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Abstract
Density functional theory (DFT), a relatively simple and successful theory of
an inhomogeneous fluid, is applied to an electrolyte adsorbed into a charged
cylindrical pore that is a model of the filter of a physiological calcium channel.
This may be the first application of DFT in cylindrical geometry, especially for
the case of charged particles. Because of the high density of charge in the pore,
this pore behaves as a calcium channel filter and selectively adsorbs calcium ions
in preference to sodium ions. This selectivity is due to the fact that a calcium
ion delivers twice the charge of a sodium ion while occupying the same space,
and so satisfies local charge neutrality more efficiently in the confined space
of the filter. The applicability of this mechanism has been confirmed earlier in
Monte Carlo simulations.

1. Introduction

Ion channels are proteins with a hole in their centre that allow ions to pass through an otherwise
impermeable membrane. They permit the passage of signals that are responsible for many
physiological functions. Generally, ion channels are selective and favour the passage of a
particular species of ion. For example, the calcium channel will accept sodium ions but
favours calcium ions even when the concentration of calcium ions in the bathing or surrounding
homogeneous electrolyte is very much less than the concentration of sodium ions in this
inhomogeneous electrolyte in the filter.

The structure of the calcium channel has not yet been determined in detail. However, an
important structural element of the calcium channel is a ring of four glutamate side chains [1],
the so-called EEEE locus, in a roughly symmetrical ring in a region of the channel. Mutation

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of the EEEE locus into a DEKA locus (aspartate (D), glutamate (E), lysine (K), and alanine (A) side chains) changes the calcium channel into a sodium channel that, under physiological conditions, favours the passage of sodium ions [2]. Thus, the EEEE locus of glutamates is the important structural element of the calcium channel and the region that contains these side chains is called the selectivity filter of the calcium channel. In the absence of calcium ions, the calcium channel can pass tetramethylammonium ions, indicating that the calcium channel is at least 7–8 Å in diameter [3].

Recently, Nonner *et al* [4] (NCE) have drawn attention to the importance of the glutamate locus and have proposed a simple model and approximation that describes the selectivity of the calcium channel filter. In their model, each glutamate side chain is replaced by a pair of oxygen 'ions' that model the two oxygen atoms that are part of the carboxylate group at the end of a glutamate chain. The carboxylate group has a negative charge equal to that of an electron; to mimic this, NCE give each oxygen a charge of $-1/2$. Since the glutamate chains are flexible, NCE allow the oxygen ions to wander nearly freely in the filter. The only constraint on their motion is that they cannot leave the filter.

To examine the selectivity of this model calcium filter, NCE consider the equilibrium between two *homogeneous* aqueous electrolytes. One represents the bathing electrolyte in which the membrane and channel have been placed. This electrolyte consists of Na^+, Ca^{2+}, and Cl^- ions; a dielectric background is used to represent the solvent (this is the so-called primitive model of an electrolyte). The other electrolyte represents the fluid in the filter. A dielectric background is also used in the filter, but the dielectric constant is lower than in the bath because of presumed incomplete solvation of the ions in the filter. The fixed concentration of the $O^{-1/2}$ ions was chosen to be that of the eight carboxylate oxygens in a cylinder whose length and radius are about 5 Å. Specifically, they took the oxygen concentration to be $8/375$ Å^3.

Since the dimensions of the filter are microscopic, the fluid in the filter is necessarily inhomogeneous (i.e., the density is dependent on position). Thus, the approximation that the electrolyte in the filter could be treated as homogeneous was, to say the least, daring. Nonner *et al* used the mean spherical approximation (MSA) to obtain the thermodynamic properties of the two fluids. This is a technical convenience and not central to their calculation. Any good theory could have been used. The MSA yields good, although not perfect, results for the thermodynamic properties of an electrolyte. The important advantage of the MSA is that it yields analytic expressions for thermodynamic functions, which facilitates the calculations.

