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Nonlinear dielectric response of dilute protein solutions

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A theory for the nonlinear dielectric response of dilute protein solutions is presented. The field-dependent dielectric function of the protein solution changes linearly with the electric field squared in the lowest order. The slope of this dependence is expressed in terms of the protein dipole moment M_0, its volume fraction in solution η_0, and the second osmotic virial coefficient. For practical conditions, the nonlinear dielectric response scales as ${\eta_0}^3{M_0}^8$. This strong dependence on the protein dipole moment and concentration establishes a sharp contrast between the nonlinear response of solvated proteins relative to the surrounding polar solvent. Nonlinear dielectric response can serve as a sensitive tool for monitoring protein conformations and physiological activity.

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1 Introduction

Consider a particle carrying the dipole moment $\mathbf{M}_0$ immersed in a polar liquid with the dielectric constant ε_s (subscript "0" stands for solute properties). When an external (Maxwell[1]) field E is applied to the solution along the laboratory axis z, the dipole moment orients along the field to allow an average dipole projection on the field $\langle M_{0z}\rangle_E$, where $\langle\ldots\rangle_E$ specifies a statistical ensemble average in the presence of the field. The Maxwell field $E = \phi/d$, given as the ratio of the voltage ϕ at a plane capacitor to the distance d between the plates, is related to the vacuum field of external charges E_{vac} trough the static dielectric constant of the solution ε_{sol} as $E_{vac} = \varepsilon_{sol}E \simeq \varepsilon_s E$.

If the response of the dipole is linear in the applied field, the standard perturbation theory yields the following result[2–4]

$$\langle M_{0z}\rangle_E = \frac{\varepsilon_s}{3}\chi_c\beta{M_0}^2E. \quad (1)$$

In this equation, χ_c is the cavity-field susceptibility[1,2,5] that connects the uniform field of external charges E_{vac} to the local field acting on the solute dipole, M_0 is the dipole moment magnitude, and $\beta = (k_BT)^{-1}$ is the inverse temperature.

Proteins typically carry large dipole moments of the order of several hundreds of Debye units due to asymmetric distributions of charged residues exposed to water and charges of the N- and C-termini.[6–8] The value of the cavity-field susceptibility was estimated as $\chi_c \simeq 1.1$–1.2 for proteins.[9,10] With these numbers, one arrives at

$$\langle M_{0z}\rangle_E/M_0 \simeq 0.23E\ (\mathrm{kV^{-1}\ cm^{-1}}), \quad (2)$$

when E is expressed in kV cm^{-1} and water at room temperature is considered as the solvent. This estimate implies that the dipole moment aligned along the field becomes a substantial portion of the dipole moment magnitude at fields exceeding $\simeq 1$ kV cm^{-1}. Such fields are available in many experimental setups, raising the question of significance of nonlinear dielectric susceptibilities extending beyond the linear term in eqn (1). This study offers an analytical theory of the nonlinear dielectric effect (NDE) of dilute protein solutions limited to the lowest-order nonlinear term quadratic in the applied field E.

Measuring NDE in bulk polar liquids requires much stronger electric fields, ~100 kV cm^{-1}.[11] The gap in field magnitudes between bulk liquids and protein solutions suggests that NDE can be used to probe the protein component separately from a much weaker nonlinear response of the surrounding solvent. Given that the dipole moment is sensitive to protein's conformations and physiological activity,[12–16] NDE can potentially monitor alterations in the protein structure, phosphorylation, and redox reactions. Despite some preliminary reports on cells[17,18] and membrane-bound proteins,[19] NDE of proteins in solution has not been measured and the formalism proposed here remains a theoretical prediction at this moment.

