z_i\) are different, the electrophoretic mobilities
of the species \(\mu_i = z_i e u\) are not identical. Then the only
parameters in the problem are \(Pe = w_0/N/D\), which may
be regarded as a “Péclet number” based on the electromi-
gration velocity \(v\), and the two valence ratios \(z_n/z, z_p/z\,
where \(z_p, z_n\) and \(z\) respectively the valence of cations,
anions and sample. We present results for two values of
the Péclet number: \(Pe = 100\) and 200 and fix the val-
ence ratio at \(z : z_p : z_n = 1 : 2 : −1\). For other values
of these parameters the results are qualitatively similar.
The parameter of greatest interest is the degree of sam-
ple loading or the amplitude of the initial peak. The
shape of the wave is insensitive to initial conditions, so
for convenience we take the initial peak shape to have a
rectangular \[19\] profile of height \(c_n\) and width \(2w_0\) cen-
tered at \(x = 10w_0\). This is also the most common initial
shape encountered in practice where the sample is intro-
duced by electrokinetic injection. The degree of sample
loading is conveniently characterized \[8\] in terms of the
quantity
\[\Gamma = \int_{-\infty}^{+\infty} \phi(x, t) \, dx = \int_{-\infty}^{+\infty} c_n \, dx,\] (1)
which has units of length. The length scale \(\Gamma\) may be used
to define a second Péclet number \(Pe = v\Gamma/D\) which may
be treated as a dimensionless measure of sample loading.
A series of simulations are conducted with peak heights
in the range \(\phi_{in} = c_n/c_n^\infty = 0.01\) (low sample loading)
to 0.8 (high sample loading). The initial co-ion concen-
tration \(c_p\) is assumed constant throughout the domain.
Then the counter-ion concentration is determined by the
local electro-neutrality constraint, Eq. \[9\]. An infinite
domain is approximated by a finite computational box of
length much greater than \(w_0\). The values of the con-
centrations are held fixed at the domain boundaries and \(\partial\phi/\partial x\) is set to the constant value \(−E^\infty\). The domain is
chosen to be sufficiently large that the perturbations of
the concentrations and fields are always negligible near
the domain boundaries.

B. Numerical Method

We will solve the governing equations for ion transport
in solution, which are
\[\frac{\partial c_i}{\partial t} + \frac{\partial}{\partial x} \left[-\mu_i c_i \frac{\partial \phi}{\partial x} - D_i \frac{\partial c_i}{\partial x}\right] = 0\] (2)
where \(c_i\) is the concentration of species \(i\) \((i = 1, 2, \ldots, N)\)
with electrophoretic mobility \(\mu_i\) and diffusivity \(D_i\).
Electro-osmotic flow is neglected so that the problem
is one dimensional and may be described using the co-
ordinate \(x\) along the capillary and time since injection,
\(t\). On account of the requirement of local electroneutral-
FIG. 1: Time evolution of the profiles of the normalized sample ion concentration ($\phi$), electric field ($E/E_{\infty}$) and Kohlrausch function ($K/K_{\infty}$) in the case of weak sample loading ($\phi \ll \phi_c$). The Kohlrausch function spreads only by diffusion so that the sample peak rapidly migrates into the zone where $K = K_{\infty}$ (movie online).

FIG. 2: The same as in Fig. 1 except here the amplitude of $\phi$ exceeds $\phi_c$. Here the part of the peak above the value $\phi = \phi_{\text{inter}}$ appears to be effectively immobilized. The middle panel shows that the stagnant zone is due to a sharp reduction in the electric field caused by the very high electrical conductivity in this zone. The assumption $K = K_{\infty}$ is clearly invalid as a part of the peak remains trapped in the injection zone (movie online).

