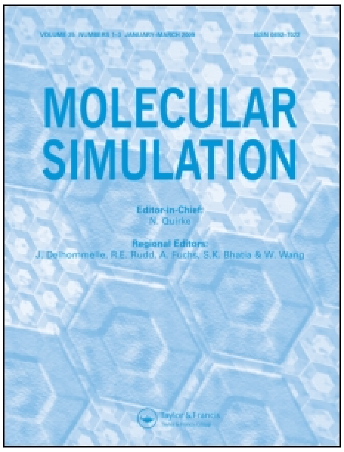

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Estimation of Free Energy Systematic Errors in Molecular Simulations of Globular Proteins Surrounded by Finite Water Clusters. One Center Multipole Expansion of Reaction Field Differences

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ESTIMATION OF FREE ENERGY SYSTEMATIC ERRORS IN MOLECULAR SIMULATIONS OF GLOBULAR PROTEINS SURROUNDED BY FINITE WATER CLUSTERS. ONE CENTER MULTIPOLE EXPANSION OF REACTION FIELD DIFFERENCES

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Free energy calculated in simulations on the atomic level (Monte Carlo or Molecular Dynamics) has a systematic error, if the water shell surrounding a globular protein is finite. The error ("cluster error") is equal to a difference of free energies obtained in simulations with an infinite and finite water shell. In this work a continuum dielectric model was used to estimate the "cluster error". A multipole expansion of the estimate was performed for a water shell with a spherical outer boundary. The expansion has very simple form. Each term is a product of two functions, one of them depending only on the charge's conformation, and the other one only on dielectric properties of the system. There are two practical uses of the expansion. First, it may be used to estimate the "cluster error" in a simulation already made; second, it may be used to plan a simulation in such a way that the "cluster error" is minimal. Numerical values of the largest terms in the multipole expansion corresponding to a typical system in simulations of globular proteins are given.

KEY WORDS: Molecular Dynamics Simulations, Monte Carlo Simulations, Globular Proteins, Reaction Field, Free Energy, Systematic Error

INTRODUCTION

In a computer modelling of globular proteins on the atomic level a simulated system is built up from a protein molecule and molecules representing its polar environment (water, ions, etc.). Simulations where only part of the environment was taken into account were reported in the literature [1]. In cluster simulations, a radius of solvent shell is of the order of 10 Å. On the other hand, the solvent is formally infinite, if periodic boundary conditions [2] are applied. In order to properly simulate a globular protein in its natural environment the modelled system should contain a substantial amount of molecules representing the bulk solvent, apart from tightly bound solvent molecules. Free energy results depend, however, on the size and dielectric properties of the solvent shell included in the simulation. Fortunately, the difference between free energy obtained in simulations with an infinite and a finite water shell (the "cluster error") can be estimated in a framework of a continuum dielectric model (CDM). CDM estimations are widely used in simulations of polar liquids for corrections due to molecules beyond cut-off radius [3–6]. Results obtained in molecular dynamics simulations of charged objects surrounded by finite amount of water molecules [7] support the use of CDM as a tool for estimation of the bulk solvent energy.

Many simulations of globular proteins are aimed on a calculation of free energy differences. In this case it is particularly important to check if "cluster errors" do cancel out if only a finite water shell is included in the simulation. A difference of "cluster errors" cannot be neglected if the longest range interactions differ. This may occur, because of:

— creation/annihilation of electric moments within the protein (by means of the perturbation technique),

— change of positions of electric charges in relation to the protein/solvent boundary and/or to each other.

In the above cases significant differences of water shell polarization may be expected.

The continuum dielectric system studied here corresponds to Monte Carlo or molecular dynamics simulations where a protein environment is represented by a finite and electrically neutral shell with a spherical outer boundary. Systems without bulk water shell, contianing a "dry" protein or a protein surrounded solely by tightly bounded water molecules are not investigated.

DIELECTRIC MODEL

The continuum dielectric system studied here is presented on Figure 1. It consists of (N) electric charges (q_i) distributed within the inner sphere surrounded by a charge free outer shell. The inner sphere is characterized by dielectric constant (ε_1) and radius (a). The outer shell has radius (b) and dielectric constant (ε_2), and it is surrounded by vacuum with $\varepsilon_3 = 1$.

