Effects of large permanent charges on ionic flows via Poisson-Nernst-Planck models

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Abstract

Permanent charge is the major structural quantity of an ion channel. It defines the ion channel and its interaction with boundary conditions plays the predominate role for ionic flow properties or functions of the ion channel. In this work, we investigate effects of *large magnitude* permanent charges of a simple form on the ionic flow of a 1:1 solution (an ionic mixture with one positive charged ion species and one negatively charged ion species). The analysis is based on a quasi-one-dimensional classical Poisson-Nernst-Planck model. Our findings include, (i) large permanent charges produce flux and current saturations at large transmembrane electric potentials; (ii) large permanent charges inhibit the flux of co-ions (ions with the same charge sign) but could either enhance or reduce the flux of counter-ion (ions with opposite charge signs), depending on boundary conditions and the channel geometry; (iii) the magnitude of the co-ion flux decreases with increases in magnitude of the large permanent charge but the counter-ion flux could either decease or increase in large permanent charge, depending on boundary conditions and the channel geometry, and quite significantly, (iv) large permanent charges are responsible for the counter-intuitive declining phenomenon – an increase in the electrochemical potential of counter-ion species in a particular manner leads to decreasing of counter-ion flux. Our work should be viewed as the first step of future analyses/numerics with more structural detail and more correlations between ions included. The basic findings in this work should provide a guidance for further investigation.

Keywords: Large permanent charge, current saturation, declining phenomenon

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1 Introduction

Ion channels provide pathways for transport of ions between inside and outside of cells that produce electric signals for cells to communicate with each other and to conduct biological functions of living organisms ([6, 7, 10, 11, 12, 13, 14]). Ion channels are defined by their main structural characteristics: the permanent charge distributions and channel shapes. They can be conveniently viewed as nano-devices ([4, 35]) and the

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ultimate interest is functions of ion channels ([3, 15, 16, 31, 33, 36, 37]). Permanent charge is the major structural quantity and plays the predominate role for functions of an ion channel. In addition to the permanent charge, other important physical parameters/quantities such as (transmembrane) electrical potential and boundary concentrations of ionic mixtures are crucial for ionic flow properties. Those boundary conditions interact nonlinearly with channel structures and together they produce rich properties that an ion channel could have. Due to the multi-scale feature and multiple parameters involved in ionic flow, a suitable mathematical analysis with a physically sound model plays critical and unique roles for a possible comprehensive understanding of ion channel problems.

Poisson-Nernst-Planck (PNP) systems serve as basic primitive models for ionic motion through ion channels. The inhered nature of multi-scale with multiple physical parameters of ion channel problems in PNP models presents a great challenge for a mathematical analysis toward concrete properties that are relevant and central to biological concerns. The present experimental techniques allow measurements of mainly the I-V relation – far away from measurements of internal dynamics of ionic flows. Not knowing what is going on in any detail adds another level of difficulty for an understanding of ion channel properties. Generally speaking, the best hope is to first understand key features and robust phenomena of ion channel problems for a certain extremal parameter values in simple biological setups. From a body of extensive researches, two parameters are arguably among the most important ones for properties of ionic flows: one is the dimensionless parameter ε as the ratio of Debye length over a characteristic length (e.g., the distance between two applied electrodes, or the channel length) of the problem, and the other is, of course, the permanent charge Q (scaled by a characteristic concentration) that includes its density and its spatial distribution. The parameter ε could vary in several orders of magnitudes depending on the setup of electrochemical problems; for ion channel problems, it is typically small, for example, it could be of order 10^{-3} or smaller. With the assumption that ε is small, a geometric singular perturbation framework was developed specifically for an analysis of classical Poisson-Nernst-Planck (cPNP) models for ion channel problems in [8, 24, 25, 28]. The specifics lie in the two critical structures of the PNP system that allows one to reduce the boundary value problem of the PNP model to an algebraic system – the governing system ([25]). The upshot of the governing system is two folds: it includes more or less all relevant physical parameters and, once a solution of the governing system is obtained, the singular orbit (the zeroth order in ε approximation solution of the boundary value problem) can be readily determined. The framework was extended to PNP with hard-sphere potentials to account for ion size effects to some extents in [18, 22, 27, 34] and a number of important applications on ionic flow has been obtained in [1, 2, 5, 17, 19, 38].

One can examine approximated solutions, extract information about interplays among multiple physical quantities, and even discover new phenomena. For example, in [19], based on the governing system obtained in [8] for a mixture of a cation (positively charged ion) and an anion (negatively charged ion), effects of small (relative to boundary concentrations) permanent charges were systematically investigated. In particular, it was shown that (Theorem 4.8 in [19]), depending on the boundary conditions, a small positive permanent charge can *(i) enhance the flux of anion and reduce that of cation,*

(ii) enhance the fluxes of both cation and anion,

(iii) reduce the fluxes of both cation and anion,

(iv) but cannot enhance the flux of cation while reducing that of anion.

Furthermore, it was shown that, to optimize the effect of (small) permanent charge, *the channel neck within which the permanent charge distributes, should be "short" and "narrow"* (Proposition 4.11 and Remark 4.12 in [19]).

Inspired by the above possibilities of (small) permanent charge effects, a flux ratio was introduced in [29] and was shown to have a universal property that is a common feature of all the above specifics (i)—(iv).

In this work, we examine effects of large permanent charges on ionic flow. Our study is based on the aforementioned geometric singular perturbation analysis for cPNP. This setup would raise a concern about the feasibility. Indeed, cPNP is known to be reliable when the ionic mixture is dilute but, with a large permanent charge, the ionic mixture would be crowded. On the other hand, the setup is reasonable for semi-conductor problems and for synthetic channels. More relevantly, the study in this paper is the first step for analysis of realistic models with large permanent charge. There are serious reasons and merits for us to take the simple model in this paper. It allows us to get more explicit expressions of the ionic fluxes in terms of the physical parameters of the problem so we are able to extract concrete information on large permanent charge effects. Furthermore, the analysis in this simpler setting provides important insights for the analysis of more realistic models.

We take the permanent charges in a special form: $Q(x) = 0$ for $x \notin [a, b]$ and $Q(x) = 2Q_0$ for $x \in [a, b]$ and assume $|Q_0|$ is large. We are able to derive expansions of fluxes in $\nu = 1/|Q_0| \ll 1$ and to extract/analyze effects of large permanent charge on fluxes. Among others, *large* permanent charges are shown to play a role for saturations of flux and current in large transmembrane electric potential; large *positive* permanent charges inhibit the flux of cation relative to the flux with no permanent charge but could either enhance or reduce the flux of anion, depending on boundary conditions and channel geometry; the magnitude of the cation flux is decreasing in large permanent charge but the anion flux could either decease or increase in large permanent charge, depending on boundary conditions and channel geometry. More interesting is a mechanism for the declining curve phenomenon: *As transmembrane electrochemical potential increases, in a particular way, the flux decreases (without a lower bound).* One mechanism for this counterintuitive phenomenon is that the permanent charge is **Large**.

The rest of the paper is organized as follows. In Section 2, we recall the quasi-one-dimensional PNP model taken in this study, the key assumptions in terms of the dimensionless version of the model, and a previous result from [8] that the analysis of this paper will be based on. Our main analysis for approximations of fluxes in large Q is conducted in Section 3. Section 4 is devoted to several biological interpretations/consequences based on the approximations of fluxes. Section 5 contains a conclusion remark. Two appendixes are provided. Appendix A (Section 6) contains an analysis for a degenerate case of the problem and Appendix B (Section 7) is a rigorous justification for the existence of a solution for small $\varepsilon > 0$ as an application of the Exchange Lemma to the singular orbit.

2 Basic setup and relevant results

2.1 A quasi-one-dimensional PNP model

Our analysis of large permanent charge effects on ionic flows is based on a quasi-one-dimensional PNP model first proposed in [30] and, for a special case, rigorously justified in [26]. For a mixture of n ion species, the model is

$$\begin{aligned}
&\frac{1}{A(X)}\frac{d}{dX}\Big(\varepsilon_r(X)\varepsilon_0 A(X)\frac{d\Phi}{dX}\Big) = -e_0\Big(\sum_{s=1}^{n} z_s C_s + \mathcal{Q}(X)\Big),\\
&\frac{d\mathcal{J}_k}{dX} = 0, \quad -\mathcal{J}_k = \frac{1}{k_B T}\mathcal{D}_k(X)A(X)C_k\frac{d\mu_k}{dX}, \quad k = 1, 2, \cdots, n
\end{aligned} \tag{2.1}$$

where $X \in [a_0, b_0]$ is the coordinate along the axis of the channel and baths of total length $b_0 - a_0$, $A(X)$ is the cross-section area of the channel over the longitudinal location X, e_0 is the elementary charge (we reserve the letter e for the Euler's number – the base for the natural exponential function), ε_0 is the vacuum permittivity, $\varepsilon_r(X)$ is the relative dielectric coefficient, $\mathcal{Q}(X)$ is the permanent charge density, k_B is the Boltzmann constant, T is the absolute temperature, Φ is the electrical potential, and, for the kth ion species, C_k is the concentration, z_k is the valence, $\mathcal{D}_k(X)$ is the diffusion coefficient, μ_k is the electrochemical potential and $\mathcal{J}_k$ is the flux density.

We will take the following boundary conditions, for $k = 1, 2, \cdots, n$,

$$\Phi(a_0) = \mathcal{V}, \quad C_k(a_0) = \mathcal{L}_k > 0; \quad \Phi(b_0) = 0, \quad C_k(b_0) = \mathcal{R}_k > 0. \tag{2.2}$$

The boundary conditions are directly related to typical experimental designs with two electrodes that are applied to control or drive the ionic flow. The positions $X = a_0$ and $X = b_0$ represent the locations of the two electrodes inside the baths separated by the channel. One would hope to have the electroneutral boundary conditions

$$\sum_{s=1}^{n} z_s\mathcal{L}_s = 0 = \sum_{s=1}^{n} z_s\mathcal{R}_s.$$

This is because, otherwise, there will be sharp boundary layers that could cause non-trivial uncertainties in experimental measurements (see [38] for more discussions).

For the kth ion species, the electrochemical potential $\mu_k(X)$ consists of the ideal component μ_k^{id} and the excess component μ_k^{ex} where the ideal component

$$\mu_k^{id}(X) = z_k e_0\Phi(X) + k_B T \ln\frac{C_k(X)}{C_0}$$

is the point-charge contribution where C_0 is a characteristic concentration, and $\mu_k^{ex}(x)$ accounts for ion size effects. As explained above, although not totally physical for ion channel problems in general, we will consider only the ideal component in this work as a starting point and hope some of the features revealed for this case can be treated as a guidance for further studies of more accurate models with excess component.

The permanent charge $\mathcal{Q}(X)$ is a mathematical model for ion channel (protein) structure that will be assumed to be given thanks to the advances of cryo-electron microscopy recognized in the 2018 Nobel Prize. We will take a simple description of permanent charges to capture some essence of the effects. More precisely, we take $\mathcal{Q}(X)$ as in ([8]), for some $a_0 < A < B < b_0$,

$$\mathcal{Q}(X) = \begin{cases} 0, & X \in (a_0, A) \cup (B, b_0) \\ 2\mathcal{Q}_0, & X \in (A, B). \end{cases} \tag{2.3}$$

We will be interested in the case where $|\mathcal{Q}_0|$ is large relative to $\mathcal{L}_k$'s and $\mathcal{R}_k$'s.

The cross-section area $A(X)$ typical has the property that $A(X)$ is much smaller for $X \in (A, B)$ (the neck region of the channel) than that for $X \notin [A, B]$.

2.2 Dimensionless of the quasi-one-dimensional PNP model

First of all, we assume

Assumption 2.1. *$\mathcal{D}_k(X) = \mathcal{D}(X)\mathcal{D}_k$ for some dimensionless function $\mathcal{D}(X)$ and dimensional constant $\mathcal{D}_k$ and $\varepsilon(X) = \varepsilon_r$ is a constant.*

Let C_0 be a characteristic concentration of the ion solution. Recall that the Debye screening length is

$$\lambda_D = \sqrt{\frac{\varepsilon_r \varepsilon_0 k_B T}{e_0^2 C_0}}.$$

We now make a dimensionless re-scaling of the variables in system (2.1) as follows.

$$\begin{aligned} &\varepsilon = \frac{\lambda_D}{b_0 - a_0}, \quad x = \frac{X - a_0}{b_0 - a_0}, \quad h(x) = \frac{A(X)}{(b_0 - a_0)^2}, \\ &Q(x) = \frac{\mathcal{Q}(X)}{C_0}, \quad \phi(x) = \frac{e_0}{k_B T}\Phi(X), \quad c_k(x) = \frac{C_k(X)}{C_0}, \\ &\bar{\mu}_k(x) = \frac{1}{k_B T}\mu_k(X), \quad D(x) = \mathcal{D}(X), \quad J_k = \frac{\mathcal{J}_k}{(b_0 - a_0)C_0\mathcal{D}_k}. \end{aligned} \tag{2.4}$$

In terms of the new variables and with the introduction of $u = \varepsilon\phi$ and $w = x$, system (2.1) is recast to

$$\begin{aligned} \varepsilon\dot{\phi} =& u, \quad \varepsilon\dot{u} = -\sum_{s=1}^{n} z_s c_s - Q(w) - \frac{\varepsilon h'(w)}{h(w)}u, \\ \varepsilon\dot{c}_k =& - z_k c_k u - \frac{\varepsilon J_k}{D(w)h(w)}, \quad \dot{J}_k = 0, \quad \dot{w} = 1, \end{aligned} \tag{2.5}$$

where the symbol dot denotes the derivative with respect to the x-variable. The autonomous system (2.5) can be then treated as a dynamical system with phase space $\mathbb{R}^{2n+3}$ and state variables $(\phi, u, c_1, \cdots, c_n, J_1, \cdots, J_n, w)$.