The NDE is quantified by the dielectric function of the solution $\varepsilon_{sol}(E)$ depending on the applied electric field, in contrast to the linear dielectric constant (a material property) ε_{sol} independent of the field. The difference $\varepsilon_{sol}(E) - \varepsilon_{sol}$ is linear in E^2 in the lowest order. The proportionality constant a is the Piekara coefficient[11,20–22]

$$\Delta\varepsilon(E) = \varepsilon_{sol}(E) - \varepsilon_{sol} = aE^2. \quad (3)$$

The theory presented here calculates a for low-concentration protein solutions when interaction between individual protein molecules are sufficiently weak to be viewed as perturbations

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(see below). It shows that $\Delta\varepsilon(E)$ changes its scaling from $\propto c_p M_0^4$ at low concentrations to $\propto c_p^3 M_0^8$ at larger protein concentrations c_p (in g L^{-1}). The slope of the dielectric constant *vs.* c_p at $c_p \to 0$ provides access to the ratio, M_0^4/M_p, of the fourth power of the protein dipole moment M_0 and the protein molar mass M_p.

A strong dependence of the protein NDE on the protein dipole moment provides high contrast of solvated proteins relative to a much weaker background signal from the solvent. Linear dielectric spectroscopy of solutions also allows[6] access to M_0^2 from the slope of the solution dielectric increment $\varepsilon_{sol} - \varepsilon_s$ *vs.* the protein concentration (Oncley's formula[23]). A much stronger scaling $\Delta\varepsilon(E) \propto c_p^3 M_0^8$ compared to $\varepsilon_{sol} - \varepsilon_s \propto c_p M_0^2$ grants a much higher sensitivity of the NDE to the presence of proteins in solution compared to linear dielectric spectra.

2 Model

A general formulation of the problem of nonlinear dielectric polarization[24] represents the Piekara coefficient in eqn (3) in terms of the parameter describing non-Gaussian fluctuations of the dipole moment projection M_{0z}

$$a \propto N\left[1 - \frac{\langle M_{0z}^4\rangle}{3\langle M_{0z}^2\rangle^2}\right]. \tag{4}$$

Here, en ensemble average $\langle\ldots\rangle$ is taken over the sample configurations in the absence of the applied field and $\langle M_{0z}\rangle = 0$ is assumed.

The term in the brackets in eqn (4) describes non-Gaussian statistics of the dipole moment projection. It vanishes for a macroscopic sample with a large number of dipoles N as stipulated by the central limit theorem. This is avoided by multiplying the bracket term with N thus resulting in a finite value of the Piekara coefficient a.

Non-Gaussian statistics of the dipole moment can arise from both internal protein motions, such as conformational transitions, and from correlated rotations of the protein dipoles in solution. The present formulation considers only the latter mechanism, leaving the possibility of intrinsic conformations and field-induced opening of membrane-bound protein pumps[18,25,26] as a source of non-Gaussian statistics to future studies. Nevertheless, conformational transitions altering the protein dipole moment should project to an altering NDE.

Assuming that proteins behave as rigid dipoles, the fourth-order statistical central moment in eqn (4) introduces dipolar correlations of up to the fourth order. Some of these correlations decouple, allowing one to cast the Piekara coefficient in terms of the second, third, and fourth-order correlations of the protein dipoles[24,27]

$$a = \frac{\pi\beta^3 M_0^4 \rho_0}{10}\left[H^{(2)} + H^{(3,4)}\right]. \tag{5}$$

Here, $\rho_0 = N_0/V$ is the number density of N_0 solutes in the solution volume V and the two correlation terms, $H^{(2)}$ and $H^{(3,4)}$, describe binary and higher-order (three- and four-particle) dipolar correlations, respectively. The term $H^{(3,4)}$ vanishes at low concentrations and only binary correlations survive. We will therefore drop $H^{(3,4)}$ in eqn (5) and focus solely on the binary term[24,27]

$$H^{(2)} = 6(g_K - 1) + \frac{5\chi_T}{2\chi_T^{id}} - 1. \tag{6}$$

In this equation, g_K is the Kirkwood factor of protein dipoles describing short-ranged binary orientational correlations and defined by the following expression

$$g_K = 1 + \sum_{j>1}\langle \hat{\mathbf{e}}_1 \cdot \hat{\mathbf{e}}_j\rangle. \tag{7}$$