They determined the concentration of Na^+, Ca^{2+}, and Cl^- ions in the filter electrolyte by allowing this electrolyte to be in equilibrium with a bathing NaCl electrolyte that has a concentration of 0.1 M NaCl to which a specified amount of $CaCl_2$ had been added. They found that there was very little Cl^- in the filter. Perhaps this is to be expected given the large negative charge of the oxygens in the filter. More importantly, they found that the filter had a marked preference for Ca^{2+} ions over Na^+ ions. Even when the concentration of Ca^{2+} ions in the bath was orders of magnitude smaller than that of the Na^+ ions, Ca^{2+} ions were adsorbed by the filter in preference to Na^+ ions.

Since the Na^+ and Ca^{2+} ions have nearly identical diameters, the selectivity of the calcium filter, at least in their calculation, is due to the fact that a Ca^{2+} ion delivers twice the charge of an Na^+ ion while occupying about the same space, and so satisfies local charge neutrality in the crowded channel more efficiently. Boda *et al* [5] have called this mechanism charge/space competition. Eisenberg [6] has called it a crowded charge effect.

One is entitled to be skeptical of the use of a theory of a homogeneous fluid to describe the electrolyte in the filter that is clearly inhomogeneous. However, the predictions of NCE have been confirmed by the Monte Carlo (MC) simulations of Boda *et al* [5, 7–9].

The purpose of this study is to formulate density functional theory (DFT) for the cylindrical geometry of a channel and obtain some results. Density functional theory is a relatively simple theory of an inhomogeneous electrolyte that generally gives good results. Previous applications of DFT have been for planar geometries. There have been few applications of DFT to cylindrical geometries. We know only of the DFT studies of Goulding *et al* [10] for hard spheres in an infinite cylinder. To our knowledge, there have been no DFT studies for cylindrical geometries involving Coulombic forces.

2. Theoretical considerations

The electrolyte is modelled as a system of charged hard spheres in a dielectric background that is placed within a cylinder that represents the filter. The electrolyte in the cylinder is in equilibrium with a distant bulk electrolyte. The interaction between a pair of ions is

$$u_{ij}(r) = \begin{cases} \infty, & r < d \\ \dfrac{q_i q_j}{\epsilon r}, & r > d, \end{cases} \tag{1}$$

where q_i is the charge of an ion of species i, r is the separation of the pair of ions, and ϵ is the dielectric constant of the electrolyte. All ions are assumed to be of equal diameter, d. Nonner *et al* used a different dielectric constant in the filter and in the bulk. It is not convenient to do this here. In conventional applications of DFT, the inhomogeneous fluid is presumed to be the same as the bulk fluid with which it is in equilibrium. It may be possible to deal with this limitation. However, we will defer this to the future. Our main purpose here is to formulate DFT for electrolytes in cylindrical geometry with the numerical results being secondary.

In the NCE theory, oxygens are in the filter but not in the bulk electrolyte. It is difficult in DFT to have preadsorbed particles in the filter that are not in the bulk fluid since all the ions in the filter are assumed to be in equilibrium with ions in the bulk. Therefore, we smear the positive charge of the glutamates uniformly over the surface of the cylinder and give the inside surface of the cylinder a charge density, σ, that, when integrated over the cylinder surface, is equal to the total charge of the glutamates. A smaller value of the cylinder radius, R, than that used in Boda *et al* simulations, is used in an attempt to compensate for the space occupied by the glutamates.

There are three species of ions in the filter and bulk fluid: Na^+, Ca^{2+}, and Cl^-. In the bulk fluid, the densities of the ions satisfy the charge neutrality condition,

$$\rho_{Na^+} + 2\rho_{Ca^{2+}} = \rho_{Cl}. \tag{2}$$

Because the bulk and filter electrolytes are electrostatically isolated, charge neutrality is satisfied exactly in the channel. This differs from the Boda *et al* simulations, where there were electrostatic interactions between the filter and bulk electrolytes, and charge neutrality was satisfied only globally.