The boundary condition (2.2) becomes

$$\phi(0) = V, \ c_k(0) = L_k; \ \phi(1) = 0, \ c_k(1) = R_k, \tag{2.6}$$

where

$$V := \frac{e_0}{k_B T}\mathcal{V}, \quad L_k := \frac{\mathcal{L}_k}{C_0}, \quad R_k := \frac{\mathcal{R}_k}{C_0}.$$

The permanent charge $Q(x)$ is now

$$Q(x) = \begin{cases} 0, & x \in (0,a) \cup (b,1) \\ 2Q_0, & x \in (a,b), \end{cases} \tag{2.7}$$

where

$$0 < a = \frac{A - a_0}{a_1 - a_0} < b = \frac{B - a_0}{a_1 - a_0} < 1.$$

In the sequel, we will take the ideal component μ_k^{id} only for the electrochemical potential μ_k. In terms of the new variables, it becomes

$$\bar{\mu}_k(x) = \bar{\mu}_k^{id}(x) = z_k\phi(x) + \ln c_k(x).$$

There are two distinguished singular parameters in this problem: ε and Q_0. Our goal is to obtain an expansion of the solution of the boundary value problem in small ε and large Q_0. The correct order to treat this two limiting process is, firstly, to take large Q_0, and then, for fixed Q_0, to take ε small; that is, we will assume

Assumption 2.2. *The dimensionless parameters $|Q_0|$ is large and ε is small so that $\varepsilon|Q_0|$ is small.*

Interested readers may check Remark 2.1 in [38] for a detailed discussion on physical basis of Assumption 2.2. The mathematical consequence of the key assumption of smallness of ε is that the boundary value problem (BVP) (2.5) and (2.6) can be treated as a *singularly perturbed problem.* A general geometric framework for analyzing the singularly perturbed BVP of PNP type systems has been developed in [8, 19, 24, 25, 28] for classical PNP systems and in [18, 22, 27] for PNP systems with finite ion sizes.

In this work, we will consider the BVP for $1:1$ ionic mixtures, that is, one cation of valence $z_1 = 1$ and one anion of valence $z_2 = -1$. We will be interested in properties of fluxes J_k for large $|Q_0|$.

2.3 A relevant result: governing system $F(A) = 0$ for $n = 2$

We first recall relevant results in [8] that our work will be based on. For $n = 2$ with $z_1 > 0 > z_2$, the authors of [8] applied geometric singular perturbation theory to a construction of the singular orbit of the BVP (2.5) and (2.6). The BVP is reduced to a connecting problem: *finding an orbit of (2.5) from*

$$B_0 = \{(V, u, L_1, L_2, J_1, J_2, 0) : \text{ arbitrary } u, J_1, J_2\}$$

to

$$B_1 = \{(0, u, R_1, R_2, J_1, J_2, 1) : \text{ arbitrary } u, J_1, J_2\}.$$

Due to the jumps of permanent charge $Q(x)$ at $x = a$ and $x = b$, the construction of singular orbits is naturally split into three intervals $[0,a]$, $[a,b]$, $[b,1]$ as follows. To do so, one introduces (unknown) values of (ϕ, c_1, c_2) at $x = a$ and $x = b$:

$$\phi(a) = \phi^a,\ c_1(a) = c_1^a,\ c_2(a) = c_2^a; \quad \phi(b) = \phi^b,\ c_1(b) = c_1^b,\ c_2(a) = c_2^b. \tag{2.8}$$

Then these values determine (boundary) conditions at $x = a$ and $x = b$ as

$$B_a = \{(\phi^a, u, c_1^a, c_2^a, J_1, J_2, a) : \text{ arbitrary } u, J_1, J_2\},$$

and

$$B_b = \{(\phi^b, u, c_1^b, c_2^b, J_1, J_2, b) : \text{ arbitrary } u, J_1, J_2\}.$$

On each interval, a singular orbit typically consists of two singular layers and one regular layer. See Figure 1 (a modification of Figure 1 in [38]) for an illustration.

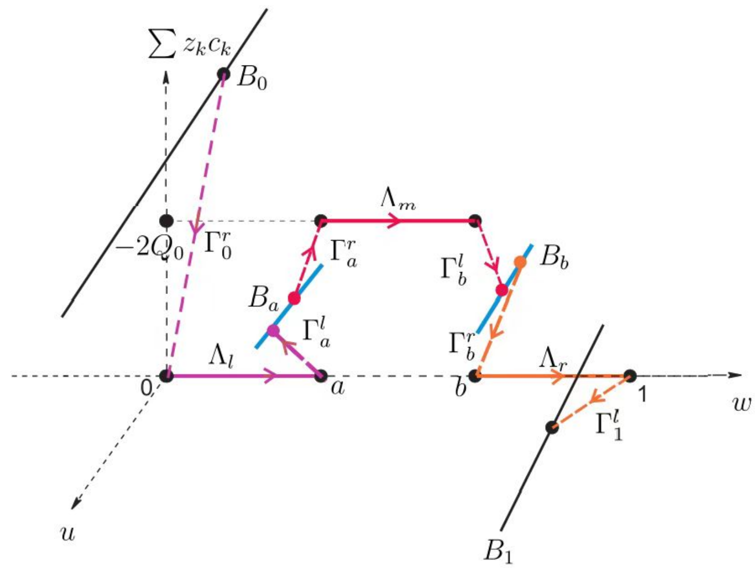

Figure 1: *An illustration of a singular connecting orbit projected to the space of* $(u, z_1c_1 + z_2c_2, w)$. *Boundary layers* Γ_0^r *and* Γ_1^l *at* $w = 0$ *and* $w = 1$ *exist if electroneutrality boundary conditions are not assumed.*

(i) On interval $[0, a]$, a singular orbit from B_0 to B_a consists of two singular layers located at $x = 0$ and $x = a$, denoted as Γ_0^l and Γ_a^l, and one regular layer Λ_l. Furthermore, with the preassigned values ϕ^a, c_1^a and c_2^a, the flux J_k^l and $u_l(a)$ are uniquely determined so that

$$(\phi^a, u_l(a), c_1^a, c_2^a, J_1^l, J_2^l, a) \in B_a.$$

(ii) On interval $[a, b]$, a singular orbit from B_a to B_b consists of two singular layers located at $x = a$ and $x = b$, denoted as Γ_a^r and Γ_b^l, and one regular layer Λ_m. Furthermore, with the preassigned values (ϕ^a, c_1^a, c_2^a) and $(\phi^b, c_1^b, c_2^b, b)$, the flux J_k^m, $u_m(a)$ and $u_m(b)$ are uniquely determined so that

$$(\phi^a, u_m(a), c_1^a, c_2^a, J_1^m, J_2^m, a) \in B_a \;\text{ and }\; (\phi^b, u_m(b), c_1^b, c_2^b, J_1^m, J_2^m, b) \in B_b.$$

(iii) On interval $[b, 1]$, a singular orbit from B_b to B_1 consists of two singular layers are located at $x = b$ and $x = 1$, denoted as Γ_b^r and Γ_1^l, and one regular layer Λ_r.

Furthermore, with the preassigned values ϕ^b, c_1^b and c_2^b, the flux J_k^r and $u_r(b)$ are uniquely determined so that

$$(\phi^b, u_r(b), c_1^b, c_2^b, J_1^r, J_2^r, b) \in B_b.$$

Requiring the singular orbit of the connecting problem to be connected leads to the matching conditions

$$J_k^l = J_k^m = J_k^r \text{ for } k = 1, 2, \; u_l(a) = u_m(a) \text{ and } u_m(b) = u_r(b). \tag{2.9}$$

The number of matching conditions is six, which is exactly the same number of unknowns preassigned in (2.8). The singular connecting problem is then reduced to *the governing system* (2.9) (see [8] for an explicit form of the governing system).

As an application of the governing system (2.9), ion channel problems for two ion species with small permanent charge was treated in [19] as mentioned in the introduction. We comment that it is much harder to analyze the situation with large permanent charge treated in this work. In [19], the permanent charge is assumed to be small (in absolute value) so the singular solution has a regular Taylor expansion in Q_0. For large Q_0 or small $\nu = 1/Q_0$, regular expansions of a singular solution in ν would not work and it was not clear how the expansion in ν should look like. It is the further reduction of the governing system (2.9) in [8] that allows us to start the analysis for large permanent charge. The expansion of a singular solution in small ν turns out to be quite irregular (see Corollary 3.4 and Remark 3.2). We now recall the further reduction of the governing system (2.9) in [8] for the special case considered in this work.

In [8], for $z_1 = 1$ and $z_2 = -1$, the governing system (2.9) is reduced to an equation with only one unknown; more precisely, set $c_1^a c_2^a = A^2$ and $c_1^b c_2^b = B^2$ with $A > 0$ and $B > 0$. Denote $L_1 L_2 = L^2$, $R_1 R_2 = R^2$, and

$$\alpha = \frac{H(a)}{H(1)} \text{ and } \beta = \frac{H(b)}{H(1)} \text{ where } H(x) = \int_0^x \frac{1}{D(s)h(s)} ds.$$

(Strictly speaking, in [8], $D(s) = 1$, but the proof there works for general $D(s)$ with only minor changes.) Then, the governing system is reduced to $F(A) = 0$ with

$$F(A) = e^{-(J_1+J_2)y}\Big(\sqrt{Q_0^2 + A^2} - \frac{J_2 - J_1}{J_1 + J_2} Q_0\Big) + \frac{J_2 - J_1}{J_1 + J_2} Q_0 - \sqrt{Q_0^2 + B^2}, \tag{2.10}$$

where B, y, J_1 and J_2 are determined in terms of the variable A by

$$\begin{aligned} B =& \frac{1-\beta}{\alpha}(L - A) + R, \quad J_1 + J_2 = 2\frac{L - A}{H(a)}, \\ J_2 - J_1 =& \frac{2(L - A)}{H(a) \ln \frac{BL}{AR}} \Bigg(\ln \frac{BL}{AR} - V - \ln \frac{L_1(\sqrt{Q_0^2 + B^2} - Q_0)}{R_1(\sqrt{Q_0^2 + A^2} - Q_0)} \\ & - \frac{\sqrt{Q_0^2 + B^2} - \sqrt{Q_0^2 + A^2}}{Q_0} - \frac{(\beta - \alpha)(L - A)}{\alpha Q_0} \Bigg), \\ (J_2 - J_1)y =& \frac{(\beta - \alpha)(L - A)}{\alpha Q_0} + \frac{\sqrt{Q_0^2 + B^2} - \sqrt{Q_0^2 + A^2}}{Q_0}. \end{aligned} \tag{2.11}$$

For a given Q_0, once a positive root A of $F(A) = 0$ is determined, a singular orbit can be constructed, in particular, the preassigned quantities in (2.8) are given by ([8])

$$
\begin{aligned}
c_1^a =& \Big(\sqrt{Q_0^2+A^2}-Q_0\Big)\exp\Big\{\frac{\sqrt{Q_0^2+A^2}-A}{Q_0}\Big\}, \quad c_2^a = \frac{A^2}{c_1^a},\\
c_1^b =& \Big(\sqrt{Q_0^2+B^2}-Q_0\Big)\exp\Big\{\frac{\sqrt{Q_0^2+B^2}-B}{Q_0}\Big\}, \quad c_2^b = \frac{B^2}{c_1^b},\\
\phi^b =& \frac{\ln\frac{B}{R}}{\ln\frac{BL}{AR}}\Big(V+\ln\frac{L_1(\sqrt{Q_0^2+B^2}-Q_0)}{R_1(\sqrt{Q_0^2+A^2}-Q_0)}+\frac{\sqrt{Q_0^2+B^2}-\sqrt{Q_0^2+A^2}}{Q_0}\\
&+\frac{(\beta-\alpha)(L-A)}{\alpha Q_0}\Big)+\ln\frac{R_1}{\sqrt{Q_0^2+B^2}-Q_0}-\frac{\sqrt{Q_0^2+B^2}-B}{Q_0},\\
\phi^a =& \phi^b-\frac{(1-\alpha)L+\alpha R-A}{\alpha Q_0}.
\end{aligned}
\tag{2.12}
$$

Working with this further reduction (2.10), we are able to show that, for $\nu = 1/Q_0$ small, there is a smooth function $A = A(\nu) = A_0 + A_1\nu + O(\nu^2)$ so that $F(A) = 0$. The expansions for the singular orbit can then be derived directly (see Section 7).

3 Expansion of singular orbits in large Q_0

Recall that we are interested in large $|Q_0|$ or small $\nu = 1/Q_0$. For definiteness, we will consider the case where $Q_0 > 0$ so that $\nu > 0$.

In replacing Q_0 with $1/\nu$ in (2.10), we have, viewing F as a function of A and ν,

$$
F(A,\nu) = \frac{1}{\nu}e^{-(J_1+J_2)y}\Big(\sqrt{1+\nu^2A^2}-\frac{J_2-J_1}{J_1+J_2}\Big)-\frac{1}{\nu}\Big(\sqrt{1+\nu^2B^2}-\frac{J_2-J_1}{J_1+J_2}\Big), \tag{3.1}
$$

where the quantities in (2.11) are now given by

$$
\begin{aligned}
B =& \frac{1-\beta}{\alpha}(L-A)+R, \quad J_1+J_2 = 2\frac{L-A}{H(a)},\\
J_2-J_1 =& \frac{2(L-A)}{H(a)\ln\frac{BL}{AR}}\bigg(\ln\frac{BL}{AR}-V-\ln\frac{L_1}{R_1}-\ln\frac{\sqrt{1+\nu^2B^2}-1}{\sqrt{1+\nu^2A^2}-1}\\
&-\sqrt{1+\nu^2B^2}+\sqrt{1+\nu^2A^2}-\frac{\beta-\alpha}{\alpha}(L-A)\nu\bigg),\\
(J_2-J_1)y =& \frac{\beta-\alpha}{\alpha}(L-A)\nu+\sqrt{1+\nu^2B^2}-\sqrt{1+\nu^2A^2}.
\end{aligned}
\tag{3.2}
$$

We will show, in Proposition 3.2, that there is $\nu_0 > 0$ small such that $F(A,\nu) = 0$ has a unique smooth solution $A = A(\nu)$ for $\nu \in [0,\nu_0)$. We will be interested in the expansions

$$
\begin{aligned}
A(\nu) =& A_0+A_1\nu+O(\nu^2), \quad B(\nu) = B_0+B_1\nu+O(\nu^2),\\
J_1(\nu) =& J_{10}+J_{11}\nu+O(\nu^2), \quad J_2(\nu) = J_{20}+J_{21}\nu+O(\nu^2),\\
y(\nu) =& y_0+y_1\nu+y_2\nu^2+O(\nu^3),
\end{aligned}
\tag{3.3}
$$

and, based on these expansions, effects of small $\nu > 0$ on the fluxes $J_1(\nu)$ and $J_2(\nu)$ will be examined. The $y_2\nu^2$ term is need in Appendix B (Section 7).