Here, $\hat{\mathbf{e}}_j = \mathbf{M}_{0j}/M_0$ is the unit vector specifying the orientation of jth protein dipole. Further, χ_T in eqn (6) is the isothermal osmotic compressibility[28–31] scaled with the ideal-gas compressibility $\chi_T^{id} = (\rho_0 k_B T)^{-1}$. The ratio of two compressibilities in eqn (6) can be expressed in terms of the $k = 0$ value of the density–density structure factor $S_{00}(k)$ of proteins in solution. It represents thermal fluctuations of the local protein density

$$\frac{\chi_T}{\chi_T^{id}} = S_{00}(k=0) = V\frac{\left\langle(\delta\rho_0)^2\right\rangle}{\rho_0}. \tag{8}$$

When the density of dipoles is low, the Kirkwood factor g_K in eqn (6) can be calculated as a series expansion in the dimensionless density of protein dipoles[2,3] commonly appearing in dielectric theories

$$y = \frac{4\pi}{9}\beta\rho_0 M_0^2 = y_0\eta_0, \tag{9}$$

where $\eta_0 = N_0\Omega_0/V$ is the volume fraction (packing fraction[32]) of proteins in solution and $y_0 = (4\pi/9\Omega_0)\beta M_0^2$ is the effective dipolar strength defined for a single protein molecule with the volume Ω_0. Gaussian electrostatic units are used here and one gets $y_0 = \beta M_0^2/(9\varepsilon_0\Omega_0)$ in SI units, where ε_0 is the vacuum permittivity. Similarly, the transformation to SI units in eqn (5) is achieved by the replacement $M_0^2 \to M_0^2/(4\pi\varepsilon_0)$.

The lowest-order perturbation expansion of g_K in terms of $y_0\eta_0$ reads[33]

$$g_K = 1 + \frac{17}{16}(y_0\eta_0)^2 + \ldots. \tag{10}$$

From eqn (9) and (10), one obtains for the Piekara coefficient

$$a = \frac{9\beta^2 M_0^2}{40} y_0\eta_0\left[\frac{51}{8}(y_0\eta_0)^2 + \frac{5}{2}S_{00}(\eta_0) - 1\right], \tag{11}$$

where we have explicitly indicated the dependence of $S_{00}(\eta_0) = S_{00}(\eta_0, k = 0)$ on the protein density.

The ideal-gas limit for $S_{00}(\eta_0)$ is the Poisson fluctuations of the protein density leading to $S_{00} = 1$ at $\eta_0 \to 0$ in eqn (8). This limit does not, however, apply to charged proteins in electrolyte solutions: the ideal-gas limit is not reached even when there are no interactions between the protein molecules.[34] The reason is that electroneutrality condition imposes a constrain on the protein density fluctuations, which become coupled to corresponding density fluctuations of the electrolyte. Following

Stockmayer[34] and Asthagiri *et al.*[35] one can calculate S_{00} from the derivative of the protein density over the protein chemical potential μ_0

$$S_{00} = \left(\frac{\partial \ln \rho_0}{\partial \beta \mu_0}\right)_{T,p,\rho_1}, \quad (12)$$

where the derivative is taken at the constant particle density ρ_1 of the electrolyte ions. Assuming that the protein carries the charge z_0 and is placed in the 1 : 1 electrolyte (*e.g.*, NaCl), one can write the equations for the chemical potentials of the protein and electrolyte ions, which are coupled to each other through the electroneutrality condition. Finding the derivative in eqn (12) becomes a matrix inversion problem.[34] Assuming ideal electrolyte on non-interacting ions, one arrives at

$$S_{00} = \left[1 + \frac{{z_0}^2\rho_0}{2\rho_1 + z_0\rho_0} + 2B_{00}\rho_0\right]^{-1}, \quad (13)$$

where B_{00} is the second osmotic virial coefficient. The Donnan term[34,35] ${z_0}^2/(2\rho_1 + z_0\rho_0)$ does not allow reaching the ideal-gas limit for S_{00} even with no inter-protein interactions, $B_{00} \to 0$. The physiological concentration of electrolyte, ~0.1 M, substantially exceeds the protein concentration, ~1 mM, at typical experimental conditions an one can re-write eqn (13) as[28,30,31]

$$S_{00} = [1 + 2B_0\eta_0]^{-1}, \quad (14)$$

where

$$B_0 = 4B_{00}/B_{00}^{HS} + {z_0}^2/(\rho_1 B_{00}^{HS}). \quad (15)$$

The first term in this equation is the osmotic coefficient reduced with its hard-sphere (HS) value $B_{00}^{HS} = 4\Omega_0$.[36–39]