ity [9]

$$\sum_{i=1}^{N} z_i c_i = 0$$

($z_i$ is the valence of the $i$th species). The electric potential $\phi_e$ may be found from the equation of current conservation:

$$\frac{\partial}{\partial x} \left[ - \sum_{i=1}^{N} z_i \mu_i c_i \frac{\partial \phi_e}{\partial x} - \sum_{i=1}^{N} z_i D_i \frac{\partial c_i}{\partial x} \right] = 0. \quad (4)$$

Eq. (4) may be readily integrated to yield the local electric field, $E = -\partial_x \phi_e$:

$$E(x, t) = \frac{E_{\infty} \sum_{i=1}^{N} z_i \mu_i c_i^\infty + \sum_{i=1}^{N} z_i D_i \partial_x c_i}{\sum_{i=1}^{N} z_i \mu_i c_i}, \quad (5)$$

where the superscript $\infty$ indicates the value of the respective variable far away from the peak and the summation is over all species.

A finite volume method is used to discretize equations (2) and (4) in space using an adaptive grid refinement algorithm that is enabled by applying the Matlab library “MatMOL” [10]. The spatially discretized system of equations is then integrated in time using the Matlab solver “ode45” [11] which is based on an explicit Runge-Kutta (4,5) formula. Equations (2) and (4) automatically ensure that the electro-neutrality condition, Eq. (3), is satisfied and this is verified at each time step.

C. Results

Figure 1(a) shows the profiles of the normalized sample concentration $\phi(x, t) = c_n/c_n^\infty$ at fixed times $vt/w_0 = 0, 0.5, 2.0, 4.0$ and 8.0 for the case of low sample loading. Fig. 1(b) and (c) show respectively the profiles of the corresponding electric field $E(x, t)$ and the Kohlrausch regulating function $K(x, t) = (c_p + c_n + c)/u$. The Kohlrausch regulating function is a useful quantity for describing electrophoretic transport. If all ionic species have the same diffusivity, $K(x, t)$ evolves as a passive scalar [12]. If ionic diffusivities are treated as zero, then $K(x, t)$ is a conserved quantity [12]. It is seen that $K(x, t)$ remains localized near the injection zone and spreads only slowly by molecular diffusion. The sample peak on the other hand moves to the right and after a short time, the sample peak essentially lies in a zone where $K = K_{\infty}$. This illustrates the behavior postulated earlier that makes possible the predictions of positivity of co- and counter-ion concentrations implies that only sample profiles satisfying the condition $\phi < \phi_c$, where $\phi_c$ is a positive number, may be described by the theory. We will call such profiles “realizable”. The critical concentration, $\phi_c$, is given by $\phi_c = (z_p - z_n)/(z_p - z)$ when $z < 0$ and $\phi_c = -[z_n(z_p - z_n)/z_p(z - z_n)]$ when $z > 0$. When the parameter $\alpha > 0$, it may be shown with some
simple algebra that $\phi_c < \phi'_c \equiv \alpha^{-1}$ (see Appendix), so that the singularity implicit in Eq. (1) when $\phi = \phi'_c$ is never reached for realizable solutions. Fig. 2 shows the behavior of the system for initial conditions that are not realizable. In this situation, a stationary “barrier” develops at a fixed spatial location corresponding to a certain value $\phi = \phi^{\text{inter}}_c < \phi_c$. The sample ions move more or less freely on crossing the barrier but are effectively immobilized on the left of the barrier. This is due to the greatly reduced strength of the electric field in the injection zone where the electrical conductivity is high. This is clearly seen in Fig. 2(b) which shows a sharp reduction in the electric field in the injection zone. Only sample ions near the edges of the zone are able to “leak out” and are carried to the right as an advancing wave. Since part of the sample profile remains quasi stationary, the assumption of the constancy of the Kohlrausch function, $K = K_\infty$ can no longer be made for non-realizable concentrations. Thus, Eq. (6), which would have led to unphysical negative concentrations for such non-realizable profiles, is not applicable until after a sufficient time has evolved so that $\phi$ is reduced to a value below $\phi^{\text{inter}}_c$ throughout the domain.