3.1 Zeroth order terms A_0 and B_0

In Appendix A (Section 6), we show that if $A = L$ (so that $J_1 + J_2 = 0$ from (3.2)), then, for a given (Q_0, L_k, R_k), there is a unique V so that $J_1 + J_2 = 0$. Thus, $A = L$ does not provide a singular orbit in general. Also, it was shown (Proposition 4.1 in [9]) that, for a given (V, L_k, R_k), there is at most one Q_0 such that $J_1 - J_2 = 0$. Thus, in general, $J_1 - J_2 \neq 0$. Since we are interested in the process of large Q_0, in the following, we will assume $J_1 \pm J_2 \neq 0$.

Proposition 3.1. *Under the assumption that $J_1 \pm J_2 \neq 0$, the function $F(A, \nu)$ is defined at $\nu = 0$, and*

$$F(A,0) = \frac{\beta-\alpha}{\alpha}(L-A)\frac{V + \ln\frac{L_1}{R_1} - 2\ln\frac{A}{B}}{V + \ln\frac{L_1}{R_1} - \ln\frac{AL}{BR}}. \tag{3.4}$$

Furthermore, $F(A, 0) = 0$ has a unique solution $A = A_0$ (and $B = B_0$) given by

$$\begin{aligned} A_0 &= \frac{\sqrt{e^V L_1}}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}}\big((1-\beta)L + \alpha R\big), \\ B_0 &= \frac{\sqrt{R_1}}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}}\big((1-\beta)L + \alpha R\big). \end{aligned} \tag{3.5}$$

Consequently,

$$\begin{aligned} y_0 &= 0, \quad y_1 = \frac{\beta-\alpha}{2}H(1), \\ J_{10} &= 0, \quad J_{20} = \frac{2\sqrt{L_1R_1}}{H(1)}\frac{\sqrt{L_2} - \sqrt{e^V R_2}}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}}. \end{aligned} \tag{3.6}$$

In particular, large positive permanent charge inhibits the flux of cations.

Proof. Using the expansions of $\sqrt{1+x}$ and $\ln(1+x)$ about $x = 0$, one has, from the last two equations in (3.2),

$$\begin{aligned} J_2 - J_1 &= \frac{2(L-A)}{H(a)\ln\frac{BL}{AR}}\left(\ln\frac{AL}{BR} - V - \ln\frac{L_1}{R_1} - \frac{\beta-\alpha}{\alpha}(L-A)\nu - \frac{B^2-A^2}{4}\nu^2 + O(\nu^4)\right), \\ (J_2 - J_1)y &= \frac{\beta-\alpha}{\alpha}(L-A)\nu + \frac{B^2-A^2}{2}\nu^2 + O(\nu^4). \end{aligned}$$

Thus, $y = Y_1(A)\nu + O(\nu^2)$ where

$$Y_1(A) = \frac{\beta-\alpha}{2}\frac{H(1)\ln\frac{BL}{AR}}{\ln\frac{AL}{BR} - V - \ln\frac{L_1}{R_1}}. \tag{3.7}$$

In particular, $y_0 = 0$. It then follows from (3.1) and (3.2) that

$$\begin{aligned} F(A,\nu) &= \frac{1}{\nu}\Big(1 - (J_1+J_2)Y_1(A)\nu + O(\nu^2)\Big)\Big(1 - \frac{J_2-J_1}{J_2+J_1} + \frac{1}{2}A^2\nu^2 + O(\nu^4)\Big) \\ &\quad - \frac{1}{\nu}\Big(1 - \frac{J_2-J_1}{J_2+J_1} + \frac{1}{2}B^2\nu^2 + O(\nu^4)\Big) \\ &= ((J_2-J_1) - (J_2+J_1))Y_1(A) + O(\nu) \\ &= \frac{\beta-\alpha}{\alpha}(L-A)\frac{V + \ln\frac{L_1}{R_1} - 2\ln\frac{A}{B}}{V + \ln\frac{L_1}{R_1} - \ln\frac{AL}{BR}} + O(\nu). \end{aligned} \tag{3.8}$$

Formula (3.4) for $F(A,0)$ then follows.

Hence, $A = A_0$ is a solution of $F(A,0) = 0$ if and only if

$$V + \ln\frac{L_1}{R_1} - 2\ln\frac{A_0}{B_0} = 0.$$

The latter together with $B_0 = (1-\beta)(L-A_0)/\alpha + R$ yield formulas for A_0 and B_0 in (3.5). The formula for y_1 then follows from (3.7).

It follows from (3.2) that $J_{20} - J_{10} = J_{20} + J_{10} = \frac{2(L-A_0)}{H(a)}$. One then has $J_{10} = 0$ and the formula for J_{20} using that of A_0. □

In order to show that, for ν near zero, $F(A,\nu) = 0$ has a unique smooth solution $A = A(\nu)$ with $A(0) = A_0$, a natural approach is to applied the Implicit Function Theorem. Given the singular appearance of ν in $F(A,\nu)$, we will take a different approach to avoid the complication about the smoothness of F at $\nu = 0$ (see Remark 3.1). We introduce $G(A,\nu) = \nu F(A,\nu)$; that is,

$$G(A,\nu) = e^{-(J_1+J_2)y}\Big(\sqrt{1+\nu^2A^2} - \frac{J_2-J_1}{J_1+J_2}\Big) + \frac{J_2-J_1}{J_1+J_2} - \sqrt{1+\nu^2B^2}. \tag{3.9}$$

Note that $G(A,\nu)$ is smooth near $(A_0,0)$ and, for $\nu \neq 0$, $G(A,\nu) = 0$ is equivalent to $F(A,\nu) = 0$.

Proposition 3.2. *There exists $\nu_0 > 0$ such that $F(A,\nu) = 0$ has a unique smooth solution $A(\nu) = A_0 + A_1\nu + O(\nu^2)$ for $\nu \in [0,\nu_0)$ with*

$$\begin{aligned} A_1 =& \frac{\alpha(\beta-\alpha)e^V L_1R_1((1-\beta)L+\alpha R)}{2\big((1-\beta)\sqrt{e^VL_1}+\alpha\sqrt{R_1}\big)^3}\big(\sqrt{e^{-V}L_2}-\sqrt{R_2}\big) \\ &+ \frac{\alpha(V-\ln L_2+\ln R_2)((1-\beta)L+\alpha R)^3}{8(\beta-\alpha)(\sqrt{e^{-V}L_2}-\sqrt{R_2})\big((1-\beta)\sqrt{e^VL_1}+\alpha\sqrt{R_1}\big)^3}\big(e^VL_1-R_1\big). \end{aligned} \tag{3.10}$$

Accordingly, $B_1 = -\frac{1-\beta}{\alpha}A_1$.

Proof. Recall that, for $\nu \neq 0$, $G(A,\nu) = 0$ and $F(A,\nu) = 0$ have the same solution. It follows from (3.9) that $G(A,0) = 0$, and

$$\begin{aligned} G_\nu(A,\nu) =& -ye^{-(J_1+J_2)y}(\sqrt{1+\nu^2A^2} - \frac{J_2-J_1}{J_2+J_1})\partial_\nu(J_1+J_2) \\ &- (J_1+J_2)e^{-(J_1+J_2)y}(1+\sqrt{1+\nu^2A^2} - \frac{J_2-J_1}{J_2+J_1})\partial_\nu y \\ &+ e^{-(J_1+J_2)y}(\frac{A^2\nu}{\sqrt{1+\nu^2A^2}} - \partial_\nu\frac{J_2-J_1}{J_2+J_1}) + \partial_\nu\frac{J_2-J_1}{J_2+J_1} - \frac{B^2\nu}{\sqrt{1+\nu^2B^2}} \\ =& \big((J_2-J_1)-(J_2+J_1)\big)Y_1(A) + O(\nu). \end{aligned}$$

One has, from (3.8), that $G_\nu(A,0) = F(A,0)$, particularly, $G_\nu(A_0,0) = F(A_0,0) = 0$. Furthermore, a direct calculation gives

$$\begin{aligned} G_{A\nu}(A_0,0) =& -\frac{4(\beta-\alpha)}{\alpha^2}\frac{(1-\beta)L+\alpha R}{V-\ln L_2+\ln R_2}\frac{L-A_0}{A_0B_0}, \\ G_{\nu\nu}(A_0,0) =& \frac{4(\beta-\alpha)^2}{\alpha^2}\frac{(L-A_0)^2}{V-\ln L_2+\ln R_2} + A_0^2 - B_0^2. \end{aligned}$$

Substitute (3.5) for A_0 and B_0 to get

$$\begin{aligned}
G_{A\nu}(A_0,0) =& \frac{4(\beta-\alpha)}{\alpha}\frac{(1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1}}{(1-\beta)L+\alpha R}\frac{\sqrt{e^{-V}L_2}-\sqrt{R_2}}{-V+\ln L_2-\ln R_2}>0,\\
G_{\nu\nu}(A_0,0) =& \frac{4(\beta-\alpha)^2}{V-\ln L_2+\ln R_2}\frac{e^V L_1 R_1(\sqrt{e^{-V}L_2}-\sqrt{R_2})^2}{((1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1})^2}\\
&+\frac{(e^V L_1-R_1)((1-\beta)L+\alpha R)^2}{((1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1})^2}.
\end{aligned}\tag{3.11}$$

We now consider the Hamiltonian system

$$A' = G_\nu(A,\nu), \quad \nu' = -G_A(A,\nu) \tag{3.12}$$

with the Hamiltonian function $G(A,\nu)$. Note that $(A,\nu)=(A_0,0)$ is an equilibrium of (3.12) and the linearization at $(A_0,0)$ is

$$\begin{pmatrix} G_{A\nu}(A_0,0) & G_{\nu\nu}(A_0,0) \\ 0 & -G_{A\nu}(A_0,0) \end{pmatrix},$$

which is hyperbolic with eigenvalues $\pm G_{A\nu}(A_0,0)$. The eigenvector associated to $G_{A\nu}(A_0,0)>0$ is $(1,0)^T$ and that associated to $-G_{A\nu}(A_0,0)<0$ is $(\rho,1)^T$ with

$$\rho = -\frac{G_{\nu\nu}(A_0,0)}{2G_{A\nu}(A_0,0)}.$$

Therefore, there are exactly two invariant manifolds through $(A_0,0)$, the stable manifold W^s and the unstable manifold W^u, of $(A_0,0)$. Furthermore, W^s is tangent to $(1,0)^T$ at $(A_0,0)$ and W^s is tangent to $(\rho,1)^T$ at $(A_0,0)$.

Note that $\{\nu=0\}$ is invariant and is tangent to $(1,0)^T$. Thus, W^u is determined by $\nu=0$ which is clearly a solution of $G(A,\nu)=\nu F(A,\nu)=0$ but is not a solution of $F(A,\nu)=0$ from (3.8). Since W^s is tangent to $(\rho,1)^T$ at $(A_0,0)$, locally near $(A,\nu)=(A_0,0)$, W^s is determined by a smooth function $A(\nu)=A_0+\rho\nu+O(\nu^2)$. Hence,

$$A_1=\rho=-\frac{G_{\nu\nu}(A_0,0)}{2G_{A\nu}(A_0,0)}. \tag{3.13}$$

Substituting (3.11) into (3.13) yields (3.10). □

Remark 3.1. The advantage of the approach in Proposition 3.2 is the bypass of checking the smoothness of $F(A,\nu)$ near $(A_0,0)$. Should one establish the smoothness of $F(A,\nu)$ near $(A_0,0)$, then $F_A(A_0,0)=G_{A\nu}(A_0,0)\neq 0$, and hence, by the Implicit Function Theorem,

$$A_1=-\frac{F_\nu(A_0,0)}{F_A(A_0,0)}.$$

As expected, this agrees with (3.13); indeed, since $G(A,\nu)=\nu F(A,\nu)$, one has

$$G_{\nu\nu}(A,\nu)=2F_\nu(A,\nu)+vF_{\nu\nu}(A,\nu) \;\text{ and }\; G_{A\nu}(A,\nu)=F_A(A,\nu)+vF_{A\nu}(A,\nu),$$

and hence, $G_{\nu\nu}(A_0,0)=2F_\nu(A_0,0)$ and $G_{A\nu}(A_0,0)=F_A(A_0,0)$. □

We are now ready to determine J_{11}, J_{21} and y_2 in (3.3).

Proposition 3.3. *One has*

$$
\begin{aligned}
J_{11} =& \frac{1}{2H(1)(\beta-\alpha)} \left(\frac{(1-\beta)L+\alpha R}{(1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1}} \right)^2 (e^V L_1 - R_1), \\
J_{21} =& -\frac{(\beta-\alpha)e^V L_1 R_1\big((1-\beta)L+\alpha R\big)}{H(1)\big((1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1}\big)^3}(\sqrt{e^{-V}L_2}-\sqrt{R_2}) \\
&+ \frac{(e^V L_1 - R_1)\big(-V+\ln L_2-\ln R_2\big)\big((1-\beta)L+\alpha R\big)^3}{4(\beta-\alpha)H(1)(\sqrt{e^{-V}L_2}-\sqrt{R_2})\big((1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1}\big)^3} \\
&- \frac{e^V L_1 - R_1}{2(\beta-\alpha)H(1)} \left(\frac{(1-\beta)L+\alpha R}{(1-\beta)\sqrt{e^V L_1}+\alpha\sqrt{R_1}} \right)^2,
\end{aligned}
\tag{3.14}
$$

and $y_2 = 0$.