The dielectric system is a model of a molecular dynamics or Monte Carlo system with the following properties:

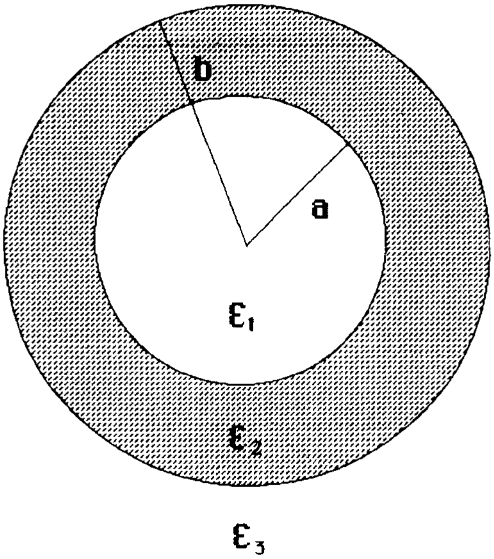

Figure 1 Schematic representation of the dielectric system in considertion: (a) is the radius of the inner dielectric sphere, (b) is the radius of the outer dielectric sphere, ε_1, ε_2, and $\varepsilon_3 = 1$ are dielectric constants of the inner sphere, the outer shell, and vacuum, respectivley. Charges are situated in the inner sphere only.

- a simulated system contains bulk solvent molecules with uniform dielectric properties;
- an outer boundary of the bulk solvent part is a sphere with the radius (b);
- there exists a sphere with the radius (a, $a < b$) outside of which there are only bulk solvent molecules.

Simulations of globular proteins surrounded by a finite, sufficiently large water shell fulfill the above requirements. The radius (a) of the inner sphere should be understood as a radius of a minimal sphere containing the whole protein and bound water molecules. For a protein with an arbitrary shape the inner sphere contains also some part of bulk water molecules. Charges of the inner sphere correspond to all charges included explicitly in the simulation, and dielectric constant (ε_1) is equal to the screening factor used in the simulation for Coulombic interactions. The conformation of charges in the dielectric model corresponds to the mean conformation of the charges in a thermodynamic state analyzed in the simulation. The dielectric constant of the outer shell (ε_2) corresponds to a static dielectric constant characterizing the water model used. The static dielectric constants of water models most widely used in simulations were studied systematically [8–10] by means of Kirkwood theory [11].

The dielectric model presented on Figure 1 can be further simplified for a spherical protein with a uniform static dielectric constant. In this case reorientable dipoles can be excluded from the set of charges (q_i). The charges (q_i) correspond then to the charges of ionized groups and bound ions. The inner sphere can be identified with the protein and the dielectric constant (ε_1) is equal to the static dielectric constant of the protein. Theoretical estimations of static dielectric constants of proteins interiors, based on an analysis of the proteins' reorientable dipoles fluctuations were done, however, for few proteins only [13,14], leading to the values between 2.5 and 4. Moreover, in the simplified dielectric model the inner sphere radius (a) cannot be determined accurately. As reorientable dipole fluctuations in the interfacial region are not uniform, an assumption about its homogeneous properties does not seem justified. For example it was demonstrated for the protein BPTI that the local static dielectric constant of the protein depends strongly on the size of the sample volume used for its determination, and it increases up to 20 in the vicinity of the protein surface [14]. In the presented model the interfacial area can be characterized by a bulk water dielectric constant or by a protein interior dielectric constant.

A CDM estimate of the "cluster error" is denoted below as $\Delta U(b)$ and it is defined as a difference between electrostatic energies calculated for the radius (b) finite ad approaching infinity.