Fig. 3 shows the variation in time of the quantity $(2D)^{-1} d\sigma^2/dt$ for a series of different values of the diffusivity and sample loading characterized by the pair of Péclet numbers $(Pe, P)$. If the profile spread purely by molecular diffusivity, this quantity should approach one asymptotically. However, it is seen that the long time asymptotic value is not one but rather $D_{\text{eff}}$ which depends solely on $P$. The dashed line shows the theoretical value of $D_{\text{eff}}$ predicted by the weakly nonlinear theory based on solutions of the Burgers’ equation [2]. Thus, once the system has evolved long enough, and dispersion has caused the amplitude to drop sufficiently, Burgers’ equation provides a valid description of the peak evolution. However, a real separation happens in a finite capillary and the long time limit may not necessarily apply. A quantity of interest is the timescale characterized by $t_\ast$: the time needed for the quantity $(2D)^{-1} d\sigma^2/dt$ to relax to 0.95 of its asymptotic value $D_{\text{eff}}$. If the separation is conducted in a capillary of length $L$, the question of interest is whether $t_\ast$ is small or large compared to the total separation time $T = L/v$. In Fig. 4 we show the normalized time $vt_\ast/w_0$ from a series of simulations with different values of $(Pe, P)$. Clearly, $vt_\ast/w_0$ is a monotonically decreasing function of $P$. This can be anticipated from the theory of nonlinear waves [2]: the higher the amplitude, the quicker a shock or shock like structure is formed. In contrast to the effective diffusivity shown in Fig. 3 which depends on $P$ but not on $Pe$, the time to reach the asymptotic state does depend on $Pe$. In fact, as Fig. 4 shows, the curve $vt_\ast/w_0$ as a function of $P$ is shifted upwards as $Pe$ is increased. Indeed, larger $Pe$ corresponds to lower diffusivity and therefore a longer time for the peak to spread and its amplitude to fall sufficiently for the weakly nonlinear description to be valid. Typical values of the physical parameters in a microchip based system may be $w_0 \sim 100\mu \text{m}$, $L \sim 5 \text{ cm}$, so that $vT/w_0 \sim 500$. Thus, Fig. 4 suggests that the Burgers’ solution does describe the peak dynamics for most of the separation time except for possibly a relatively short initial transient.
D. Analysis

An approximate theoretical determination of the concentration \( \phi^{\text{inter}} \) may be provided using the conservation equations. The method of doing this is in fact entirely analogous to the “Moving Boundary Equations” (MBE) \(^{12}\) for describing advancing fronts (e.g. in isostachophoresis), except, in this case, the front happens to be quasi stationary. The conceptual framework is illustrated in Fig. 3. The domain is decomposed into three parts: the “Initial Zone” where the sample is injected, the “Background Zone” ahead of the advancing wave where all concentrations equal their initial values and an “Interzone” between them. All variables are assumed constant within each zone but undergo a discontinuous change across zone boundaries. The values of the variables in each zone are indicated in Fig. 3. The boundary between the Initial Zone and the Interzone is stationary whereas the boundary between the Interzone and the Background Zone moves to the right. The arrows indicate fluxes of ions across the stationary zone boundary. Conservation of these ionic fluxes require

\[
E^{\text{ini}} \phi^{\text{ini}} = E^{\text{inter}} \phi^{\text{inter}} \\
E^{\text{ini}} \phi^{\text{ini}} = E^{\text{inter}} \phi^{\text{inter}}
\]

where \( E \) represents the electric field and \( \phi \) represents the concentration (normalized by \( c_{\infty} \)). The superscript (“ini” for the Initial Zone, “inter” for the Interzone and “\( \infty \)” for the Background Zone) indicates the zone in which the variable is evaluated and the subscript (\( p \) for cation, \( n \) for anion and no subscript for the sample) identifies the species. Therefore,

\[
\phi^{\text{inter}} = \left( \frac{\phi^{\text{ini}}}{\phi^{\text{ini}}} \right) \phi^{\text{inter}} \\
\phi^{\text{inter}} = \left( \frac{\phi^{\text{ini}}}{\phi^{\text{ini}}} \right) \phi^{\text{inter}}
\]