Proof. We first derive the formula for J_{11}. It follows from (3.2) that

$$
\begin{aligned}
J_1 =& \frac{1}{2}(J_1+J_2) - \frac{1}{2}(J_2-J_1) \\
=& \frac{L-A}{H(a)\ln\frac{BL}{AR}} \Bigg(V + \ln\frac{L_1}{R_1} + \ln\frac{\sqrt{1+\nu^2B^2}-1}{\sqrt{1+\nu^2A^2}-1} \\
&+ \sqrt{1+\nu^2B^2} - \sqrt{1+\nu^2A^2} + \frac{\beta-\alpha}{\alpha}(L-A)\nu \Bigg).
\end{aligned}
$$

Note that

$$
\begin{aligned}
\sqrt{1+\nu^2A^2} =& 1 + \frac{1}{2}(A_0^2 + 2A_0A_1\nu)\nu^2 + O(\nu^4), \\
\sqrt{1+\nu^2B^2} =& 1 + \frac{1}{2}(B_0^2 + 2B_0B_1\nu)\nu^2 + O(\nu^4).
\end{aligned}
$$

One has $\sqrt{1+\nu^2B^2} - \sqrt{1+\nu^2A^2} = O(\nu^2)$ and

$$
\begin{aligned}
\ln\frac{\sqrt{1+\nu^2B^2}-1}{\sqrt{1+\nu^2A^2}-1} =& \ln\frac{B_0^2+2B_0B_1\nu+O(\nu^2)}{A_0^2+2A_0A_1\nu+O(\nu^2)} \\
=& 2\ln\frac{B_0}{A_0} + 2\Big(\frac{B_1}{B_0} - \frac{A_1}{A_0}\Big)\nu + O(\nu^2).
\end{aligned}
$$

Note also that $V + \ln\frac{L_1}{R_1} + 2\ln\frac{B_0}{A_0} = 0$, which is equivalent to $J_{10} = 0$. Thus,

$$
J_{11} = \frac{L-A_0}{H(a)\ln\frac{B_0L}{A_0R}} \left(2\Big(\frac{B_1}{B_0} - \frac{A_1}{A_0}\Big) + \frac{\beta-\alpha}{\alpha}(L-A_0) \right).
$$

Substituting the formulas in (3.5) and (3.10) for A_0, B_0, A_1 and B_1, one gets the formula for J_{11}. The formula for J_{21} then follows from $J_{11} + J_{21} = -2A_1/H(a)$.

Now, using the last two equations in (3.2) to rewrite y as

$$
y = \frac{\frac{\beta-\alpha}{\alpha}(L-A_0)\nu - \frac{\beta-\alpha}{\alpha}A_1\nu^2 + \frac{B_0^2-A_0^2}{2}\nu^2 + O(\nu^3)}{J_{20} + (J_{21}-J_{11})\nu + O(\nu^2)},
$$

one recovers $y_0 = 0$ and $y_1 = \frac{\beta-\alpha}{2}H(1)$ in Proposition 3.1, and obtains

$$y_2 = \frac{1}{J_{20}}\Big(\frac{B_0^2 - A_0^2}{2} - \frac{\beta-\alpha}{\alpha}A_1 - \frac{\beta-\alpha}{2}H(1)(J_{21} - J_{11})\Big).$$

One can then verify that $y_2 = 0$. $\square$

From the construction of singular orbits of system (2.1), the unknowns of the singular orbits are $\phi^a, \phi^b, c_k^a, c_k^b, J_k$, $k = 1, 2$. We now provide expansions of $\phi^a, \phi^b, c_k^a, c_k^b$ in ν and analyze the interplays between other parameters for large permanent charge.

Corollary 3.4. *The quantities c_1^a, c_2^a, c_1^b, and c_2^b in (2.8) have the following expansions in ν in terms of A_0, A_1, B_0 and B_1*

$$\begin{aligned} c_1^a =& \frac{e}{2}A_0^2\nu + \frac{1}{2}e(2A_0A_1 - A_0^3)\nu^2 + O(\nu^3), \quad c_2^a = \frac{2}{e\nu} + \frac{2}{e}A_0^2 + O(\nu),\\ c_1^b =& \frac{e}{2}B_0^2\nu + \frac{1}{2}e(2B_0B_1 - B_0^3)\nu^2 + O(\nu^3), \quad c_2^b = \frac{2}{e\nu} + \frac{2}{e}B_0^2 + O(\nu); \end{aligned} \tag{3.15}$$

The quantities ϕ^a and ϕ^b in (2.8) have the following expansions in ν

$$\phi^a = -\ln\nu + \phi_0^a + \phi_1^a\nu + O(\nu^2), \quad \phi^b = -\ln\nu + \phi_0^b + \phi_1^b\nu + O(\nu^2),$$

where

$$\begin{aligned} \phi_0^a &= \phi_0^b = \ln\frac{2}{e} - 2\ln\frac{(1-\beta)L + \alpha R}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}},\\ \phi_1^a &= \frac{2\ln\frac{B_0}{R}}{\ln\frac{L_2}{R_2} - V}\left(2\Big(\frac{B_1}{B_0} - \frac{A_1}{A_0}\Big) + \frac{\beta-\alpha}{\alpha}(L - A_0)\right) - 2\frac{B_1}{B_0} - \frac{\beta}{\alpha}(L - A_0) + L,\\ \phi_1^b &= \frac{\ln\frac{B_0}{R}}{\ln\frac{L_2}{R_2} - V}\left(2\Big(\frac{B_1}{B_0} - \frac{A_1}{A_0}\Big) + \frac{\beta-\alpha}{\alpha}(L - A_0)\right) - 2\frac{B_1}{B_0} + B_0, \end{aligned} \tag{3.16}$$

where A_k and B_k for $k = 0, 1$ are as provided in Propositions 3.1 and 3.2.

Proof. These can be obtained directly from (2.12) and the expansion for $A(\nu)$. $\square$

Remark 3.2. Note that, the expansions of c_1^a and c_1^b in ν start with first order terms in ν and those for c_2^a and c_2^b have singular terms. Furthermore, the expansions for ϕ^a and ϕ^b involve the term $\ln\nu$. These forms of expansions in ν are harder to guess from the governing system (2.9). It is the further reduction to (2.10) in [8] that makes our initial guess for $A(\nu)$ in ν possible. In turn, the expansions for c_k^a, c_k^b, ϕ^a and ϕ^b are derived automatically. $\square$

We end this section with two more comments.

(i) As $Q_0 \to \infty$, or equivalently, $\nu \to 0$, from equations (3.15), (3.16), (3.6) and (3.14), the concentrations c_1^a and c_1^b of cation at $x = a$ and $x = b$ as well as the flux J_1 of the cation tend to zero. On the other hand, the concentrations c_2^a and c_2^b of anion and the electric potentials ϕ^a and ϕ^b at $x = a$ and $x = b$ tend to infinity. However, the flux J_2 of anion tends to J_{20}, a finite value.

(ii) Notice that there is a symmetry for the present problem. If we flip the ion channel, then the parameter $(V, L_k, R_k; a, b)$ converts to $(-V, R_k, L_k; 1-b, 1-a)$ and (α, β) converts to $(1-\beta, 1-\alpha)$. From biological consideration, one would have the fluxes J_k's become $-J_k$'s. Our formulas (3.6) for J_{k0}'s and (3.14) for J_{k1}'s are indeed consistent with this symmetry.

4 Effects of the large permanent charge Q_0 on fluxes

The result $J_{10} = 0$ implies that large positive permanent charge inhibits the flux of cation. We now analyze effects of large permanent charge on fluxes based on formulas for J_{20}, J_{11} and J_{21}.

4.1 Signs and magnitudes of fluxes

Recall that the leading term for J_1 is $J_{11}\nu$ since $J_{10} = 0$ and the leading term for J_2 is J_{20}. An observation is made in [9] (see the discussion following formula (1.5) in [9]), that is, the *sign* of J_k is the same as that of $\bar{\mu}_k(0) - \bar{\mu}_k(1)$. The latter is determined by the boundary conditions – *independent* of the channel structure such as the channel geometry and the distribution of the permanent charge $Q(x)$.

The next result shows the consistence with the aforementioned observation on the signs for large permanent charge.

Proposition 4.1. *The sign of J_{11} is the same as the sign of the (scaled) transmembrane electrochemical potential $\bar{\mu}_1(0) - \bar{\mu}_1(1) = V + \ln L_1 - \ln R_1$. The sign of J_{20} is the same as the sign of the (scaled) transmembrane electrochemical potential $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L_2 - \ln R_2$. The sign of J_{21} may not be the same as the sign of $\bar{\mu}_2(0) - \bar{\mu}_2(1)$ but, as expected, $J_{21} = 0$ if $\bar{\mu}_2(0) - \bar{\mu}_2(1) = 0$.*

Proof. The statements about J_{20} and J_{11} are clearly true from (3.6) and (3.14).

The sign of J_{21} will be treated in more details in Proposition 4.8. Note that $J_2 = J_{20} + J_{21}\nu + O(\nu^2, \varepsilon) = 0$ if $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L_2 - \ln R_2 = 0$. It follows from (3.6) that the zeroth order flux $J_{20} = 0$ if $\bar{\mu}_2(0) - \bar{\mu}_2(1) = 0$. Thus, it is expected that $J_{21} = 0$ if $\bar{\mu}_2(0) - \bar{\mu}_2(1) = 0$. This is indeed the case. In fact, a direct calculation gives that, as $e^{-V}L_2 \to R_2$,

$$
\begin{aligned}
J_{21} \to & \frac{\big((1-\beta)L + \alpha R\big)^3}{2(\beta-\alpha)H(1)\sqrt{R_2}\big((1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}\big)^3}\big(e^V L_1 - R_1\big) \\
& - \frac{1}{2(\beta-\alpha)H(1)}\left(\frac{(1-\beta)L + \alpha R}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}}\right)^2 (e^V L_1 - R_1) \\
= & \frac{\big(e^V L_1 - R_1\big)\big((1-\beta)L + \alpha R\big)}{2(\beta-\alpha)H(1)((1-\beta)\sqrt{e^V R_2 L_1} + \alpha\sqrt{R_2 R_1})}\left(\frac{(1-\beta)L + \alpha R}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}}\right)^2 \\
& - \frac{e^V L_1 - R_1}{2(\beta-\alpha)H(1)}\left(\frac{(1-\beta)L + \alpha R}{(1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}}\right)^2 = 0.
\end{aligned}
$$

This completes the proof. ∎

On the other hand, the channel structure affects the magnitudes of fluxes J_k's in general. For large permanent charge, the leading term of J_k is J_{k0}. Notice that $J_{10} = 0$, independent of the channel geometry. However,

$$
J_{20}(\alpha, \beta) = \frac{2\sqrt{e^V L_1 R_1}}{H(1)\big((1-\beta)\sqrt{e^V L_1} + \alpha\sqrt{R_1}\big)}\big(\sqrt{e^{-V}L_2} - \sqrt{R_2}\big).
$$

The next result follows easily.

Proposition 4.2. *If $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L_2 - \ln R_2 \neq 0$, then $|J_{20}(\alpha, \beta)| \to \infty$ as $(\alpha, \beta) \to (0, 1)$.*

Similar to the discussion for Proposition 4.11 in [19], $(\alpha, \beta) \approx (0, 1)$ implies that, *to optimize the effect of large permanent charge Q_0, the channel neck, within where the permanent charge is constraint, should be "short" ($b - a \ll 1$) and "narrow" (the value of $h(x)$ is much smaller for $x \in (a, b)$ than that for $x \notin [a, b]$).* The same conclusion on the channel neck property is obtained in [19] for the case of small Q_0.

4.2 Saturation, monotonicity in V and scaling laws

The next two properties follow from (3.6) for J_{k0}'s and (3.14) for J_{k1}'s directly.

Proposition 4.3. *[Flux and Current Saturations] For large permanent charge Q_0 (small $\nu = 1/Q_0$) and to the leading order terms in ν, individual fluxes J_k's, and hence, the total current I saturate in $|V|$; more precisely, one has J_{20} is decreasing in V, concave downward for $V < V_0^*$ and concave upward for $V > V_0^*$ for some V_0^*, and J_{11} is increasing in V, concave upward for $V < V_1^*$ and concave downward for $V > V_1^*$ for some V_1^*. Furthermore, $|J_{20}|$ and $|J_{11}|$ are bounded in V with a bound that can be determined from the limits*

$$\begin{aligned}
&\lim_{V\to+\infty} J_{20} = -\frac{1}{1-\beta}\frac{2R}{H(1)}, \quad \lim_{V\to-\infty} J_{20} = \frac{1}{\alpha}\frac{2L}{H(1)},\\
&\lim_{V\to+\infty} J_{11} = -\lim_{V\to+\infty} J_{21} = \frac{1}{(1-\beta)^2}\frac{((1-\beta)L+\alpha R)^2}{2H(1)(\beta-\alpha)},\\
&\lim_{V\to-\infty} J_{11} = -\lim_{V\to-\infty} J_{21} = -\frac{1}{\alpha^2}\frac{((1-\beta)L+\alpha R)^2}{2H(1)(\beta-\alpha)}.
\end{aligned} \tag{4.1}$$

Proposition 4.4. *[Scaling Laws] For $k = 1, 2$ and for $s > 0$, one has*

$$\begin{aligned}
J_{k0}(V; sL_1, sR_1, sL_2, sR_2) =& sJ_{k0}(V; L_1, R_1, L_2, R_2),\\
J_{k1}(V; sL_1, sR_1, sL_2, sR_2) =& s^2 J_{k1}(V; L_1, R_1, L_2, R_2).
\end{aligned}$$

The linear scaling law for J_{k0}'s is natural. The quadratic scaling law for J_{k1}'s indicates that large permanent charges significantly increase the effect of boundary concentrations on the fluxes.