SOLUTION OF DIELECTRIC PROBLEM

Electrostatic Energy in the Presence of Dielectric Boundaries

Electrostatic energy of a system containing (N) electric charges (q_i) in the presence of dielectric boundaries can be generally expressed as a sum of Coulombic (ϕ^0) and reaction field (ϕ^R) terms [15]:

$$U = \frac{1}{2}\sum_{m=1}^{N} \phi^R(m, m) q_m + \frac{1}{2}\sum_{n=1}^{N} \sum_{\substack{m=1 \\ m \neq n}}^{N} \{\phi^R(n,m) + \phi^0(n,m)\} q_m \qquad (1)$$

where:

$\phi^R(n,m)$ — potential of the reaction field produced by the n-th charge in the position of m-th charge,

$\phi^0(n,m)$ — potential produced by the n-th charge in the position of m-th charge in the absence of any dielectric boundaries,

The reaction field potential in equation (1) can be represented (see Appendix) as the series of Legendre polynomials (P_n):

$$\phi^R(k,m) = \sum_{n=0}^{\infty} \frac{q_k R_k^n R_m^n}{4\pi\varepsilon_0\varepsilon_1 a^{2n+1}} (n+1) H_n P_n(\cos\Theta_{mk}) \tag{2}$$

where;

P_n - the Legendre polynomial of the n-th order,

R_k, Θ_{mk}- spherical coordinates of the charge k, the Z-axis is chosen so that m-th charge is laying on it.

ε_0- permittivity of vacuum.

$H_n = H_n(\varepsilon_1, \varepsilon_2, \varepsilon_3, a, b)$ — the coefficient depending only on parameters characterizing the dielectric medium.

The analytical form of H_n has been derived in the Appendix. The formula (2) may be applied also for $m = k$. It is worth to note that if the conformation of charges of the inner sphere remains unchanged, electrostatic energy (U) depends on the outer sphere radius (b) only via H_n coefficients.

Difference Between Electrostatic Energies of a Finite and an Infinite System $\Delta U(b)$

The difference of electrostatic energies of a finite and an infinite system $\Delta U(b) = U(b = \text{infinity}) - U(b)$ is determined by reaction field potential differences ($\Delta\phi^R(k,m) = \phi^R(k,m)_{b=\text{infinity}} - \phi^R(k,m)$):

$$\Delta U(b) = \frac{1}{2} \sum_{m=1}^{N} \sum_{k=1}^{N} \Delta\phi^R(k,m)\, q_k, \tag{3}$$

where the difference $\Delta\phi^R(k,m)$ equals:

$$\Delta\phi^R(k,m) = \sum_{n=0}^{\infty} \frac{(n+1)\, q_k R_k^n R_m^n}{4\pi\varepsilon_0\varepsilon_1 a^{2n+1}} [H_n^{\text{inf}} - H_n]\, P_n(\cos\Theta_{m,k}) \tag{4}$$

H_n^{inf} — is a value of H_n for radius (b) approaching infinity.

Combining (3) with (4) one obtains a new expression for $\Delta U(b)$:

$$\Delta U(b) = \sum_{n=0}^{\infty} X_n T_n \tag{5}$$

where;

X_n— depends only on parameters characterizing the dielectric medium (ε_1, ε_2, ε_3, a, and b) and is equal to:

$$X_n = -\frac{1}{2} \frac{(n+1)(2n+1)^2 \varepsilon_2 (\varepsilon_2 - \varepsilon_3)}{4\pi\varepsilon_0 [n\varepsilon_1 + (n+1)\varepsilon_2]} \times \\ \{[n\varepsilon_1 + (n+1)\varepsilon_2][n\varepsilon_2 + (n+1)\varepsilon_3]\, b^{2n+1} \\ + n(n+1)(\varepsilon_2 - \varepsilon_3)(\varepsilon_1 - \varepsilon_2)\, a^{2n+1}\}^{-1}, \tag{6}$$

T_n — depends only on charges' conformation and equals:

$$T_n = \sum_{m=1}^{N} \left\{ q_m R_m^n \left[\sum_{l=1}^{N} q_l R_l^n P_n (\cos \Theta_{ml}) \right] \right\} \tag{7}$$

Each coefficient T_n is related to the electric n-th multipole moment of the charge distribution. For example, the coefficient T_0 equals Q^2, where Q is a total electric charge of the inner sphere i.e. the 0-th multipole moment, T_1 equals P^2, where P is the length of total dipole moment **P** of the inner sphere.