One finally arrives at the following equation for the Piekara coefficient

$$a = \frac{9\beta^2{M_0}^2}{40} y_0\eta_0\left[\frac{51}{8}(y_0\eta_0)^2 + \frac{3 - 4B_0\eta_0}{2(1 + 2B_0\eta_0)}\right]. \quad (16)$$

Below, this equation is applied to known parameters of proteins in solution to establish the relative significance of two terms in the brackets and the anticipated scaling of the protein NDE with the protein dipole moment and the solution concentration.

3 Discussion and model calculations

A positive NDE ($a > 0$) is found here for a dilute protein solution. In contrast, the NDE is typically negative ($a < 0$) for bulk polar liquids, thus leading to a dielectric decrement in the applied field.[11] While a negative NDE is often related to dielectric saturation through the Langevin equation,[2] an exact theoretical formalism[27] leading to eqn (5) assigns negative NDE to multi-dipolar correlations responsible for a negative $H^{(3,4)}$ in eqn (5), which exceeds in magnitude the typically positive binary term $H^{(2)}$. The binary term can in principle be negative for a sufficiently large positive second virial osmotic coefficient (B_0 in eqn (16)), which can lead to rather complex concentration dependencies for the NDE of binary mixtures of polar and nonpolar liquids.[22]

For typically large protein dipole moments, the polar term, 6 ($g_K - 1$), dominates in eqn (5) and (6) thus leading to a positive NDE. This result is generally consistent with an increment of dielectric constant of protein solutions over that of the solvent,[6] also arising from a large protein dipole moment. The strong polarity of proteins in solution is allowed by reorientations of the protein dipole to align along the applied field. When the protein is immobilized, its internal dielectric constant is low,[41] $\varepsilon_p \simeq 4$, because the internal dipoles are restricted, by the protein fold, from aligning along the field.

The present theory is not limited to protein solutions and can be applied to test the widely accepted dielectric saturation paradigm[2] for the NDE. The Langevin equation used to describe dipole's saturation predicts a linear scaling,[2,24] $-a \propto \beta^3{M_0}^4\rho_0 \propto \rho_0$, of a negative Piekara coefficient with the solute concentration. The derivation of the Langevin equation is performed for a single dipoles and specific assumptions need to be imposed when the theory is extended to an ensemble of dipoles. While those are often omitted, it is implicitly assumed that dipoles are placed on a rigid lattice with a low compressibility ($\chi_T \ll \chi_T^{id}$) and they do not interact ($g_K = 1$).[2] Eqn (5) then reduces to a result, $a = -\pi\beta^3{M_0}^4\rho_0/10$, very close to the result of the Langevin model, which additionally requires adopting a specific form for the cavity-field susceptibility χ_c (see eqn (1)).[24]

Saturation prescribed by the Langevin framework can be distinguished from correlations advocated here by measuring NDE of dilute solutions of dipolar particles (dipolar molecules, proteins, or ferroelectric nanoparticles[42]) in less polar or nonpolar solvents. A positive Piekara coefficient in the present formulation scales linearly with the concentration in the infinite dilution limit, $a = 3\pi\beta^3{M_0}^4\rho_0/20$, but becomes proportional to the third power of the concentration, $a \propto {\rho_0}^3$, when binary dipolar correlations start dominating over the compressibility term in the brackets of eqn (16). The distinction in the sign and in the concentration scaling should allow one to discriminate between saturation and binary correlations when dielectric measurement are performed at sufficiently low frequencies below the frequency of solute tumbling.