4.3 On a flux ratio for effects of permanent charges

In [29], to characterize effects of permanent charges on fluxes for given boundary conditions, the author introduced the flux ratio

$$\lambda_k(Q;\varepsilon) = \frac{J_k(Q;\varepsilon)}{J_k(0;\varepsilon)},$$

where, for the same boundary condition (2.6), $J_k(Q;\varepsilon)$ is the flux of kth ion species associated to the permanent charge $Q(x)$ and $J_k(0;\varepsilon)$ is the flux associated to zero permanent charge. Since permanent charges cannot change the sign of flux, one has $\lambda_k(Q;\varepsilon) > 0$.

If $\lambda_k(Q;\varepsilon) > 1$, then the permanent charge Q *enhances* the flux in the sense that $|J_k(Q;\varepsilon)| > |J_k(0;\varepsilon)|$; if $\lambda_k(Q;\varepsilon) < 1$, then the permanent charge Q *reduces* the flux in the sense that $|J_k(Q;\varepsilon)| < |J_k(0;\varepsilon)|$.

In [29], the following *universality of a permanent charge effect* is established. For ionic flow with one cation and one anion, let $\lambda_1(Q;\varepsilon)$ be the flux ratio associated to the cation and let $\lambda_2(Q;\varepsilon)$ be the flux ratio associated to the anion. Under some general conditions, one has, independent of boundary conditions,

$$\textit{if } \ Q(x) \geq 0, \ \textit{then, for } \ \varepsilon > 0 \textit{ small, } \lambda_1(Q;\varepsilon) \leq \lambda_2(Q;\varepsilon). \tag{4.2}$$

Furthermore, the statement (4.2) is sharp in the sense that, depending on the boundary conditions, each one of the followings is possible ([19])

(i) $1 < \lambda_1(Q;\varepsilon) < \lambda_2(Q;\varepsilon)$ (both cation and anion fluxes are enhanced);

(ii) $\lambda_1(Q;\varepsilon) < 1 < \lambda_2(Q;\varepsilon)$ (cation flux is reduced but anion flux is enhanced);

(iii) $\lambda_1(Q;\varepsilon) < \lambda_2(Q;\varepsilon) < 1$ (both cation and anion fluxes are reduced).

For our case where the permanent charge $Q = Q(x) \geq 0$ is given in (2.7) with $\nu = 1/Q_0 \ll 1$, we will consider the leading terms J_{k0} for $J_k(\nu)$ in (3.3). For cations, we have

$$\lambda_1(Q_0) = \frac{J_1(Q_0)}{J_1(0)} \approx \frac{J_{10}}{J_1(0)} = 0 < 1 \ \text{ if } \ V + \ln\frac{L_1}{R_1} \neq 0 \ \text{ (so that } \ J_1(0) \neq 0). \tag{4.3}$$

Thus, large positive permanent charges inhibit the flux of cations. This contrasts sharply to the effect of small positive permanent charge where, under some boundary conditions, it could enhance the flux of cations (statement (i) above).

We now consider $\lambda_2(Q_0)$ for the counter-ions. For simplicity, we assume electroneutrality boundary conditions $L_1 = L_2 = L$ and $R_1 = R_2 = R$ in the following.

For $Q_0 = 0$, the formula

$$J_2(0) = \frac{L-R}{H(1)(\ln L - \ln R)}(-V + \ln L - \ln R)$$

is provided in equation (4.4) in [19]. Using (3.6) for J_{20}, one has, for $Q_0 \gg 1$,

$$\lambda_2(Q_0) - 1 = \frac{J_2(Q_0)}{J_2(0)} - 1 \approx \frac{J_{20}}{J_2(0)} - 1 = \frac{\sqrt{R} f_K(\alpha,\beta)}{((1-\beta)\sqrt{e^V L} + \alpha\sqrt{R})}, \tag{4.4}$$

where, for a given $K = (V, L, R)$ with $\rho = L/R$,

$$f_K(\alpha,\beta) = \frac{2(\rho - \sqrt{e^V\rho})\ln\rho}{(\ln\rho - V)(\rho - 1)} - \big((1-\beta)\sqrt{e^V\rho} + \alpha\big), \tag{4.5}$$

which is linear in (α,β). Now for a given $K = (V, L, R)$, let

$$\Omega_+^K = \big\{(\alpha,\beta) \in \mathbb{R}^2 : \ 0 < \alpha < \beta < 1, \ f_K(\alpha,\beta) > 0\big\},$$
$$\Omega_-^K = \big\{(\alpha,\beta) \in \mathbb{R}^2 : \ 0 < \alpha < \beta < 1, \ f_K(\alpha,\beta) < 0\big\}.$$

Immediately, one has

Proposition 4.5. *Fix $K=(V,L,R)$ with $L>0$ and $R>0$. If $(\alpha,\beta)\in\Omega_+^K$, then, for $Q_0>0$ large, $\lambda_2(Q_0)>1$, and hence, the flux of J_2 is enhanced by the large positive permanent charge. If $(\alpha,\beta)\in\Omega_-^K$, then, for $Q_0>0$ large, $\lambda_2(Q_0)<1$, and hence, the flux of J_2 is reduced by the large positive permanent charge.*

Note that, for any $K=(V,L,R)$, $f_K(0,1)>0$ so $(\alpha,\beta)=(0,1)\in\Omega_+^K$. Thus, if (α,β) is close to $(0,1)$, then the large Q_0 enhances the flux of anion.

Next, we characterize the regions $\Omega_\pm^K$. For fixed $K=(V,L,R)$, denote the line given by $f_K(\alpha,\beta)=0$ by L_K.

Lemma 4.6. *The β-intercept of the line L_K is strictly increasing in V and the α-value of the intersection of L_K with $\{\beta=1\}$ is increasing in V too.*

As $V\to-\infty$, the line L_K approaches the β-axis.

As $V\to-\ln(L/R)$ (or $e^VL\to R$), the line L_K approaches the line $\{\alpha=\beta\}$.

As $V\to\infty$, the line approaches $\{\beta=1\}$.

In particular, for any K with $e^VL\neq R$, the line L_k intersect $\{\alpha=\beta\in(0,1)\}$ at a unique point, and both Ω_+^K and Ω_-^K are not empty.

Proof. Let $\rho=L/R$. The line L_K is given by

$$\beta=\frac{1}{\sqrt{e^V\rho}}\alpha-\frac{2(\sqrt{e^{-V}\rho}-1)\ln\rho}{(\ln\rho-V)(\rho-1)}+1.$$

The β-intercept of the line L_K is given by

$$\beta(V)=-\frac{2(\sqrt{e^{-V}\rho}-1)\ln\rho}{(\ln\rho-V)(\rho-1)}+1.$$

Thus,

$$\beta'(V)=\frac{\ln\rho}{\rho-1}\frac{-\rho^{1/2}Ve^{-V/2}+\rho^{1/2}\ln\rho e^{-V/2}-2\rho^{1/2}e^{-V/2}+2}{(\ln\rho-V)^2}.$$

Introduce $x=\rho^{1/2}$. The numerator of the second factor above becomes

$$g_1(x)=-Ve^{-V/2}x+2e^{-V/2}x\ln x-2e^{-V/2}x+2,\ x\in(0,\infty)$$

It follows from $g_1'(x)=e^{-V/2}(2\ln x-V)$ and $g_1''(x)=2e^{-V/2}/x$ that $g_1(x)$ is concave upward and its minimum is $g_1(x_c)=0$ with $x_c=e^{V/2}$ being the only critical point of $g_1(x)$. It is easy to see that $\lim_{x\to0}g_1(x)=2>0$ and $\lim_{x\to\infty}g_1(x)=\infty$. Thus, $g_1(x)\ge0$ for $x\in(0,\infty)$ and the equal sign occurs only at $x=e^{V/2}$. Therefore, the β-intercept $\beta(V)$ of the line L_K is strictly increasing in V.

The α-value $\alpha(V)$ of the intersection of the line L_K with $\{\beta=1\}$ is

$$\alpha(V)=\frac{2\sqrt{e^V\rho}(\sqrt{e^{-V}\rho}-1)\ln\rho}{(\ln\rho-V)(\rho-1)}=\frac{2\ln\rho}{\rho-1}\frac{\rho-\sqrt{e^V\rho}}{(\ln\rho-V)}.$$

Thus,

$$\alpha'(V)=\frac{\ln\rho}{\rho-1}\frac{g_2(V)}{(\ln\rho-V)^2},$$

where

$$g_2(V)=2\rho-2\rho^{1/2}e^{V/2}-e^{V/2}\rho^{1/2}\ln\rho+\rho^{1/2}Ve^{V/2}.$$

Note that

$$g_2'(V) = -\frac{1}{2}e^{V/2}\rho^{1/2}\ln\rho + \frac{1}{2}\rho^{1/2}Ve^{V/2}.$$

Thus, $g_2(V)$ has exactly one critical point $V_c = \ln\rho$. It follows from

$$g_2(V_c) = 0, \ \lim_{V\to-\infty} g_2(V) = 2\rho, \ \lim_{V\to\infty} g_2(V) = \infty$$

that $g_2(V) \geq 0$ and the equal sign occurs only for $V = \ln\rho$. We thus conclude that the α-value of the intersection of L_K with $\{\beta = 1\}$ is increasing in V too.

Note that, as $e^V\rho \to 1$, $\beta(V) \to 0$. The rest statement follows directly. □

4.4 Monotonicity of J_2 in Q_0: Sign of J_{21}

For simplicity, we assume electroneutrality boundary conditions $L_1 = L_2 = L$ and $R_1 = R_2 = R$ in the following.

Note that $J_1 = J_{11}\nu + O(\nu^2)$ and J_{11} has the same sign as that of $\bar{\mu}_1(0) - \bar{\mu}_1(1) = V + \ln L - \ln R$. The next statement is straightforward.

Proposition 4.7. *Up to the leading order $O(\nu)$, J_1 increases in ν (decreases in Q_0) if $V + \ln L - \ln R > 0$ and decreases in ν (increases in Q_0) if $V + \ln L - \ln R < 0$.*

Recall that J_{20} has the same sign as that of $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L - \ln R$. But J_{21} may not have the same sign as that of $-V + \ln L - \ln R$. The geometry of the channel and the boundary conditions work together to influence the sign of J_{21}. Since $J_2 = J_{20} + J_{21}\nu + O(\nu^2)$. Up to $O(\nu)$, the monotonicity of J_2 in ν (hence in Q_0) is determined by the sign of J_{21}.

Note that, if $V + \ln L - \ln R = 0$, then, from (3.14),

$$J_{21} = -\frac{(\beta-\alpha)e^V LR\big((1-\beta)L + \alpha R\big)}{H(1)\big((1-\beta)\sqrt{e^V L} + \alpha\sqrt{R}\big)^3}\left(\sqrt{e^{-V}L} - \sqrt{R}\right),$$

which has opposite sign as that of $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L - \ln R$, and hence, $J_{20}J_{21} < 0$. In this case, up to the leading order $O(\nu)$, $J_2 = J_{20} + J_{21}\nu$ is decreasing in ν (increasing in Q_0) if $-V + \ln L - \ln R > 0$ and is increasing in ν (decreasing in Q_0) if $-V + \ln L - \ln R < 0$.

In general, for fixed (α, β), J_{21} in (3.14) can be rewritten as

$$J_{21} = \frac{((1-\beta)s + \alpha)R^2}{2H(1)(\beta-\alpha)\left((1-\beta)s + \alpha t\right)^3}G(s,t), \tag{4.6}$$

where $s = L/R$, $t = \sqrt{e^{-V}L/R}$, and

$$\begin{aligned} G(s,t) = &-2s^2(\beta-\alpha)^2 t(t-1) \\ &+ ((1-\beta)s + \alpha)(s^2 - t^2)\left((1-\beta)s + \alpha)\frac{t\ln t}{t-1} - ((1-\beta)s + \alpha t)\right). \end{aligned} \tag{4.7}$$

It is complicated to determine the sign of J_{21}. We will provide a partial result to indicate that the sign of J_{21} can be the same or opposite as that of $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L - \ln R$.

Notice that if $t = 1$, then $J_{21}(t) = 0$. In a neighborhood of $t = 1$, we have the following result.