For the dielectric system in consideration, i.e. for $b > a$, $\varepsilon_2 > \varepsilon_3$, and $\varepsilon_2 > \varepsilon_1$ equation (6) leads to the following properties of X_n:

— coefficients X_n are negative for all n, consequently all multipole contributions to ΔU (b) are negative (or zero);

— coefficient X_n decreases with the increase of solvent shell radius (b) for any n;

— for $b >> a$ X_n is proportional to $b^{-(2n+1)}$ and does not depend on the inner sphere radius (a);

— for a given n (except $n = 0$) X_n decrease monotonically with the increase of the inner sphere radius (a);

— X_n does not depend on ε_1 if $\varepsilon_2 >> \varepsilon_1$;

— the $n = 0$ contribution to the $\Delta U(b)$ (see equation 5 and 6) has a simple analytical form:

$$\Delta U_0 (b) = - \frac{1}{4\pi \varepsilon_0} \frac{\varepsilon_2 - \varepsilon_3}{\varepsilon_2 \varepsilon_3} \frac{Q^2}{2b} \tag{8}$$

and does not depend on the conformation of charges within the inner sphere, but only on its total charge.

It was worth to note that as far as only 0-th order terms are discussed, the "cluster error" estimate depends *neither on the dielectric constant of the inner sphere nor on its size.* For simulations with constant total electric charge $n = 0$ contribution to the "cluster error" does not vary.

A NUMERICAL EXAMPLE

In the following the dielectric system characterized by parameters: $a = 12.5$ or 15 Å, $\varepsilon_1 = 3$, $\varepsilon_2 = 80$, $\varepsilon_1 = 1$, and a variable radius (b), is studied.

The values of X_n T_n (for $n = 0,1,2$) for different radii (b) and an arbitrarily chosen value of $T_n = (10\,\text{Å})^{2n} e^2$ are presented on Figure 2. Coefficients T_n correspond to a unit electric charge situated 10 Å from the cluster center.

The 0-th order term does not vanish for charged systems, even at the radius (b) as large as several hundred Ångstrems. It reaches the value of 1 kT ($T = 300$ K) at the radius (b) close to 270 Å (Figure 2a). This value indicates that the free energy difference obtained for two thermodynamic states differing in total electric charges, has a serious systematic error for all presently possible cluster sizes. Since this contribution can be estimated without any assumptions about the conformation of charges and the inner sphere properties, the results of any given simulation can be easily corrected.

The first order term was calculated assuming $T_1 = (10\,\text{Å})^2 e^2$. The value of T_1 corresponds to the dielectric moment of the inner sphere equal to 10 Åe, or to the

