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COMMENTS

Comments on "Anomalous Dielectric Relaxation of Aqueous Protein Solutions" by Nilashis Nandi and Biman Bagchi (*J. Phys. Chem. A* 1998, *102,* 8217)

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In a Letter entitled "Anomalous Dielectric Relaxation of Aqueous Protein Solutions" Nandi and Bagchi (denoted as NB) claim to present the "first unified, microscopic theory of the dielectric relaxation spectra of an aqueous protein solution".[1] However, several of their conclusions are inconsistent with the standard viewpoint found both in the experimental literature[2,3] and in recent molecular dynamics simulation studies.[4,5] The completely negligible contribution of the biomolecule to the frequency dependent dielectric constant (DC) $\epsilon(\omega)$ of the solution, often referred to as α-relaxation by experimentalists,[2] is particularly striking. In an effort to resolve these contradictions, we analyzed NB's approach and found the following severe shortcomings in the underlying theory.

In the following we restrict ourselves to the static case $\epsilon_0 = \epsilon(\omega = 0)$, which suffices to demonstrate the general problem. The connection between the static DC of a system ϵ_0 and its (fluctuations of the) mean square dipole moment $\langle \vec{M}^2 \rangle$ is always of the general form[6−8]

$$Q(\epsilon_0) = \frac{\langle \vec{M}^2 \rangle}{V k_B T} \tag{1}$$

where $\vec{M}$ is the total dipole moment of the system, V is its volume, k_B is the Boltzmann constant, and T is the temperature. $Q(\epsilon_0)$ is a rational function of no more than second degree; its form depends on the boundary conditions used. For example, for an isolated sphere in vacuum, $Q(\epsilon_0) = 3(\epsilon_0 - 1)/4\pi(\epsilon_0 + 2)$ (this is the case considered in ref 1).[7] Other widely used cases are a spherical region embedded in an infinite dielectric ($Q(\epsilon_0) = (\epsilon_0 - 1)(2\epsilon_0 + 1)/4\pi\epsilon_0$)[7] and the so-called tinfoil boundary conditions, which are applicable in computer simulations when Ewald summation is employed ($Q(\epsilon_0) = (\epsilon_0 - 1)/4\pi$).[9] When comparing eq 1 with the starting equation of NB (eq 2.1, with $\omega = 0$), one notices an additional scaling by the number of particles $1/N$. However, this factor of $1/N$ is nowhere to be found in the complete body of literature over the last half century, and it is physically meaningless since it would render $Q(\epsilon_0)$ (and, hence, ϵ_0) dependent on the system size. Thus, the incorrect eq 2.1 is the most fundamental problem of ref 1.

Inspection of NB's eq 2.3 reveals another deviation from the established theory. It clearly shows that the authors operate with a modified Kirkwood g-factor $g_{K,NB}$ defined by the relationship

$$N^2 \mu^2 g_{K,NB} = \langle \vec{M}^2 \rangle \tag{2}$$

(where μ is the dipole moment of a single molecule), as opposed to the correct expression[8−10]

$$N \mu^2 g_K = \langle \vec{M}^2 \rangle \tag{3}$$

A possible explanation for this wrong N^2 dependence in $g_{K,NB}$ may be a confusion of the Kirkwood g-factor with spatial pair correlation functions. For the latter, an N^2 weighting (and, hence, an ansatz like eq 2.3) would indeed be correct. However, g_K is not a pair property, but a measure for the orientational correlation between a reference dipole with its complete environment (i.e., all other $N - 1 \approx N$ dipoles in the system) and, thus, implicitly already contains a factor of N. Consequently, the connection between g_K and $\langle \vec{M}^2 \rangle$ is given by eq 3, and *not* by eq 2; for the details, see, e.g., refs 8 and 10. Before we turn to the consequences that eq 2 (rather than eq 3) has for the interpretation of the frequency dependent DC of protein solutions, we mention two additonal inconsistencies that follow from it. First, eq 2 necessitates the incorrect eq 2.1 of ref 1 to recover the correct DC for a pure liquid (NB mention this themselves to demonstrate the soundness of their decomposition). Second, NB utilize 2.8 for the g_K of water (see Table 1 of ref 1), a value that was certainly obtained with the correct definition eq 3.

The twofold incorrect dependence on N used by NB (their eq 2.1 and eq 2) fortuitously cancels for a neat system, such as pure water. For mixtures, however, it leads to incorrect conclusions. Instead of a cancellation, one obtains (as in eq 2.3 of NB) ratios of the form $N_i N_j / N$, where N_i and N_j denote the number of molecules of the respective component, such as protein (p), hydration water (h), and bulk water (w). Thus, any contribution from the protein is suppressed since $N_p \ll N_w$ whereas the components involving bulk water N_w, which has by far the largest number of particles, dominate. This is best seen by the results of NB listed in Table 3 of the Supporting Information, where only the bulk−bulk (w−w) self-contribution and the hydration water−bulk (h−w) cross term are relevant. Thus, it becomes also clear why the results of NB for *protein solutions* depend so crucially on the value of the dipole moment of water (as they point out themselves). Since any contributions involving the protein (p) are negligible, their role has to be taken over by components of higher weight, in particular the h−w cross term, which, in consequence, leads to the surprising quantitative importance attributed to it. Further, for the frequency dependent properties, this makes it necessary to assume extremely long relaxation times (30−50 ns, i.e., values characteristic for rotational tumbling of proteins) for the exchange between hydration water and bulk water. Such time scales, at least to us, are physically not intuitive. Finally, we note that the linear concentration dependence demonstrated by Figure 2 of NB does by no means require the problematic eq 2.3; it also follows from the standard approach of describing dielectric

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properties of solutions,[11−13] where the weight of each component i is proportional to its concentration $c_i = N_i/V$.

To sum up, the alleged rationalization of dielectric spectra of protein solutions provided by NB relies on a wrong starting equation (eq 2.1; see, e.g., refs 6−8) combined with an incorrect definition of the Kirkwood *g*-factor (eq 2; see, e.g., refs 8−10). Furthermore, it deviates from the established theoretical treatment of the dielectric properties of mixtures, which is immediately applicable to protein solutions as well.[11−13] In principle, an analytical approach as presented by NB would be highly welcome and useful since it could aid considerably in the interpretation of experimental dielectric spectra. However, because of its severe theoretical weaknesses, any conclusions of ref 1 seem to be doubtful. The excellent agreement with experimental data reported by the authors does not change this verdict.

References and Notes

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