Proposition 4.8. *Let $s = L/R$ and $t = \sqrt{e^{-V}L/R}$. For fixed (α, β), there are two positive functions $s_1^* = s_1^*(\alpha, \beta)$ and $s_2^* = s_2^*(\alpha, \beta)$ with $s_1^* < 1 < s_2^*$ such that*

- *(i) if $s \in (0, s_1^*) \bigcup (s_2^*, \infty)$, then there exists a small positive number θ_1 such that, for $t \in (1 - \theta_1, 1 + \theta_1)$ and $t \neq 1$, $J_{20}J_{21} > 0$, and hence, $J_{20} + J_{21}\nu$ is increasing in ν for small ν (decreasing in Q_0 for large $Q_0 > 0$);*
- *(ii) if $s \in (s_1^*, s_2^*)$, then there exists a small positive number θ_2 so that, for $t \in (1 - \theta_2, 1 + \theta_2)$ and $t \neq 1$, $J_{20}J_{21} < 0$, and hence, $J_{20} + J_{21}\nu$ is decreasing in ν for small $\nu > 0$ (increasing in Q_0 for large $Q_0 > 0$).*

Proof. For function $G(s, t)$ in (4.7), let

$$g(s) = \lim_{t \to 1} \frac{G(s, t)}{t - 1}. \tag{4.8}$$

Then, $g(s) = (1 - \beta)^2 s^4 - \left((1 - \beta)^2 + \alpha^2 + 4(\beta - \alpha)^2\right) s^2 + \alpha^2$. Note that, $g(s)$ is even in s, $g(0) = \alpha^2 > 0$, $g(s) > 0$ if $s \gg 1$, and $g(1) = -4(\beta - \alpha)^2 < 0$. Thus, $g(s)$ has exactly two distinct positive roots $s_1^* = s_1^*(\alpha, \beta)$ and $s_2^* = s_2^*(\alpha, \beta)$ with $0 < s_1^* < 1 < s_2^*$. In particular, if $s \in (0, s_1^*) \bigcup (s_2^*, \infty)$, then $g(s) > 0$, and hence, for t near 1, $\frac{G(s,t)}{t-1} > 0$ from (4.8); and if $s \in (s_1^*, s_2^*)$, then $g(s) < 0$, and hence, for t near 1, $\frac{G(s,t)}{t-1} < 0$ from (4.8). All conclusions follow since $\mu_2(0) - \mu_2(1)$ and J_{20} have the same sign as that of $(t - 1)$. □

4.5 The declining phenomenon

We recall the so-called *declining phenomenon*: *For fixed V and L, as R decreases to zero, the (scaled) transmembrane electrochemical potential for the counter-ion $\bar{\mu}_2(0) - \bar{\mu}_2(1) = -V + \ln L - \ln R$ increases to infinity but the magnitude of counterion flux ($|J_2|$ in the setting since $Q_0 > 0$) decreases monotonically to zero.*

Remark 5.1 from [38]: The phenomenon was well-known in the physiology community. Unfortunately, we could not find references stating precisely this phenomenon. We have contacted many leading experts who are all recognizing this phenomenon. Some experts mention this phenomena as an example of 'exchange diffusion' and/or long channel phenomena.

This phenomenon is rather counterintuitive. Recall the Nernst-Planck equation

$$-J_2 = D_2(x)h(x)c_2(x; \nu)\frac{d}{dx}\bar{\mu}_2(x; \nu).$$

Since $D_2(x)$ and $h(x)$ are fixed, we will treat them as of order $O(1)$ quantities so that they do not contribute much to the near zero flux scenario when R is small. Thus, as far as the near zero flux mechanism is concerned, one has

$$-J_2 \approx c_2(x; \nu)\bar{\mu}_2'(x; \nu). \tag{4.9}$$

One sees that the gradient $\bar{\mu}_2'(x; \nu)$ of the electrochemical potential is the main driving force for the flux J_2. Intuitively, large drop of (or transmembrane) electrochemical potential $\bar{\mu}_2(0) - \bar{\mu}_2(1)$ of $\bar{\mu}_2$ produces large flux J_2. In this sense, the declining curve phenomenon is rather counterintuitive. A careful look at (4.9) reveals that there is only one possibility for the declining curve phenomenon; that is, whenever $\bar{\mu}_2'(x;\nu)$ is large, $c_2(x;\nu)$ has to be much smaller in order to produce a small flux $|J_2|$. In [38], the analytical results of the internal dynamics from this work is applied to show that this is indeed the case. We refer the readers to [38] for detailed discussions.

5 Conclusion remarks

In this work, for a simple form of the permanent charge distribution, we investigate effects of *large magnitude* of permanent charges on the ionic flow. The analysis is based on a quasi-one-dimensional classical Poisson-Nernst-Planck model. Our result provides expansions for ionic fluxes, concentrations and electric potential in the reciprocal of the large permanent charge. The explicit leading terms of the expansions allow one to analyze concrete effects of large permanent charges and their nonlinear interplay with boundary conditions. As expected, the effects are significantly different from those of small permanent charges. Among others, we find

(i) *large permanent charges* produce flux and current saturations as transmembrane electric potential increases (Proposition 4.3);

(ii) *large permanent charges* inhibit the flux of co-ions (Display (4.3)) but, depending on boundary conditions and channel geometry, either enhance or reduce the flux of counter-ion (Proposition 4.5);

(iii) the magnitude of the co-ion flux is decreasing in large permanent charge (Proposition 4.7) but, depending on boundary conditions and channel geometry, the counter-ion flux could either decease or increase in large permanent charge (Proposition 4.8);

(iv) *large permanent charges* are responsible for the counter-intuitive declining phenomenon – increasing of electrochemical potential of counter-ion species leads to decreasing of counter-ion flux (Section 4.5 and detailed discussion in [38]).

6 Appendix A. On $A = L$

We will establish the statement used in the beginning of Section 3.1; that is, $A = L$ *(so that $J_1 + J_2 = 0$ from (3.2)) does not provide a singular orbit in general in the sense that, for a given (Q_0, L_k, R_k), there is a unique V so that $J_1 + J_2 = 0$.*

For simplicity, we assume $L_1 = L_2 = L$ and $R_1 = R_2 = R$.

It follows from Corollary 3.3 in [8] that, if $A = L$, then $c_1^{a,l} = c_2^{a,l} = L$ and

$$\phi^{a,l} = \phi^a - \frac{1}{2}\ln\frac{c_2^a}{c_1^a} = \phi^a - \ln\frac{L}{c_1^a}. \tag{6.1}$$

For $x \in (0, a)$, it then follows from system (19) in [8] that

$$\begin{aligned} &J_1 + J_2 = 0, \quad c_1(x) = c_2(x) = L;\\ &\phi(x) = V - \frac{J_1}{L}H(x), \quad \phi^{a,l} = V - \frac{J_1}{L}H(a). \end{aligned} \tag{6.2}$$

For $x \in (b, 1)$, it then follows from equation (41) in [8] and $J_1 + J_2 = 0$ that

$$\begin{aligned} c_k^{b,r} =& c_k(x) = R = B; \\ \phi(x) =& \phi^{b,r} - \frac{J_1}{R}(H(x) - H(b)), \quad \phi^{b,r} = -\frac{J_1}{R}(H(1) - H(b)), \end{aligned} \tag{6.3}$$

and

$$\phi^{b,r} = \phi^b - \ln \frac{R}{c_1^b}. \tag{6.4}$$

For $x \in (a, b)$, it then follows from equation (33) in [8] and $J_1 + J_2 = 0$ that

$$\begin{aligned} (c_1(x) + Q_0)^2 =& (c_1^{a,m} + Q_0)^2 - 2Q_0 J_1 (H(x) - H(a)), \\ Q_0\phi(x) - c_1(x) =& Q_0\phi^{a,m} - c_1^{a,m} = Q_0\phi^{b,m} - c_1^{b,m}. \end{aligned}$$

Therefore,

$$J_1 = -J_2 = \frac{(c_1^{a,m} + Q_0)^2 - (c_1^{b,m} + Q_0)^2}{2Q_0(H(b) - H(a))}, \quad \phi^{b,m} - \phi^{a,m} = \frac{c_1^{b,m} - c_1^{a,m}}{Q_0}. \tag{6.5}$$

The first two equations of (43) in [8] are definitions of $\phi^{a,m}$ and $\phi^{b,m}$, and the next two equations correspond to $u_l(a) = u_m(a)$ and $u_m(b) = u_r(b)$, respectively. They give rise to, with $A = L$ and $B = R$,

$$\begin{aligned} c_1^a =& \Big(\sqrt{Q_0^2 + L^2} - Q_0\Big) \exp\Big\{\frac{\sqrt{Q_0^2 + L^2} - L}{Q_0}\Big\}, \\ c_1^b =& \Big(\sqrt{Q_0^2 + R^2} - Q_0\Big) \exp\Big\{\frac{\sqrt{Q_0^2 + R^2} - R}{Q_0}\Big\}; \\ c_2^a =& \frac{L^2}{\sqrt{Q_0^2 + L^2} - Q_0} \exp\Big\{-\frac{\sqrt{Q_0^2 + L^2} - L}{Q_0}\Big\}, \\ c_2^b =& \frac{R^2}{\sqrt{Q_0^2 + R^2} - Q_0} \exp\Big\{-\frac{\sqrt{Q_0^2 + R^2} - R}{Q_0}\Big\}. \end{aligned}$$

Hence,

$$\begin{aligned} \phi^a - \phi^{a,m} =& \ln \frac{\sqrt{Q_0^2 + L^2} - Q_0}{c_1^a} = -\frac{\sqrt{Q_0^2 + L^2} - L}{Q_0}, \\ \phi^b - \phi^{b,m} =& \ln \frac{\sqrt{Q_0^2 + R^2} - Q_0}{\ln c_1^b} = -\frac{\sqrt{Q_0^2 + R^2} - R}{Q_0}, \\ c_1^{a,m} =& e^{\phi^a - \phi^{a,m}} c_1^a = \sqrt{Q_0^2 + L^2} - Q_0, \quad c_2^{a,m} = \sqrt{Q_0^2 + L^2} + Q_0, \\ c_1^{b,m} =& e^{\phi^b - \phi^{b,m}} c_1^b = \sqrt{Q_0^2 + R^2} - Q_0, \quad c_2^{b,m} = \sqrt{Q_0^2 + R^2} + Q_0. \end{aligned} \tag{6.6}$$

Therefore, from (6.5),

$$J_1 = -J_2 = \frac{L^2 - R^2}{2Q_0(H(b) - H(a))}, \quad \phi^{b,m} - \phi^{a,m} = \frac{\sqrt{Q_0^2 + R^2} - \sqrt{Q_0^2 + L^2}}{Q_0}.$$

Note also, from (6.6), that

$$\phi^{b,m} - \phi^{a,m} = \phi^b - \phi^a + \frac{\sqrt{Q_0^2 + R^2} - R}{Q_0} - \frac{\sqrt{Q_0^2 + L^2} - L}{Q_0}.$$

Thus, agreeing with (46) in [8],

$$\phi^b - \phi^a = \frac{R - L}{Q_0}. \tag{6.7}$$

But, from (6.1) and (6.2) for $\phi^{a,l}$ and from (6.3) and (6.4) for $\phi^{b,r}$, one has

$$\begin{aligned}
\phi^a + \ln\frac{\sqrt{Q_0^2 + L^2} - Q_0}{L} + \frac{\sqrt{Q_0^2 + L^2} - L}{Q_0} &= V - \frac{H(a)(L^2 - R^2)}{2(H(b) - H(a))LQ_0},\\
\phi^b + \ln\frac{\sqrt{Q_0^2 + R^2} - Q_0}{R} + \frac{\sqrt{Q_0^2 + R^2} - R}{Q_0} &= \frac{(H(1) - H(b))(L^2 - R^2)}{2(H(b) - H(a))RQ_0}.
\end{aligned}$$

It follows that,

$$\begin{aligned}
\phi^b - \phi^a = &- V + \frac{(H(1) - H(b))L - H(a)R}{H(b) - H(a)}\frac{L^2 - R^2}{2LRQ_0}\\
&- \ln\frac{\sqrt{Q_0^2 + R^2} - Q_0}{R} - \frac{\sqrt{Q_0^2 + R^2} - R}{Q_0}\\
&+ \ln\frac{\sqrt{Q_0^2 + L^2} - Q_0}{L} + \frac{\sqrt{Q_0^2 + L^2} - L}{Q_0}.
\end{aligned} \tag{6.8}$$

Finally, combining (6.7) and (6.8), one has, if $A = L$ so that $J_1 + J_2 = 0$, then

$$\begin{aligned}
V = &- \ln\frac{L}{R} + \frac{(H(1) - H(b))L + H(a)R}{H(b) - H(a)}\frac{L^2 - R^2}{2LRQ_0}\\
&+ \ln\frac{\sqrt{Q_0^2 + L^2} - Q_0}{\sqrt{Q_0^2 + R^2} - Q_0} + \frac{\sqrt{Q_0^2 + L^2} - \sqrt{Q_0^2 + R^2}}{Q_0}.
\end{aligned} \tag{6.9}$$

7 Appendix B. Existence for BVP with $\varepsilon > 0$ small

For fixed large Q_0 or small ν, we will determine the singular orbit first and then apply the Exchange Lemma to show, for $\varepsilon > 0$ small, there exists a unique solution of the BVP near the singular orbit.

7.1 The singular orbit

Once $A = A_0 + A_1\nu + O(\nu^2)$ is determined, the intermediate variables introduced in (2.8) can be determined from (2.12). A singular orbit can then be found by applying the result in [8] directly. We will provide the singular slow orbits over each subinterval and refer the details to the relevant result in [8]. As a by-product, we provide details about what happens to the internal dynamics that leads to $J_{10} = 0$: over different subintervals, the causes for $J_{10} = 0$ are different (Corollaries 7.2, 7.4 and 7.6).

7.1.1 Internal dynamics over the interval $(0, a)$.

The singular slow orbit (ϕ, c_1, c_2) can be obtained from the display (24) in [8].

Proposition 7.1. *For $x \in (0, a)$, $k = 1, 2$,*

$$c_k(x;\nu) = c_{k0}(x) + c_{k1}(x)\nu + O(\nu^2), \quad \phi(x;\nu) = \phi_0(x) + \phi_1(x)\nu + O(\nu^2),$$

where

$$\begin{aligned} c_{k0}(x) =& L - \frac{J_{20}}{2}H(x), \quad c_{k1}(x) = -\frac{J_{11}+J_{21}}{2}H(x), \\ \phi_0(x) =& V + \ln L - \ln c_{10}(x), \quad \phi_1(x) = -\frac{c_{11}(x)}{c_{10}(x)} + \frac{2J_{11}}{J_{20}}\ln\frac{c_{10}(x)}{L}. \end{aligned}$$

The following is then a direct consequence.