The filter cylinder is assumed to be smooth and of uniform radius, R. In reality, the filter is of finite length. However, this would make the various profiles functions of r and z. We have found that with only one variable, r, the DFT calculations are already lengthy. As a result, we consider a cylinder of radius R and length L and periodically extend this 'unit' cylinder so that we are have an infinite cylinder whose profiles are only r dependent. The charge of the glutamates is smeared uniformly over the surface of this cylinder so that each periodic replica has a negative charge equal to that of the glutamates in the filter. Our DFT program uses the potential at the surface of the cylinder, V, as a boundary condition so that we must vary V until the charge of all the particles in the channel matches the charge density on the cylinder surface.

The potential profile, $\phi(r)$, is obtained by integrating Possion's equation,

$$\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial\phi}{\partial r}\right) = \frac{4\pi}{\epsilon}\sum_i q_i\rho_i g_i(r), \tag{3}$$

where $g_i(r)$ is the distribution function, $g_i(r) = \rho_i(r)\rho_i$. Densities have the units of number of particles/volume. The result is

$$\phi(r) = V + \frac{4\pi}{\epsilon}\sum_i q_i\rho_i\int_0^R \ln\left(\frac{R}{t}\right)g_i(t)t\,\mathrm{d}t - \frac{4\pi}{\epsilon}\sum_i q_i\rho_i\int_0^r \ln\left(\frac{r}{t}\right)g_i(t)t\,\mathrm{d}t. \tag{4}$$

Gaussian units are used.

It is convenient to introduce reduced or dimensionless units in our calculations. The reduced temperature is

$$T^* = \frac{k\epsilon d}{e^2}T, \tag{5}$$

where e is the magnitude of the charge of an electron, T is the temperature, and k is Boltzmann's constant. The reduced charge density is

$$\sigma^* = \frac{\sigma d}{e}, \tag{6}$$

and the reduced potential is

$$\phi^*(z) = \beta e\phi(z). \tag{7}$$

Distances are scaled by d.

3. Density functional theory

The version of DFT that has been employed in the present study is based on the fundamental measure theory, developed by Rosenfeld [11]. This theory is quite standard now. Moreover, it is closely related to the Kierlik–Rosinberg approach [12], which we have used recently to describe inhomogeneous ions at adsorbing surfaces [13–16]. For these reasons we give only the final equations. In particular, the density profile equation reads

$$-kT\ln[\rho_i(r)/\rho_{ib}] = v^{\mathrm{HC}}(r) + \left[\frac{\delta F_{\mathrm{HS}}^{\mathrm{ex}}}{\delta\rho_i(\mathbf{r})} - \mu_{i,\mathrm{HS}}^{\mathrm{ex}}\right] + z_i e\,\phi(r)$$
$$- kT\sum_j\int \Delta c_{ij}(|\mathbf{r}-\mathbf{r}'|)\,\Delta\rho_j(r')\,\mathrm{d}\mathbf{r}', \tag{8}$$

where

$$v^{\mathrm{HC}}(r) = \begin{cases} \infty, & r > R - d/2 \\ 0, & r < R - d/2, \end{cases} \tag{9}$$

is the hard-cylinder potential, F_{HS} is the excess free energy functional of a hard sphere system of density $\rho = \sum\rho_i$, μ_i^{ex} is the excess (apart from the ideal contribution) part of the chemical potential, $\Delta\rho_i(\mathbf{r}) = \rho_i(\mathbf{r}) - \rho_{ib}$, ρ_{ib} is the bulk density of a homogeneous neutral fluid, remaining in chemical equilibrium with the confined fluid, and Δc_{ij} are the short-ranged parts of the direct correlation functions resulting from coulombic interactions. The functional $F_{\mathrm{HS}}^{\mathrm{ex}}$ is calculated from [11]

$$F_{\mathrm{HS}}^{\mathrm{ex}}/kT = -n_0\ln(1-n_3) + \frac{n_1 n_2 - (\mathbf{n}_{v1}\cdot\mathbf{n}_{v2})}{4\pi(1-n_3)} + \frac{n_2}{24\pi}\frac{n_2^3 - 3(\mathbf{n}_{v2}\cdot\mathbf{n}_{v2})}{(1-n_3)^2}. \tag{10}$$