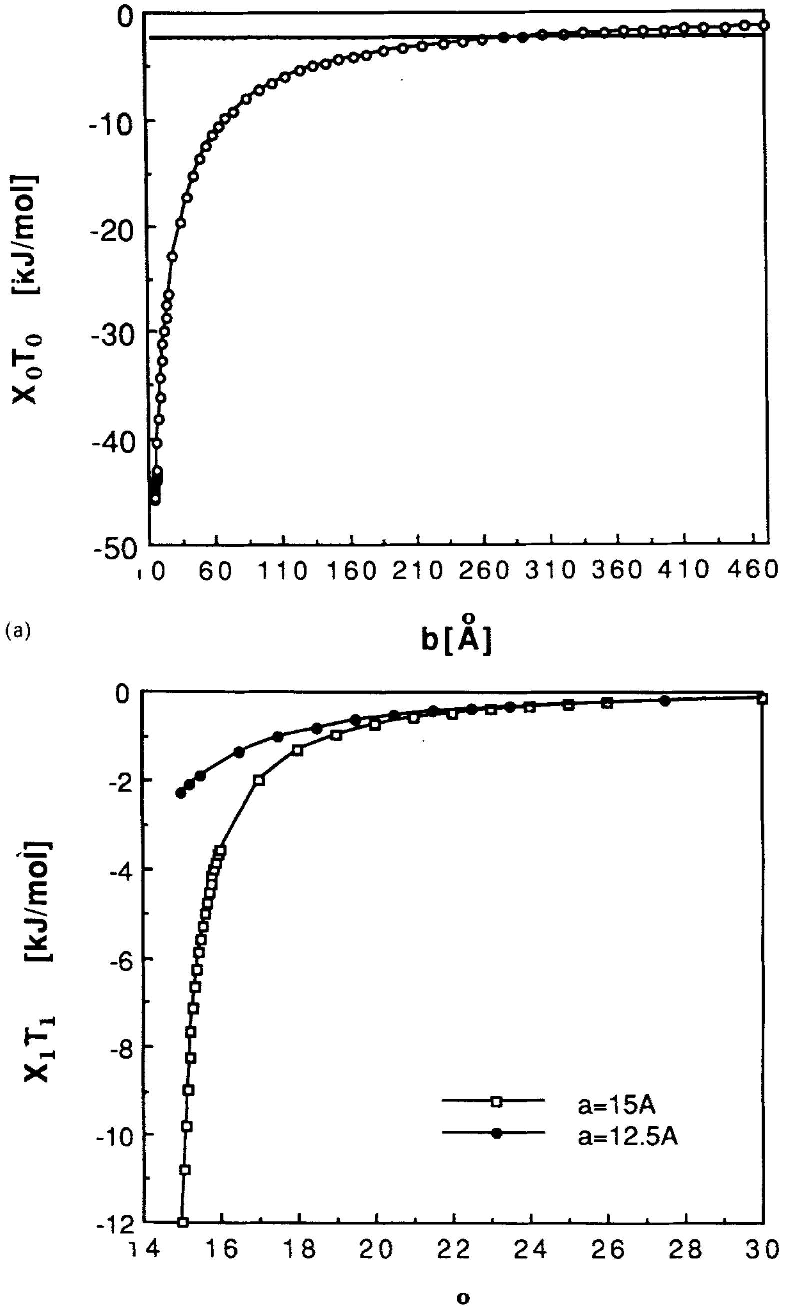

Figure 2 Terms X_n T_n (in kJ/mol]) in the "cluster error" multipole expansion calculated for $T_n = (10\,\text{Å})^{2n} e^2$, $\varepsilon_1 = 3$, $\varepsilon_2 = 80$, $\varepsilon_3 = 1$, and $a = 12.5$ or $a = 15$ Å, as a function of the bulk solvent shell radius (*b*) (in Å): a) $n = 0$, b) $n = 1$, c) $n = 2$. For $n = 0$ the energy level equal to 1 kT ($T = 300$ K) is marked.

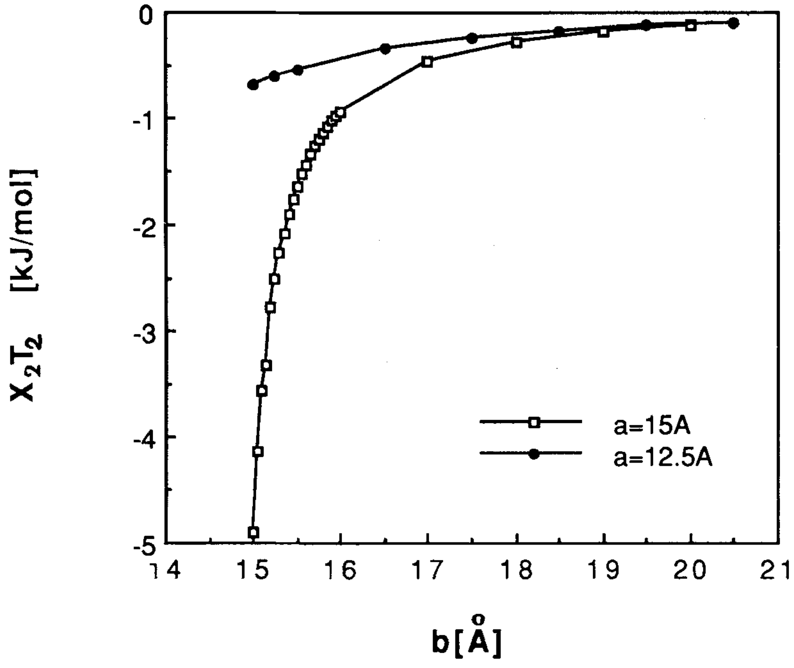

Figure 2 (continued)

difference of dipole moments' squares of two thermodynamic states equal to 100 $(\text{Å}e)^2$. It can be seen on Figure 2b (for $a = 15$ Å) that this term can be responsible for a serious systematic error, greater than 1 kT, it the radius of the solvent shell is smaller than 17 Å. This result indicates that even in simulations of neutral systems (where $n = 0$ term vanishes) the existence of non-vanishing dipoles in the non-bulk solven part of the system may cause serious systematic errors. The above concerns also calculation of the free energy difference between two systems with the same total electric charge.