For the inter zone,

\[
K^{\text{inter}} = c_{\infty} \left( \phi^{\text{inter}} + \phi^{\text{inter}} + \phi^{\text{inter}} \right) / u
\]

\[
= K_{\infty} = c_{\infty}^{\infty} \left( \phi^{\text{inter}} + 1 \right) / u \\
= c_{\infty}^{\infty} \left( 1 - z_{n}/z_{p} \right) / u.
\]

The electro-neutrality condition (valid in all zones) is:

\[
z_{p} \phi_{p} + z_{n} \phi_{n} + z \phi = 0.
\]

By combining Eq. (9) and (10) and using the electro-neutrality condition we get an equation for determining \( \phi^{\text{inter}} \)

\[
(1 - z/z_{p}) \phi^{\text{inter}} + r (1 - z_{n}/z_{p}) \phi^{\text{inter}} = 1 - z_{n}/z_{p},
\]

where the ratio \( \phi^{\text{ini}} / \phi^{\text{ini}} = r \) is a constant determined by the ionic composition of the injected zone. Solving the above linear equation for \( \phi^{\text{inter}} \) we have

\[
\phi^{\text{inter}} = \left[ r + (z_{p} - z)/(z_{p} - z_{n}) \right]^{-1}.
\]

In our numerical experiment the cation concentration was chosen to be uniform, so that \( \phi^{\text{ini}} = \phi_{\infty} = -z_{n}/z_{p} \). Since \( z : z_{p} : z_{n} = 1 : 2 : -1 \), \( r = 1/\phi^{\text{ini}} - z/z_{n} = 1.5 \), and, \( \phi^{\text{inter}} = 0.55 \). This value is indicated by the dashed line in Fig. 2(a). Clearly, it correctly describes the concentration of sample in the Interzone.

Thus, the theoretical description developed in \(^{5}\) may be used in the Interzone (\( \phi < \phi^{\text{inter}} \)) but not in the Initial Zone. In order that all ion concentrations be non-negative in the Interzone we must have \( \phi^{\text{inter}} < \phi_{c} < \phi^{\text{ini}} \). This inequality is indeed true as can be shown by some simple algebra (see Appendix).

III. CONCLUSIONS

The development of nonlinear waves in capillary electrophoresis in the limit of low as well as high concentration of sample ions was studied by numerical integration of the governing equations. An idealized minimal model was considered consisting of a three ion (sample, co-ion and counter-ion) system of strong electrolytes \(^{20}\). This study complements an earlier paper by the authors. There it was shown that, in the weakly nonlinear limit, the evolution of the sample concentration may be reduced to Burgers’ equation, which admits an exact analytical solution.

Numerical simulation revealed that the evolution of the peak proceeds in a way that is qualitatively different when the sample concentration is high. As a consequence of the sharp reduction of the electric field in the region of sample injection, the ion migration velocity in this zone is very small. Ahead of this zone the ions form a surge front with a step-like profile propagating to the right. This state of affairs continues until the dimensionless ion concentration (\( \phi \)) in the injection zone drops sufficiently so that \( \phi < \phi^{\text{inter}} \). The subsequent dynamics then proceeds in accordance with the weakly nonlinear theory \(^{5}\). The value of \( \phi^{\text{inter}} \) may be approximately calculated by using a simple model based on conservation of ionic fluxes.
This qualitative change in the dynamics of peak evolution explains the breakdown of the weakly nonlinear theory when the concentration $\phi$ exceeds the critical value $\phi_c$. When $\phi$ exceeds a certain value $\phi_{\text{inter}} < \phi_c$, part of the propagating wave is effectively immobilized in the injection zone. It is then no longer correct to assume [5] that the sample pulse would quickly move out to a region where the Kohlrausch function is constant.