The quantities n_i are the averaged densities; they are given by the following equation:

$$n_\alpha(\mathbf{r}_1) = \int \rho(\mathbf{r}_2)\omega_\alpha(r_{12})\, d\mathbf{r}_2, \tag{11}$$

where $\omega_\alpha(r)$ ($\alpha = 0, 1, 2, 3, v1, v2$) are weight functions, cf [11]. The first four weight functions (and the weighted densities) are scalars, whereas the last two weight functions are vectors. In the latter case $\mathbf{n}_{v1} \cdot \mathbf{n}_{v2}$ and $\mathbf{n}_{v2} \cdot \mathbf{n}_{v2}$ are scalar products.

The most widely used expression to evaluate the short-ranged parts of the direct correlation function, $\Delta c_{ij}(r)$, is the MSA result for charged hard spheres that are all of equal diameter, d. This result is of reasonable accuracy (especially for the electrostatic portion) and yields the following analytical expressions [17]:

$$kT\Delta c_{ij}(r) = \begin{cases} -\dfrac{q_i q_j}{\epsilon}\left[\dfrac{2A}{d} - \left(\dfrac{A}{d}\right)^2 r - \dfrac{1}{r}\right] & r < d \\ 0 & r > d. \end{cases} \tag{12}$$

In the above, $A = x^{-2}[x^2 + x - x(1 + 2x)^{1/2}]$, and $x^2 = \frac{4\pi\beta d^2}{\epsilon}\sum_i \rho_{ib}q_i^2$.

The numerical technique applied here to solve the density profile equation is quite similar to that described in [13–16]. We applied a standard Picard iteration procedure. All the integrations were carried out with $R = 1.6d$ and a grid size of $0.025d$. For a given value of V, the iterations are continued until the sum over the grid points of the absolute values of the differences between two consecutive iterations is smaller than 10^{-3}. Then, for a given Ca^{2+} concentration, the potential V is varied until the charge of the ions in the filter is equal in magnitude but opposite in sign to that on the cylinder wall, i.e., when

$$R\sigma = -\sum_i q_i \rho_i \int_0^R g_i(t)t\, dt \tag{13}$$

is satisfied.

4. Results

As we have pointed out, an expression for the direct correlation function of a uniform fluid of charged hard spheres is required by DFT. The length of the computation is greatly reduced if an analytic result can be used so that the inner integration in equation (8) can be performed analytically. Such a result is known only in the case of the MSA for charged hard spheres of equal size, equation (12). As a result, we limit our calculation to the case where all the ions have the same diameter. As in [5], we use $d = 2.5$ Å. The unit cell of the cylinder is assumed to have a length of $3d$ and a radius of $1.6d$. The charge density on the surface of the cylinder is assumed to be $\sigma^* = -0.0995$. This is equivalent to the smaller of the two sets of charges considered in [5] and corresponds to six half-charged oxygen 'ions' smeared over the surface of a cylinder of length 7.5 Å (the length of the filter is known only approximately). Six half-charged oxygen 'ions' correspond to three non-neutral glutamates, which we assume is roughly the situation for pH $= 7$; the higher charge, corresponding to four charged glutamates in a cylinder, which is surely appropriate only when pH > 9, is not considered in detail here. We use $T^* = 0.3523$, which corresponds to an aqueous electrolyte at room temperature with $\epsilon = 78.5$ and $d = 2.5$ Å.