The second order term calculated assuming $T_2 = (10\,\text{Å})^4e^2$ is less important although it is greater than 1 kT for radius (b) smaller than 15.5 Å (Figure 2c for $a = 15$ Å).

Numerical values of the first and the second order corrections calculated with the same value of T_n but $a = 12.5$ Å are significantly smaller than those obtained for $a = 15$ Å, because in this case the system contains a bigger bulk solvent part.

The value of $\Delta U(b)$ calculated according to equation (5) represents also the CDM estimate of a systematic error of free energy difference in a simulation in which a unit electric charge is created/annihilated 10 Å from the cluster center. The difference between results calculated for $a = 12.5$ Å and for $a = 15$ Å represents the difference between minimal and maximal CDM estimates of Δ U (b) for a simulation in which the interfacial area (the region between protein interior and a bulk solvent) is 2.5 Å thick. Minimal and maximal estimates coverge quickly with the increase of the radius (b). It is clear that the total "cluster error" in such simulation is minimal when the created/annihilated charge is situated in the cluster center. If the cluster center cannot be situated in this point, the multipole expansion may help to chose the optimal cluster geometry.

APPLICATION OF THE DIELECTRIC MODEL TO THE MOLECULAR DYNAMICS OR MONTE CARLO SIMULATIONS

The dielectric model presented here may be used to calculate a free energy systematic error in an earlier made molecular dynamics or Monte Carlo simulation. A calculation of mean value of T_n's of the central (non bulk water) part of the simulated system is then required. The first non-trivial term (T_1) is equal to the square of the inner sphere dipole moment. Practical evaluation of T_n's does not seem to be computer time expensive, because:

- only inner sphere charges contribute to each T_n;
- X_n T_n decreases quickly with the increase of n, and the calculation can be terminated at small n.

It is easy to check the "cluster error" cancellation in a calculation of free energy differences, because it is enough to check T_n's equivalence. In this case the inner sphere radius (a) should be the same in both states. If the perturbation technique is used, the calculation of T_n's is required only for the final and initial states.

When T_n values can be estimated prior to the simulation, the presented CDM can be used to plan the simulation. The cluster center should be positioned in a way that minimizes second non-vanishing T_n (the first one does not depend on the cluster center position). An estimated value of the second non vanishing T_n can be used to estimate the solvent shell radius (b) large enough to neglect X_n T_n term. For example, an estimation of T_n is possible for spherical proteins for which the ionization state of ionizable groups is known. It is possible also for smaller molecules when the mean structure of the first solvatation shell is known.

CONCLUSIONS

The dielectric model presented above leads to analytic formulas applicable in the analyzing or preparing Monte Carlo or molecular dynamics simulation. The multipole expansion of the "cluster error" provides a simple formalism for calculating or estimating systematic errors in simulations made in water clusters.

Numerical examples illustrate that even for neutral systems the "cluster error" may be significant, if higher electric multipole moments do not vanish. This suggests that in practical simulations of free energy differences the "cluster error" cancellation should be checked.

The proposed model includes only electric polarization as the mechanism contributing to the dependence of free energy on bulk solvent size. This mechanism is common for all simulations of polar liquids. There are, however, other long range mechanisms which may lead to this dependence. The most important one is the long range ordering of water molecules. However, an ability to form long range structures depends strongly on water model.

APPENDIX

Calculation of the Electrostatic Potential in the Inner Sphere

The electrostatic potential of the set of charges in a dielectric system is a sum of single charge contributions. The single charge contribution is calculated below. The origin of a coordinates system is chosen in the sphere center and the charge q is situated on the Z axis. Spherical coordinates of the charge are $(s, 0)$ and those of the observation point (R, Θ).