The model studied here is clearly oversimplified. In particular, real electrophoresis buffers contain many more than three ions including one or more weak acids or bases to maintain a stable pH. Further, complex effects due to inhomogeneities in the electroosmotic flow may be relevant [14]. In this paper we ignore these complexities and attempt to produce a detailed understanding of a “minimal” model problem. One may question whether the strongly nonlinear regime considered here is of relevance to actual laboratory practice. The answer depends on the numerical values of the critical concentrations $\phi_{\text{inter}} < \phi_c < \phi'_c$. If the sample and carrier ions have similar valences then all of these critical concentrations are of order unity. Thus, to exceed these critical values the sample ions in the injected plug will need to be present at concentrations approaching that of the carrier electrolyte. Such high concentrations are rarely employed in laboratory practice. However, if the sample is a macro-ion the critical values may actually be quite small. For example, at pH 2.0 Bovine serum albumin has a valence, $z \sim 55$ [15]. Then, in a univalent carrier electrolyte we have $\phi_c \sim 0.04$, so that the strongly nonlinear regime studied here may be easily reached.

Acknowledgments

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Appendix: Proof of the inequality $\phi_{\text{inter}} < \phi_c < \phi'_c$

The critical concentration $\phi_c$ is defined as [5]

$$\phi_c = \begin{cases} \frac{z_p-z_n}{z_p-z} & \text{if } z < 0 \\ \frac{-z_n z_p - z_n}{z_p - z} & \text{if } z > 0 \end{cases}$$

(A.1)

whereas

$$\phi'_c = \frac{1}{\alpha} \frac{z_n (z_p - z_n)}{(z - z_n)(z - z_p)}$$

(A.2)

We need to show that $\phi_c < \phi'_c$ when $\alpha > 0$, that is, when $z_p > z > z_n$. To do this, evaluate the ratio $\phi_c/\phi'_c$ when

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[18] The effect of a wall zeta potential has recently been investigated [15].
[19] to reduce numerical errors, the corners of the rectangle were slightly “rounded” by using a tan hyperbolic function.
[20] the situation of a weak electrolytic buffer was recently investigated by the authors [17].
\[ z_p > z > z_n: \]
\[ \frac{\phi_c}{\phi_c'} = \begin{cases} \frac{z_p - z_n (z - z_p)}{z_p (z_n - z_n)} = -\frac{z_p - z_n}{z_p} & \text{if } z < 0 \\ \frac{z_n}{z_p} - \frac{(z - z_n)(z - z_p)}{z_n (z_p - z_n)} = \frac{z_n - z}{z_p} & \text{if } z > 0 \end{cases} \]
\[ < 1 \text{ if } z < 0 \]
\[ = \frac{z_p - z_n}{z_p - z} = \phi_c \quad (A.3) \]

which completes the proof.

To prove the remaining inequality, \( \phi_{\text{inter}} < \phi_c \), we first show that \( r > -z/z_n \) when \( z < 0 \). To do this, we use the electro-neutrality condition to express \( \phi_{\text{ini}}^p \) in terms of the other variables
\[ \phi_{\text{ini}}^p = -\frac{z}{z_p} \phi_{\text{ini}}^n = -\frac{z}{z_p} \phi_{\text{ini}}^n = \phi_{\text{ini}}^n \quad (A.4) \]

Now we must have \( \phi_{\text{ini}}^p > 0 \). This is always true if \( z < 0 \), but if \( z > 0 \) then we require that \( r > -z/z_n \).

First suppose that \( z < 0 \). Then
\[ \phi_{\text{inter}} = \frac{1}{r + (z_p - z)/(z_p - z_n)} \]
\[ \phi_{\text{inter}} < 1 \]
\[ = \frac{z_p - z_n}{z_p - z} = \phi_c \quad (A.5) \]

Thus, in all cases, \( \phi_{\text{inter}} < \phi_c \) which completes the proof.