The positive Donnan term in eqn (15) can be neglected at sufficiently large electrolyte concentrations and pH close to the isoelectric point. The second osmotic coefficient becomes negative at high pH and high electrolyte concentrations.[30,31] A negative B_{00} is a good predictor of protein crystallization[36,43] or of the liquid–liquid phase separation.[38,39] For a negative value of B_0 in eqn (15), the truncation of the osmotic virial expansion produces a divergence in the structure factor S_{00} in eqn (14) at $1 + 2B_0\eta_0 \to 0$. Such a singularity, reached at the critical point or at the spinodal line, might signal the onset of the liquid–liquid phase separation of the protein solution[32,38,39] or arise from the failure of the truncated expansion for the osmotic pressure. The range of protein concentrations is chosen to ensure $1 + 2B_0\eta_0 > 0$ in the present calculations.

It is clear that the second term in the brackets in eqn (16) dominates over the first term at $\eta_0 \to 0$. Given that B_{00} is of the order of B_{00}^{HS},[36,44] and $\eta_0 < 0.2$ at typical protein concentrations <20 g L^{-1},[37,39] the first term gains in importance at

Table 1 Calculation parameters for proteins at 300 K in 0.1 M electrolyte

Protein[a]	z_0	Ω_0, nm^3	M_0, D	y_0	$B_0/4$
Lys[b]	8	24	208	61	29
Lys[c]					−0.87
BSA[d]	−8	137	384	36	4.5[e]

[a] Lys = lysozyme, BSA = bovine serum albumin. [b] Second virial coefficient at pH = 7 and c_1 = 7 mM is taken from ref. 35. [c] Data at pH = 6 and c_1 = 0.1 M from ref. 30. [d] Data taken at pH = 7 and c_1 = 15 mM from ref. 40 where corrections for the Donnan term were implemented. [e] Values $\simeq -2.4$ at c_1 = 7 mM were reported in the presence of trivalent salts.[38,39]

$\eta_0^* > 2/(\sqrt{17}\,y_0)$. With the typical values of y_0 for proteins (Table 1), this condition puts η_0^* within the range of protein concentrations studied by light scattering[35,38–40] and dielectric spectroscopy[45,46] of protein solutions. One can, therefore, anticipate a crossover from the linear scaling, $a \propto M_0{}^4 c_p$, to a cubic dependence $a \propto M_0{}^8 c_p{}^3$. However, by virtue of being multiplied with η_0 in the Piekara coefficient in eqn (16), the low-concentration range is not prominent in the overall dependence $a(c_p)$.

Fig. 1 shows $a(c_p)/|a_w|$ normalized with the Piekara coefficient for bulk water at 293 K:[47] $a_w = -0.8 \times 10^{-15}$ m^2 V^{-2}. The calculations are done for lysozyme (Lys, second line in Table 1) and bovine serum albumin (BSA) proteins (Table 1). The full calculation according to eqn (16) (solid lines) is compared to the results with the second term in the brackets, containing the second osmotic coefficient, dropped (dashed lines). At concentrations $c_p > 10$ g L^{-1}, one can neglect the virial coefficient component and approximate the Piekara coefficient by the dipolar term

$$a \simeq 0.93 \times 10^{-18} M_0{}^2 (y_0 \eta_0)^3 \left(\frac{\mathrm{m}}{\mathrm{V}}\right)^2, \qquad (17)$$

where the numerical coefficient is evaluated at $T = 300$ K and M_0 is in Debye units (y_0 is unitless, see Table 1). It is clear from the plot that the Piekara coefficient of protein solutions exceeds that of bulk water by about two orders of magnitude in the concentration range shown in the plot.