We start the calculation with a 0.1 M NaCl solution in the bulk and add incremental amounts of $CaCl_2$ to the bulk solution. A 0.1 M NaCl solution corresponds to the reduced densities, $\rho_{Na}d^3 = \rho_{Cl}d^3 = 0.00094$. As is seen in figure 1, even when the concentration of the Ca^{2+} ions is small, significant numbers of Ca^{2+} ions enter the filter. The filter is
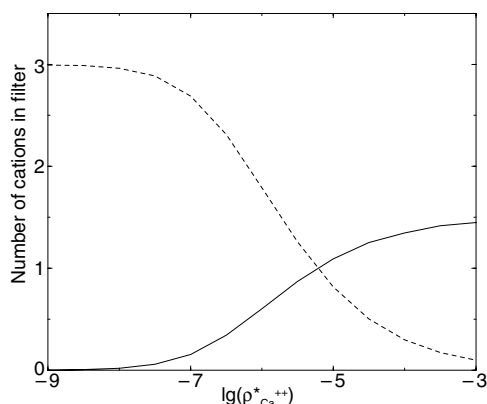
Figure 1. The number of Na$^+$ and Ca^{2+} ions in a 3d length of the cylinder as a function of the density of Ca^{2+} ions added to a 0.1 M NaCl solution in the bath. The reduced density is defined by $\rho^* = \rho d^3$.

calcium selective. In the limited volume of the filter, the divalent Ca^{2+} ions neutralize the negative charge of the glutamates more effectively than the monovalent Na$^+$ ions. This is the charge/space competition or crowded charge mechanism, already mentioned. Note that because the bulk fluid does not influence the contents of the filter, other than by determining the chemical potentials, charge neutrality is satisfied exactly in the filter.

The number of Ca^{2+} ions in the channel exceeds the number of Na$^+$ ions in the channel when the reduced density of Ca^{2+} ions in the bulk is about 5×10^{-6} or about 50 μM. This value is somewhat greater than the corresponding simulation value of about 10^{-6} (100 μM) obtained by Boda *et al* for an infinite cylinder with explicit glutamate oxygens.

Let us compare the DFT and simulation values of the Ca^{2+} reduced density in the bath at which the Ca^{2+} density in the channel has increased to half its final value (ρ_{50}) with experiment. The Ca^{2+}/Na$^+$ binding selectivity profile is expected to be directly related to the Ca^{2+} block of monovalent current profile. Although the experimental measurement of the Ca^{2+} block of monovalent current is typically made under non-equilibrium conditions, we assume for the sake of comparison to our results that the effects of the membrane potential and ion concentration gradients will be small relative to the discrepancies between experiment and theory. For simplicity, we compare the Ca^{2+} concentration at which the measured monovalent current is half-blocked (IC$_{50}$) in wild type L-type calcium channels with the simulated value of ρ_{50}. In an early well-accepted study of frog skeletal muscle fibres with a small membrane potential (-20 mV) and equal concentrations of Na$^+$ inside and outside the cell (32 mM) at about pH $= 7.4$, the IC^{50} was measured to be 0.68 μM [18]. This is comparable to the value of ρ_{50} obtained in our earliest MC study with an infinite cylinder with 4 O$^{-1/2}$/2.5 Å (corresponding to fully charged glutamates), namely, 0.7 μM, and smaller than the values of 200 and 500 μM obtained by simulation and DFT, respectively, for an infinite cylinder with 2 O$^{-1/2}$/2.5 Å (corresponding to partially protonated glutamates). Given uncertainties about the degree of protonation of the glutamates in the channel, even knowing the pH and the length of the filter, a qualitative statement is all that is possible. Nevertheless, it seems safe to say that the DFT values of ρ_{50} are greater than the simulation values, which in turn are greater than the experimental values of IC$_{50}$.