Corollary 7.2. *Over the interval $(0, a)$, the electrochemical potentials are*

$$\bar\mu_k(x;\nu) = \bar\mu_{k0}(x) + \bar\mu_{k1}(x)\nu + O(\nu^2)$$

for $k = 1, 2$, where

$$\begin{aligned} \bar\mu_{10}(x) =& \phi_0(x) + \ln c_{10}(x) = V + \ln L, \quad \bar\mu_{11}(x) = \phi_1(x) + \frac{c_{11}(x)}{c_{10}(x)} = 2\frac{J_{11}}{J_{20}}\ln\frac{c_{10}(x)}{L}, \\ \bar\mu_{20}(x) =& -V - \ln L + 2\ln c_{20}(x), \quad \bar\mu_{21}(x) = \frac{2c_{21}(x)}{c_{20}(x)} - \frac{2J_{11}}{J_{20}}\ln\frac{c_{20}(x)}{L}. \end{aligned}$$

In particular, $\bar\mu'_{10}(x) = 0$, and hence, $J_{10} = 0$ over the interval $(0, a)$.

7.1.2 Internal dynamics over the interval (a, b).

The singular slow orbit (ϕ, c_1, c_2) can be obtained from the display (35) in [8].

Proposition 7.3. *For $x \in (a, b)$,*

$$\begin{aligned} c_1(x;\nu) =& c_{10}(x) + c_{11}(x)\nu + c_{12}(x)\nu^2 + O(\nu^3), \\ c_2(x;\nu) =& \frac{2}{\nu} + \left(\frac{1}{2}A_0^2 - J_{11}(H(x) - H(a))\right)\nu + O(\nu^2), \\ \phi(x;\nu) =& -\ln\nu + \phi_0(x) + \phi_1(x)\nu + O(\nu^2), \end{aligned}$$

where

$$\begin{aligned} c_{10}(x) =& 0, \quad c_{11}(x) = \frac{1}{2}A_0^2 - J_{11}(H(x) - H(a)), \\ c_{12}(x) =& -\frac{H(x) - H(a)}{2H(a)}(L - A_0)A_0^2 + A_0A_1, \\ \phi_0(x) =& \ln\frac{2e^V L}{A_0^2}, \quad \phi_1(x) = \phi_1^a - A_0 + \frac{J_{20}}{2}(H(x) - H(a)), \end{aligned}$$

where A_0 is given in (3.5), A_1 is given in (3.10), and ϕ_1^a is in (3.16).

In particular, $c_{10}(x) = 0$ implies that $J_{10} = 0$ over the interval (a, b).

As an immediate consequence, one has

Corollary 7.4. *Over the interval (a,b), the electrochemical potentials are*

$$\bar\mu_k(x;\nu) = \bar\mu_{k0}(x) + \bar\mu_{k1}(x)\nu + O(\nu^2)$$

for $k=1,2$, where

$$\begin{aligned}
\bar\mu_{10}(x) =& \phi_0(x) + \ln c_{11}(x) = \ln\Big(\frac{H(b)-H(x)}{H(b)-H(a)}e^V L + \frac{H(x)-H(a)}{H(b)-H(a)}R\Big),\\
\bar\mu_{11}(x) =& \phi_1(x) + \frac{c_{12}(x)}{c_{11}(x)},\\
\bar\mu_{20}(x) =& -\phi_0(x) + \ln 2 = \ln\frac{A_0^2}{e^V L},\\
\bar\mu_{21}(x) =& -\phi_1(x) = -\phi_1^a + A_0 - \frac{J_{20}}{2}(H(x)-H(a)).
\end{aligned}$$

Proof. For $\bar\mu_1$, one has

$$\begin{aligned}
\bar\mu_1(x) =& \phi(x) + \ln c_1(x) = -\ln\nu + \phi_0(x) + \phi_1(x)\nu + O(\nu^2)\\
&+ \ln\big(c_{11}(x)\nu + c_{12}(x)\nu^2 + O(\nu^3)\big)\\
=& \phi_0(x) + \ln c_{11}(x) + \Big(\phi_1(x) + \frac{c_{12}(x)}{c_{11}(x)}\Big)\nu + O(\nu^2).
\end{aligned}$$

For $\bar\mu_2(x)$, one has

$$\begin{aligned}
\bar\mu_2(x) =& -\phi(x) + \ln c_2(x) = \ln\nu - \phi_0(x) - \phi_1(x)\nu + \ln\frac{2}{\nu} + O(\nu^2)\\
=& -\phi_0(x) + \ln 2 - \phi_1(x)\nu + O(\nu^2).
\end{aligned}$$

All claims on components of electrochemical potentials then follow. □

7.1.3 Internal dynamics over the interval $(b,1)$.

The singular slow orbit (ϕ, c_1, c_2) can be obtained from the display below (41) in [8].

Proposition 7.5. *For $x\in(b,1)$ and for $k=1,2$,*

$$c_k(x;\nu) = c_{k0}(x) + c_{k1}(x)\nu + O(\nu^2), \quad \phi(x;\nu) = \phi_0(x) + \phi_1(x)\nu + O(\nu^2),$$

where

$$\begin{aligned}
c_{10}(x) =& c_{20}(x) = R + \frac{H(1)-H(x)}{2}J_{20},\ c_{11}(x) = c_{21}(x) = \frac{H(1)-H(x)}{2}\big(J_{11}+J_{21}\big);\\
\phi_0(x) =& \ln R - \ln c_{10}(x), \quad \phi_1(x) = -\frac{c_{21}(x)}{c_{20}(x)} + \frac{2J_{11}}{J_{20}}\ln\frac{c_{20}(x)}{R},
\end{aligned}$$

where ϕ_1^b is given in (3.16).

Corollary 7.6. *Over the interval $(b,1)$, the electrochemical potentials are*

$$\bar{\mu}_k(x;\nu) = \bar{\mu}_{k0}(x) + \bar{\mu}_{k1}(x)\nu + O(\nu^2)$$

for $k = 1,2$, where

$$\begin{aligned}
\bar{\mu}_{10}(x) =&\phi_0(x) + \ln c_{10}(x) = \ln R, \quad \bar{\mu}_{11}(x) = \phi_1(x) + \frac{c_{11}(x)}{c_{10}(x)} = \frac{2J_{11}}{J_{20}} \ln \frac{c_{20}(x)}{R},\\
\bar{\mu}_{20}(x) =&2\ln c_{20}(x) - \ln R, \quad \bar{\mu}_{21}(x) = -\phi_1(x) + \frac{c_{21}(x)}{c_{20}(x)} = \frac{2c_{21}(x)}{c_{20}(x)} - \frac{2J_{11}}{J_{20}} \ln \frac{c_{20}(x)}{R};
\end{aligned}$$

In particular, $\bar{\mu}_{10}'(x) = 0$, and hence, $J_{10} = 0$ over the interval $(b,1)$.

7.2 An orbit near the singular orbit for small $\varepsilon > 0$

We now provide a proof for the existence and uniqueness of an orbit for $\varepsilon > 0$ small, near the singular orbit, of the connecting problem associated to the BVP. This will be accomplished in several steps and by an application of the Exchange Lemma ([20, 21, 23, 32]).

Step 1. Exchange Lemma over $[0,a]$ along $\Gamma_0^r \cup \Lambda_l \cup \Gamma_a^l$. Recall that

$$B_0 = \{(V, u, L_1, L_2, J_1, J_2, 0) : \text{ arbitrary } u, J_1, J_2\}.$$

Let M_ε^0 be the (positively) invariant manifold consisting of forward orbits of (2.5) from B_0. Due to the nonzero w-component, the vector field of (2.5) is not tangent to B_0, and hence, $\dim M_\varepsilon^0 = \dim B_0 + 1 = 4$. Let

$$P_0 = (V, u^0, L_1, L_2, J_1^0, J_2^0, 0) \in B_0 \cap W^s(\mathcal{Z}_l)$$

be the initial point of the singular orbit.

Lemma 7.7. *The intersection of M_0^0 and $W^s(\mathcal{Z}_l)$ at P_0 is transversal.*

Proof. We will show that B_0 intersect $W^s(\mathcal{Z}_l)$ transversally at P_0. Since $W^s(\mathcal{Z}_l)$ is co-dimension one, it suffices to find one non-zero vector in $T_{P_0}B_0$ but not in $T_{P_0}W^s(\mathcal{Z}_l)$. Note that the unit vector in the u-direction $e_u = (0,1,0,0,0,0,0) \in T_{P_0}B_0$. We claim that $e_u \notin T_{P_0}W^s(\mathcal{Z}_l)$. Suppose, on the contrary, that $e_u \in T_{P_0}W^s(\mathcal{Z}_l)$. Then there is a smooth curve $P(s) \in W^s(\mathcal{Z}_l)$ parameterized by $s \in (-1,1)$, such that,

$$P(0) = P_0 \text{ and } \frac{d}{ds}P(0) = e_u. \tag{7.1}$$

Let $(\phi(\xi;s), u(\xi;s), C(\xi;s), J(s), w(s))$ be the solution of the limit fast system with $(\phi(0;s), u(0;s), C(0;s), J(s), w(s)) = P(s)$. (Note that J and w are conserved for the limit fast system.) Then

$$\lim_{\xi\to\infty} (\phi(\xi;s), u(\xi;s), C(\xi;s), J(s), w(s)) = (\phi^L(s), 0, C^L(s), J(s), w(s)) \in \mathcal{Z}_l.$$

where, following from Proposition 3.2 and Corollary 3.3 in [8] that,

$$\begin{aligned}
\phi^L(s) =&\phi(0;s) - \frac{1}{2}\ln\frac{c_2(0;s)}{c_1(0;s)},\\
c_1^L(s) =&c_1(0;s)e^{\phi(0;s)-\phi^L(s)} = \sqrt{c_1(0;s)c_2(0;s)},\\
c_2^L(s) =&c_2(0;s)e^{\phi^L(s)-\phi(0;s)} = \sqrt{c_1(0;s)c_2(0;s)}.
\end{aligned} \tag{7.2}$$

and

$$\begin{aligned} \frac{1}{2}u^2(0;s) =& c_1(0;s) + c_2(0;s) - c_1^L(s) - c_2^L(s) \\ =& c_1(0;s) + c_2(0;s) - 2\sqrt{c_1(0;s)c_2(0;s)}. \end{aligned} \tag{7.3}$$

The second condition in (7.1) gives

$$\frac{\partial}{\partial s}u(0;0) = 1, \quad \frac{\partial}{\partial s}c_1(0;0) = \frac{\partial}{\partial s}c_2(0;0) = 0.$$

Taking the derivative of (7.3) with respect to s and applying $\frac{\partial}{\partial s}c_k(0;0) = 0$, one gets $\frac{\partial}{\partial s}u(0;0) = 0$, which contradicts to $\frac{\partial}{\partial s}u(0;0) = 1$. Thus, B_0 intersects $W^s(\mathcal{Z}_l)$ transversally at P_0. $\square$

Next, recall that, for $N_0 = M_0^0 \cap W^s(\mathcal{Z}_l)$, with $\phi^L = V - \frac{1}{2}\ln\frac{L_2}{L_1}$,

$$\omega(N_0) = \Big\{(\phi^L, 0, c_1^L, c_2^L, J_1, J_2, 0) : \text{ arbitrary } \ J_1, J_2\Big\}.$$

For $\delta > 0$ small and $I_a^\delta = (a-\delta, a+\delta)$, let $W_a = W^u(\omega(N_0)\cdot I_a^\delta)$. Applying the Exchange Lemma together with Lemma 7.7 along $\Gamma_0^r \cup \Lambda_l \cup \Gamma_a^l$, one concludes that M_ε is $\mathcal{C}^1$ $O(\varepsilon)$-close to W_a around Γ_a^l. Note that

$$\omega(N_0)\cdot I_a^\delta = \Big\{(\phi(x;\nu), 0, c_1(x;\nu), c_2(x;\nu), J_1, J_2, x) : \ x \in I_a^\delta, \ \text{ any } \ J_1, J_2\Big\},$$

where $(\phi(x;\nu), 0, c_1(x;\nu), c_2(x;\nu))$ is given in Proposition 7.1 and

$$c_k(x;\nu) = L - \frac{J_{20}}{2}H(x) + O(\nu), \quad \phi(x;\nu) = V + \ln L - \ln c_{10}(x) + O(\nu).$$

Step 2. Transversal intersection of W_a with $W^s(\mathcal{Z}_m)$ along Γ_a^r. Let

$$P_a = (\phi^a, u^a, c_1^a, c_2^a, J_1^0, J_2^0, a) \in W_a \cap W^s(\mathcal{Z}_m).$$

be the starting point of the layer Γ_a^r, which is also the end point of the layer Γ_a^l.