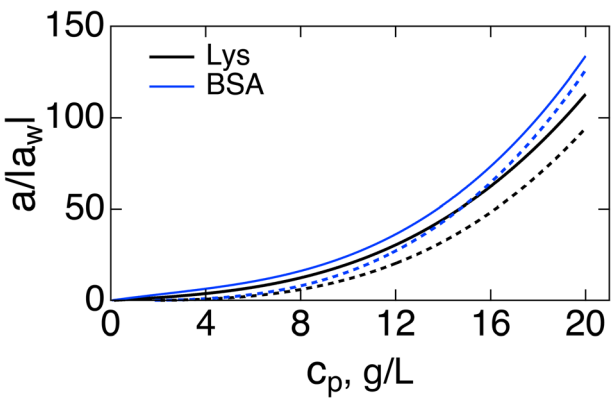

Fig. 1 Reduced Piekara coefficient $a/|a_w|$ ($a_w = -0.8 \times 10^{-15}$ m^2 V^{-2} is the Piekara coefficient for bulk water) *vs.* the protein concentration c_p (g L^{-1}) for Lys (black, second line in Table 1) and BSA (blue). The solid lines indicate calculations based on eqn (16) and dashed lines refer to calculations with the second term in the brackets (involving the second virial coefficient) dropped.

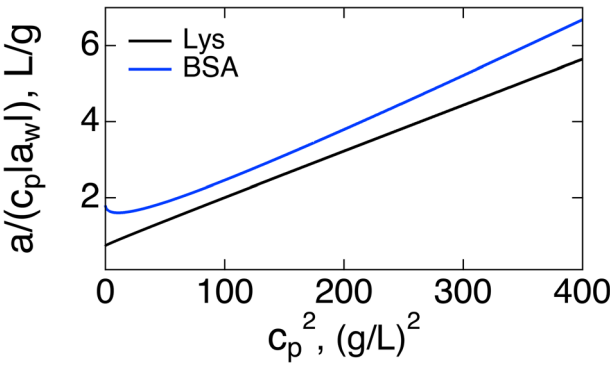

Fig. 2 $a/(|a_w|c_p)$ *vs.* $c_p{}^2$ for Lys (black, second line in Table 1) and BSA (blue).

If the purpose of measuring the Piekara coefficient is to gain access to the protein dipole moment, a better strategy might be to plot a/c_p *vs.* $c_p{}^2$. Both the slope and intercept should provide access to M_0: the intercept becomes $(3\pi/20)\beta^2 M_0{}^4 N_A/M_p$, where M_p is the protein molar mass and N_A is the Avogadro number. Extrapolation from high concentrations can be of limited value because of the curvature of the plot at $c_p \to 0$, as is seen in Fig. 2 for BSA. The slope $(17/5)\beta^5 M_0{}^8 (\pi N_A/(3M_p))^3$, provides a more robust access to M_0. A strong temperature dependence of the slope, $\propto T^{-5}$, can be used to test theory predictions.

From a general perspective, the Piekara coefficient quantifies the non-linear dielectric response and non-Gaussian statistics of the sample dipole moment[24] (eqn (4)). In bulk dipolar materials, the NDE arises from rotations of individual non-interacting dipoles (Langevin model) or from mutual correlations of dipoles (the present description). Non-Gaussian statistics of the dipole moment can also arise from intrinsic conformational transitions of the protein. Intrinsically disordered proteins or disordered domains of folded proteins[48] can potentially be good candidates for observing the NDE. The field required to observe protein NDE, $\simeq$1–10 kV cm^{-1}, is comparable to the field strength of protein capture on nanopores by another nonlinear dielectric effect, the protein dielectrophoresis.[49,50] Extending the theory to the response of entire cells[18] requires modeling the field-induced changes of the membrane-bound protein pumps.[26]

4 Conclusions

An analytical theory for the nonlinear dielectric response of protein solutions developed here shows high contrast between the nonlinear response of proteins in solution and surrounding water. The contrast arises from a strong scaling, $\propto c_p{}^3 M_0{}^8$, of the nonlinear response with the large protein dipole. The Piekara coefficient of proteins in solution exceeds that of surrounding water by two orders of magnitude at the typical protein concentrations used in light-scattering and dielectric measurements.

Conflicts of interest

There are no conflicts of interest to declare.

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