Thus, although the DFT results for IC$_{50}$ are small, they are still consistent with Ca^{2+} selectivity. It appears that smearing the glutamate charge over the surface of the cylinder
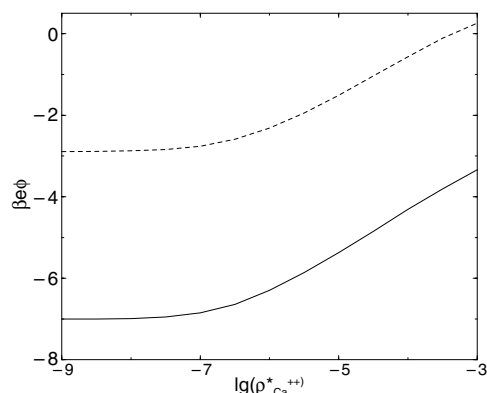
Figure 2. The electrostatic potential at the centre (broken curve) and the edge (solid curve) of the cylinder as a function of the density of Ca^{2+} ions added to a 0.1 M NaCl solution in the bath. The reduced density is defined in figure 1.
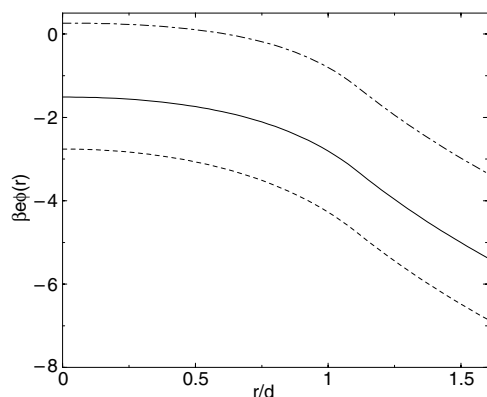

Figure 3. The electrostatic potential in the cylinder as a function of r. The broken, solid, and dot-dashed curves show the results when the density of Ca^{2+} ions in the bath is 10^{-7}, 10^{-5}, and 10^{-3}, respectively.

is a less efficient representation of the glutamates than is a collection of charged spheres. Presumably a smaller value of R would cause the DFT results to be closer to the corresponding simulation values for the corresponding σ^*.

In figure 2 the electrostatic potential at the edge and centre of the cylinder is plotted as a function of the Ca^{2+} ion concentration in the bulk. The potential in the cylinder decreases in magnitude as the concentration of Ca^{2+} ions in the bulk and, more importantly, in the filter increases. This is logical because the Ca^{2+} ion is divalent; the energy in the filter is roughly constant. The potential at the centre of the cylinder is smaller in magnitude than at the edge. Interestingly, the potential at the centre turns positive at the largest Ca^{2+} concentration.