Lemma 7.8. *The intersection of W_a and $W^s(\mathcal{Z}_m)$ at P_a is transversal.*

Proof. Note that the set $W_a \cap \{J = J^0\}$ is two dimensional, and consists of points $(\phi, u, c_1, c_2, J_1^0, J_2^0, w)$ with $w = x \in I_a^\delta$ and

$$\begin{aligned} \ln c_1 + \phi =& \ln\left(c_1^L - \frac{J_1^0+J_2^0}{2}H(x)\right) + \phi^L - \frac{J_1^0-J_2^0}{J_1^0+J_2^0}\ln\left(1 - \frac{J_1^0+J_2^0}{2c_1^L}H(x)\right), \\ \ln c_2 - \phi =& \ln\left(c_2^L - \frac{J_1^0+J_2^0}{2}H(x)\right) - \phi_L + \frac{J_1^0-J_2^0}{J_1^0+J_2^0}\ln\left(1 - \frac{J_1^0+J_2^0}{2c_2^L}H(x)\right), \\ \frac{1}{2}u^2 =& \frac{1}{2}(u^a)^2 - c_1^a - c_2^a + c_1 + c_2. \end{aligned}$$

We will parameterize $W_a \cap \{J_1 = J_1^0, J_2 = J_2^0\}$ by ϕ and x. By differentiations with respect to ϕ and x, respectively, one finds that $T_{P_a}(W_a \cap \{J = J^0\})$ is spanned by,

$$T_1 = \Big(1, (c_2^a - c_1^a)/u^a, -c_1^a, c_2^a, 0, 0, 0\Big) \ \text{ and } \ T_2 = \Big(0, (c_1^a + c_2^a)c^*/u^a, c_1^a c^*, c_2^a c^*, 0, 0, 1\Big)$$

where

$$c^* = -\frac{2J_2^0}{2c_1^L - (J_1^0 + J_2^0)H(a)}H'(a).$$

We claim that $W^s(\mathcal{Z}_m)$ is given by

$$F = F(\phi, u, c_1, c_2, J_1, J_2, w) = 0 \quad \text{for} \quad w \in [a, b]$$

where

$$F = \frac{1}{2}u^2 - c_1 - c_2 + 2\sqrt{Q_0^2 + c_1 c_2} + 2Q_0 \ln \frac{\sqrt{Q_0^2 + c_1 c_2} - Q_0}{c_1}. \tag{7.4}$$

Indeed, $W^s(\mathcal{Z}_m)$ is determined, from the integrals in Proposition 3.4 in [8], by

$$\begin{aligned} c_1 e^{\phi} = c_1^{a,m} e^{\phi^{a,m}}, \quad c_2 e^{-\phi} =& c_2^{a,m} e^{-\phi^{a,m}}, \quad c_1^{a.m} - c_2^{a,m} + 2Q_0 = 0, \\ \frac{1}{2}u^2 - c_1 - c_2 + 2Q_0\phi =& - c_1^{a,m} - c_2^{a,m} + 2Q_0 \phi^{a,m}. \end{aligned}$$

The first three equations give

$$c_1 e^{\phi - \phi^{a,m}} - c_2 e^{-\phi + \phi^{a,m}} + 2Q_0 = 0.$$

Thus,

$$\phi^{a,m} = \phi - \ln \frac{\sqrt{Q_0^2 + c_1 c_2} - Q_0}{c_1}.$$

Hence,

$$\frac{1}{2}u^2 - c_1 - c_2 + 2Q_0\phi = -2\sqrt{Q_0^2 + c_1 c_2} + 2Q_0\phi - 2Q_0 \ln \frac{\sqrt{Q_0^2 + c_1 c_2} - Q_0}{c_1},$$

which verifies the claim.

Note that, a norm vector to $W^s(\mathcal{Z}_m)$ at P_a is

$$\nabla F(P_a) = (F_\phi, F_u, F_{c_1}, F_{c_2}, F_{J_1}, F_{J_2}, F_w),$$

where, from (7.4),

$$\begin{aligned} F_\phi =& F_{J_1} = F_{J_2} = F_w = 0, \quad F_u = u^a, \\ F_{c_1} =& -1 + (Q_0^2 + c_1^a c_2^a)^{-1/2} c_2^a - Q_0(\sqrt{Q_0^2 + c_1^a c_2^a} + Q_0)^{-1}(Q_0^2 + c_1^a c_2^a)^{-1/2} c_2^a, \\ F_{c_2} =& -1 + (Q_0^2 + c_1^a c_2^a)^{-1/2} c_1^a + Q_0(\sqrt{Q_0^2 + c_1^a c_2^a} - Q_0)^{-1}(Q_0^2 + c_1^a c_2^a)^{-1/2} c_1^a. \end{aligned}$$

One has

$$\begin{aligned} T_1 \cdot \nabla F(P_a) =& c_2^a - c_1^a - c_1^a F_{c_1} + c_2^a F_{c_2} \\ =& Q_0(\sqrt{Q_0^2 + c_1^a c_2^a} - Q_0)^{-1}(Q_0^2 + c_1^a c_2^a)^{-1/2} c_1^a c_2^a \\ &+ Q_0(\sqrt{Q_0^2 + c_1^a c_2^a} + Q_0)^{-1}(Q_0^2 + c_1^a c_2^a)^{-1/2} c_1^a c_2^a \\ =& 2Q_0\sqrt{Q_0^2 + c_1^a c_2^a} \neq 0. \end{aligned}$$

Thus, $T_1 \notin W^s(\mathcal{Z}_m)$, and hence, W_a and $W^s(\mathcal{Z}_m)$ intersect transversally at P_a. □

Let $N^{a,m} = W_a \cap W^s(\mathcal{Z}_m)$. Then $\dim N^{a,m} = 4+6-7 = 3$ and $\dim \omega(N^{a,m}) = 2$. One has, from Proposition 7.3,

$$\omega(N^{a,m}) = \{(\phi^{a,m}, 0, c_1^{a,m}, c_2^{a,m}, J, a) : \ |J - J^0| < \delta\} \subset \mathcal{Z}_m, \tag{7.5}$$

where

$$\begin{aligned} \phi^{a,m} =& - \ln \nu + \ln \frac{2e^V L}{A_0^2} + (\phi_1^a - A_0)\nu + O(\nu^2), \\ c_1^{a,m} =& \frac{1}{2} A_0^2 \nu + A_0 A_1 \nu^2 + O(\nu^3), \quad c_2^{a,m} = \frac{2}{\nu} + \frac{1}{2} A_0^2 \nu + O(\nu^2). \end{aligned} \tag{7.6}$$

Step 3. M_ε^1 along $\Gamma_b^r \cup \Lambda_r \cup \Gamma_1^l$ and Γ_b^l. Let M_ε^1 be the set that consists of backward orbits from B_1. Similarly, one can apply the procedure in Steps 1 and 2 backward to M_ε^1 along $\Gamma_b^r \cup \Lambda_r \cup \Gamma_1^l$ and Γ_b^l. If the set $\alpha(N^{b,m})$ associated to M_ε^1 is the counterpart of the set $\omega(N^{a,m})$ associated to M_ε^0, then

$$\alpha(N^{b,m}) = \{(\phi^{b,m}, 0, c_k^{b.m}, J, b) : \ |J - J^0| < \delta\} \subset \mathcal{Z}_m, \tag{7.7}$$

where, from Proposition 7.3,

$$\begin{aligned} \phi^{b,m} =& - \ln \nu + \ln \frac{2e^V L}{A_0^2} + \Big(\phi_1^a - A_0 + \frac{J_{20}}{2}(H(b) - H(a))\Big)\nu + O(\nu^2), \\ c_1^{b,m} =& \Big(\frac{1}{2} A_0^2 - J_{11}(H(b) - H(a))\Big)\nu + \Big(A_0 A_1 - \frac{H(b) - H(a)}{2H(a)}(L - A_0)A_0^2\Big)\nu^2 + O(\nu^3), \\ c_2^{b,m} =& \frac{2}{\nu} + \Big(\frac{1}{2} A_0^2 - J_{11}(H(b) - H(a))\Big)\nu + O(\nu^2). \end{aligned}$$

Step 4. Transversal intersection of $\hat{M}_a$ and $\hat{M}_b$ on $\mathcal{Z}_m$. Recall the sets $\omega(N^{a,m})$ in (7.5) and $\alpha(N^{b,m})$ in (7.7). Let $\hat{M}_a$ be the collection of forward (limiting slow) orbits from $\omega(N^{a,m})$ on $\mathcal{Z}_m$ and let $\hat{M}_b$ be the collection of backward (limiting slow) orbits from $\alpha(N^{b,m})$ on $\mathcal{Z}_m$. To complete the proof, it suffices to show that

Lemma 7.9. *The intersection of $\hat{M}_a$ and $\hat{M}_b$ on $\mathcal{Z}_m$ is transversal.*

Proof. We will show that $\hat{M}_a$ and $\hat{M}_b$ intersect transversally on $\mathcal{Z}_m \cap \{w = x = b\}$. We use (ϕ, c_1, J_1, J_2) as a coordinate system on $\mathcal{Z}_m \cap \{w = x = b\}$. To characterize

$$\hat{M}_a \cap \big\{w = x = b\big\} = \{(\phi(J_1, J_2), c_1(J_1, J_2), J_1, J_2) : |J_k - J_k^0| < \delta\},$$

we recall from display (35) in [8] that the solution of the limiting slow system with the initial condition $(\phi^{a,m}, c_1^{a,m}, J_1, J_2, a)$ that corresponds to the point

$$(\phi^{a,m}, 0, c_1^{a,m}, c_2^{a,m}, J_1, J_2, a) \in \omega(N^{a,m})$$

is given by

$$\begin{aligned} \phi(y) =& \phi^{a,m} + (J_2 - J_1)y, \quad c_1(y) = e^{-(J_1+J_2)y} c_1^{a,m} - \frac{2Q_0 J_1}{J_1 + J_2}\left(1 - e^{-(J_1+J_2)y}\right), \\ \int_a^w h^{-1}(s)ds =& \frac{2c_1^{a,m}}{J_1 + J_2}\left(1 - e^{-(J_1+J_2)y}\right) \\ & - \frac{4Q_0 J_1}{J_1 + J_2}\left(y - \frac{1}{J_1 + J_2}\left(1 - e^{-(J_1+J_2)y}\right)\right) + 2Q_0 y. \end{aligned}$$

At $w=b$, the quantity y is determined by (J_1,J_2) from the last equation implicitly and, in turn, ϕ and c_1 are functions of (J_1,J_2) defined implicitly by

$$\begin{aligned} &\phi=\phi^{a,m}+(J_2-J_1)y, \quad c_1=e^{-(J_1+J_2)y}c_1^{a,m}-\frac{2Q_0J_1}{J_1+J_2}\left(1-e^{-(J_1+J_2)y}\right),\\ &\frac{H(b)-H(a)}{2}(J_1+J_2)=\left(c_1^{a,m}+\frac{2Q_0J_1}{J_1+J_2}\right)\left(1-e^{-(J_1+J_2)y}\right)+Q_0(J_2-J_1)y. \end{aligned} \tag{7.8}$$

Thus, the tangent space to $\hat{M}_a\cap\{w=x=b\}$ is spanned by the vectors

$$(\partial_{J_1}\phi,\partial_{J_1}c_1,1,0) \text{ and } (\partial_{J_2}\phi,\partial_{J_2}c_1,0,1).$$

It follows from (7.7) that the tangent space to $\alpha(N^{b,m})$ is spanned by (0,0,1,0) and (0,0,0,1). Note that $\mathcal{Z}_m\bigcap\{w=x=b\}$ is four dimensional. Thus, it suffices to show that the above four vectors are linearly independent, that is, $\partial_{J_1}\phi\partial_{J_2}c_1\neq\partial_{J_2}\phi\partial_{J_1}c_1$ at $J=J^0$. The latter will be established in the rest of this part.

Take derivative with respect to J_1 and J_2 in the last equation of (7.8) to get

$$\begin{aligned} \frac{H(b)-H(a)}{2}=&\frac{2Q_0}{J_1+J_2}\left(1-e^{-(J_1+J_2)y}\right)-\frac{2Q_0J_1}{(J_1+J_2)^2}\left(1-e^{-(J_1+J_2)y}\right)\\ &+\left(c_1^{a,m}+\frac{2Q_0J_1}{J_1+J_2}\right)\left(y+(J_1+J_2)\partial_{J_1}y\right)e^{-(J_1+J_2)y}\\ &+Q_0(J_2-J_1)\partial_{J_1}y-Q_0y,\\ \frac{H(b)-H(a)}{2}=&-\frac{2Q_0J_1}{(J_1+J_2)^2}\left(1-e^{-(J_1+J_2)y}\right)\\ &+\left(c_1^{a,m}+\frac{2Q_0J_1}{J_1+J_2}\right)\left(y+(J_1+J_2)\partial_{J_2}y\right)e^{-(J_1+J_2)y}\\ &+Q_0(J_2-J_1)\partial_{J_2}y+Q_0y. \end{aligned} \tag{7.9}$$

Recall, from Propositions 3.1 and 3.3, and display (7.6), that

$$\begin{aligned} y=&y_1\nu+O(\nu^3)=\frac{\beta-\alpha}{2}H(1)\nu+O(\nu^3), \quad c_1^{a,m}=\frac{1}{2}A_0^2\nu+A_0A_1\nu^2+O(\nu^3),\\ J_1=&J_{11}\nu+O(\nu^2), \quad J_2=J_{20}+J_{21}\nu+O(\nu^2). \end{aligned}$$

One has, from (7.9), that

$$\partial_{J_1}y=-\frac{y_1^2}{J_{20}}\nu^2+O(\nu^3) \text{ and } \partial_{J_2}y=O(\nu^3).$$

Taking the derivatives with respect to J_1 and J_2 in (7.8), one has

$$\begin{aligned}
\partial_{J_1}\phi =& - y + (J_2 - J_1)\partial_{J_1} y = -y_1^2\nu^2 + O(\nu^3),\\
\partial_{J_1} c_1 =& - (y + (J_1 + J_2)\partial_{J_1} y)e^{-(J_1+J_2)y} c_1^{a,m} - \frac{2Q_0}{J_1+J_2}\left(1 - e^{-(J_1+J_2)y}\right)\\
&+ \frac{2Q_0 J_1}{(J_1+J_2)^2}\left(1 - e^{-(J_1+J_2)y}\right) - \frac{2Q_0 J_1}{J_1+J_2}(y + (J_1+J_2)\partial_{J_1} y)e^{-(J_1+J_2)y}\\
=& - 2y_1 + O(\nu),\\
\partial_{J_2}\phi =& y + (J_2 - J_1)\partial_{J_2} y = O(\nu^3),\\
\partial_{J_2} c_1 =& - (y + (J_1 + J_2)\partial_{J_2} y)e^{-(J_1+J_2)y} c_1^{a,m}\\
&+ \frac{2Q_0 J_1}{(J_1+J_2)^2}\left(1 - e^{-(J_1+J_2)y}\right) - \frac{2Q_0 J_1}{J_1+J_2}(y + (J_1+J_2)\partial_{J_2} y)e^{-(J_1+J_2)y}\\
=& 2y_1 + O(\nu).
\end{aligned}$$

Therefore, at $(\phi^{b,m}, 0, c_k^{b.m}, J^0, b)$ and for $\nu > 0$ small,

$$\partial_{J_1}\phi\partial_{J_2} c_1 - \partial_{J_2}\phi\partial_{J_1} c_1 = -2y_1^3\nu^2 + O(\nu^3) \neq 0.$$

This completes the proof. □

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