For points with $R > s$ the solution for the potential can be expressed as a combination of Legendre polynomials [16].

$$\begin{aligned}
\phi_1 &= \sum_{n=0}^{\infty} [A_n R^n + B_n R^{-n-1}]\, P_n(\cos\Theta) \quad (R < a) \\
\phi_2 &= \sum_{n=0}^{\infty} [C_n R^n + D_n R^{-n-1}]\, P_n(\cos\Theta) \quad (a < R < b) \qquad \text{(A1)} \\
\phi_3 &= \sum_{n=0}^{\infty} [E_n R^n + F_n R^{-n-1}]\, P_n(\cos\Theta) \quad (R > b)
\end{aligned}$$

On the boundary between different dielectrics the potential and the normal component of the electric field vector are continuous. The above conditions together with the requirement the electrostatic potential should vanish in infinity are summarized in equations (A2):

$$\begin{aligned}
\phi_1(R = a) &= \phi_2(R = a) \\
\phi_2(R = b) &= \phi_3(R = b) \\
\varepsilon_1 \frac{\partial}{\partial R}\phi_1(R = a) &= \varepsilon_2 \frac{\partial}{\partial R}\phi_2(R = a) \qquad \text{(A2)} \\
\varepsilon_2 \frac{\partial}{\partial R}\phi_2(R = b) &= \varepsilon_3 \frac{\partial}{\partial R}\phi_3(R = b) \\
\phi_3 \to 0 &\text{ for } R \to \infty
\end{aligned}$$

The condition that $\phi_3 \to 0$ for $R \to \infty$ implies that $F_n = 0$ for all n. Due to the orthogonality of Legendre polynomials first four equations in (A2) can be rewritten as a set of linear equations for five variables $(A_n, B_n, C_n, D_n, E_n)$:

$$\begin{vmatrix}
a^{2n+1} & -a^{2n+1} & -1 & 0 \\
0 & b^{2n+1} & 1 & -1 \\
n\varepsilon_1 a^{2n+1} & -n\varepsilon_2 a^{2n+1} & \varepsilon_2(n+1) & 0 \\
0 & n\varepsilon_2 b^{2n+1} & -\varepsilon_2(n+1) & \varepsilon_3(n+1)
\end{vmatrix} \times \begin{vmatrix} A_n \\ C_n \\ D_n \\ E_n \end{vmatrix} = \begin{vmatrix} -B_n \\ 0 \\ (n+1)\varepsilon_1 B_n \\ 0 \end{vmatrix}$$

The above equations lead to the following relation between A_n and B_n.

$$A_n = B_n \frac{n+1}{a^{2n+1}} H_n \qquad \text{(A3)}$$

where:

$$H_n = \frac{b^{2n+1}[n\varepsilon_2 + (n+1)\varepsilon_3](\varepsilon_1 - \varepsilon_2) - a^{2n+1}[\mathrm{n}\varepsilon_2 + (n+1)\varepsilon_1](\varepsilon_3 - \varepsilon_2)}{b^{2n+1}[n\varepsilon_2 + (n+1)\varepsilon_3][n\varepsilon_1 + (n+1)\varepsilon_2] + a^{2n+1} n(n+1)(\varepsilon_2 - \varepsilon_3)(\varepsilon_1 - \varepsilon_2)}$$

Terms $B_n R^{-(n+1)} P_n$ in ϕ_1 (equation A1) can be identified with contributions to the electrostatic potential produced by the point charge q, in a medium with dielectric constant (ε_1), for $a > R > s$ [16].

$$B_n = \frac{qs^n}{4\pi\varepsilon_0\,\varepsilon_1} \tag{A4}$$

In the equation (A1) terms with A_n contribute to the reaction field within the inner sphere. The equation (A3) is then used to calculate the reaction field potential. Solution for A_n was obtained also in [12] and the results agree with the ones presented above.

For $b \to \infty$ (the outer shell is infinite) A_n equals:

$$A_n = B_n \frac{n+1}{a^{2n+1}} \frac{\varepsilon_1 - \varepsilon_2}{n\varepsilon_1 + (n+1)\varepsilon_2} \tag{A5}$$

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