The electrostatic potential is plotted in figure 3 as a function of r for three Ca^{2+} bulk densities. The potential is increasingly negative as r increases. As was seen in figure 2, the potential is more negative at small Ca^{2+} densities. The lower electrostatic potential at the cylinder surface is due to the presence of the smeared negative charge on the cylinder surface and results in a cation preference for the cylinder surface and a preference of anions for the cylinder centre. The Cl^- ions do enter the filter except in very small numbers. This was observed earlier by NCE and Boda *et al*.
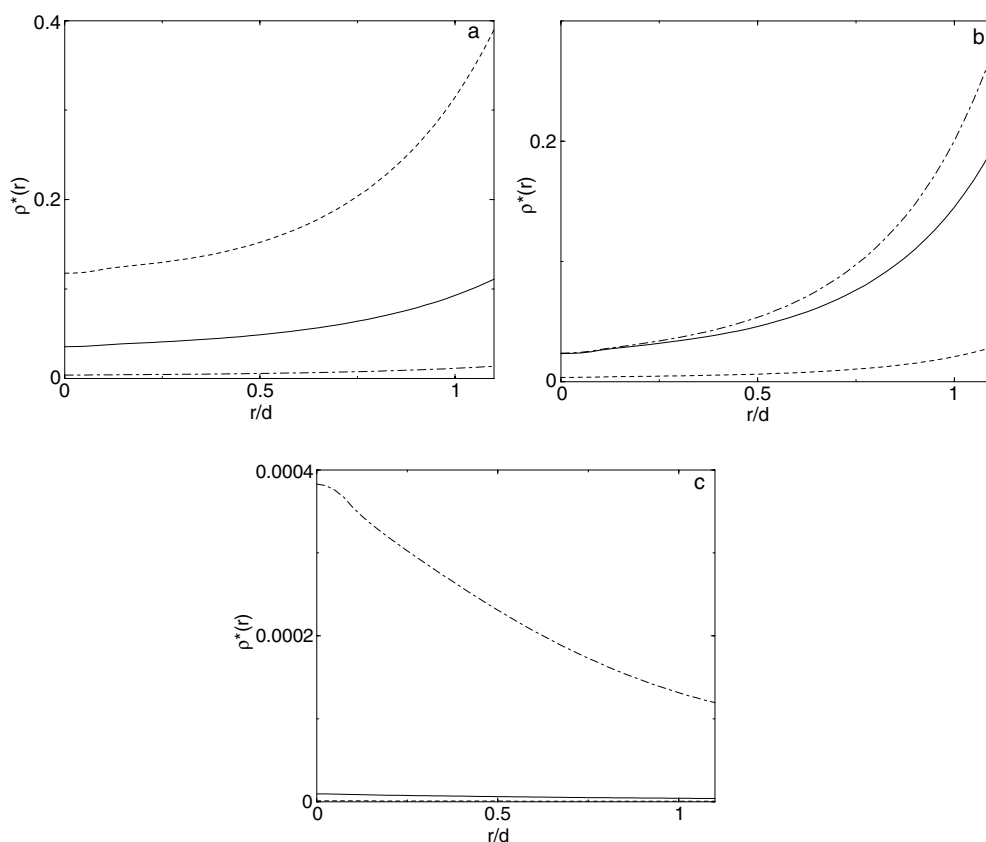
Figure 4. Density profiles of Na$^+$ (part (a)), Ca^{2+} (part (b)), and Cl$^-$ (part (c)) ions in the cylinder as a function of r. The curves have the same meaning as in figure 3. The reduced density is defined in figure 1.

The densities of the ions that are adsorbed into the filter are plotted as functions of r in figure 4. As in the simulations of Boda *et al*, the adsorption of Cl$^-$ ions is small. The Na$^+$ and Ca^{2+} ions tend to prefer to be at the outer edge of the cylinder. This is in contrast to the situation in the simulations, where the cations tended to be near the centre of the cylinder. Presumably, this is due to the smearing of the glutamates over the cylinder wall in this calculation.

5. Concluding remarks

We have adapted DFT to cylindrical geometry and have calculated the selectivity of a model calcium filter. The model used is an infinite cylinder, similar to the model used in the earliest Boda *et al* simulation [5]. The model filter is calcium selective. However, having an explicit model of the glutamate side chains is a better model than the smeared charges used here. Also, this calculation differs from that of [5] because, in this calculation, the ions in the channel are electrostatically isolated from the bulk fluid. This was not the case in the simulation; electrostatic isolation is less realistic. The DFT program could be modified to relax this constraint, as was done in [13] for planar geometry, but the range of integration must then be increased with a consequent increase in computation time.

There is no fundamental reason why ions of differing sizes cannot be used in DFT. However, to do this an analytic result for MSA direct correlation functions for charged hard spheres with different diameters would be required, or at least convenient. We are not aware of such a result in the literature. Because NCE were considering two homogeneous fluids in equilibrium, with no specified interface, they did not require correlation functions and were able to use different dielectric constants for the bathing and filter fluids without any concern about polarization effects at a dielectric interface. Things are not so simple here, since polarization effects would need to be considered. Also, DFT is a theory for identical homogeneous and inhomogeneous fluids that are in equilibrium. In this connection, we note that we have made progress towards incorporating the effects of regions of differing dielectric constant into simulations.

Acknowledgment

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