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Notes

Chain Dynamics in Poly(L-glutamic acid) Aqueous Solutions As Observed by Means of Frequency Domain Dielectric Spectroscopy

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Introduction

Dielectric spectroscopy in the radiowave frequency range has already proven to be a very useful tool of continuing importance for studying the electrical properties and the dynamics of a large variety of biological macromolecules,[1] and at present this technique has gained new interest since the development of time domain measurement[2] makes it possible to extend the dielectric investigation up to microwave frequencies. In particular, time domain reflectometry spectroscopy has been employed to investigate side-chain motion in polypeptides in organic solvents,[3,4] and well-defined dielectric dispersions with relaxation times in the range 0.1–0.5 ns were observed.

Recently, Mashimo et al.[5] have reported the existence of a secondary dielectric dispersion in aqueous solutions of poly(L-glutamic acid) centered, at room temperature, in the region around 100 MHz between the dielectric relaxation due to counterion fluctuation (from 10 kHz to 10 MHz) and that due to the dipole orientation polarization of the water molecule at frequencies higher than 1–5 GHz. This secondary dispersion, whose characteristics depend on the conformation of the polymer which undergoes an helix–coil transition at pH = 6, was attributed to internal motions of the polar side groups.

Dielectric dispersions caused by this mechanism have been previously found only in synthetic polymers in organic solvents[6–8] but have not been described so far in aqueous solutions.

On the other hand, in a recent paper, Gestblom et al.[9] have shown that these dielectric dispersions can be artificially introduced in the time domain dielectric spectroscopy (TDS) by premature truncation of the time domain line shapes when high-conductivity samples, displaying dielectric dispersions at lower frequencies, are investigated. In particular, these authors, in support of this thesis, have measured the dielectric properties of poly(L-glutamic acid) (PGA) aqueous solutions (polymer molecular weight 74 × 10^3) by means of TDS using both the transmission and reflection methods and have shown that an apparent dielectric dispersion occurs, the frequency position of which depends upon the time window employed. This artifact occurring in PGA solutions where dielectric dispersions are present at frequencies lower than 1 MHz is mainly due to the high electrical conductivity of samples, causing a marked distortion of the pulse shape. On the basis of a theoretical analysis of the TDS methods, these authors[9] conclude that there is no reliable experimental evidence for dielectric processes with relaxation frequencies of about 100 MHz in aqueous solutions of biopolymers.

In order to clarify the existence of secondary dispersions in poly(L-glutamic acid) aqueous suspensions in the 100 MHz region attributable to side-chain dynamics, we have carried out dielectric measurements by means of frequency domain spectroscopy, exploring the frequency interval from 1 MHz to 1 GHz. In this way, the above interesting contrast is completely overcome and the dielectric dispersions around 100 MHz, if they really exist, should be properly measured.

In the present paper, we report on some dielectric measurements of three molecular weight poly(L-glutamic acid) aqueous solutions, at different polymer concentrations from 0.5 to 10 mg/mL. Our measurements clearly demonstrate the existence of a dielectric dispersion around 100 MHz, whose dielectric increment is confined within some units, and the relaxation times depend on the polymer concentration. This finding may be expected to provide useful information on the structural properties of biopolymers in aqueous solution.

Experimental Section

Sodium salts of poly(L-glutamic acid) of different average molecular weights from 13 × 10^3 to 74 × 10^3 were obtained from Sigma Chemical Co. and were used without further purification. The solutions were prepared with deionized water (electrical conductivity less than 10^{-6} Ω^{-1} cm^{-1} at 20 °C).

In aqueous solution, PGA exhibits a helix–coil transition with varying pH values.[10] In the present case, the pH is higher than 8 and the polymer is completely randomly coiled. For each molecular weight, the polymer content was varied from 0.5 to about 10 mg/mL. All measurements were carried out at a temperature of 25 °C.

Dielectric measurements were made in the frequency range 1 MHz to 1 GHz by means of a Hewlett-Packard Model 4191A radio-frequency impedance analyzer. The dielectric cell consists of a short section of a coaxial line whose constants have been determined by calibration using NaCl electrolyte solutions of appropriate molarities, to cover the whole conductivity range displayed by the PGA solutions. At the salt concentration employed, the dielectric properties $\epsilon^*_{\mathrm{ref}}$ of the reference electrolyte solution as a function of the concentration C can be calculated from the expression

$$\epsilon^*_{\mathrm{ref}}(\omega) = \epsilon_{\mathrm{inf}}(C) + \frac{\epsilon_{\mathrm{s}}(C) - \epsilon_{\mathrm{inf}}(C)}{1 + i\omega\tau(C)} + \frac{\sigma(C)}{i\epsilon_0\omega}$$

where the dielectric parameters $\epsilon_{\mathrm{s}}(C)$, $\epsilon_{\mathrm{inf}}(C)$, $\tau(C)$, and $\sigma(C)$ are obtained from the Stogryn equation.[11]

The dielectric loss $\epsilon''(\omega)$ as a function of frequency was calculated from the total conductivity $\sigma(\omega)$, by subtracting the conductivity term $\sigma(0)/\epsilon_0\omega$, due to ion drift

$$\epsilon''(\omega) = \frac{\sigma(\omega) - \sigma(0)}{\epsilon_0\omega}$$

where $\sigma(0)$ is the low-frequency conductivity, ω the angular frequency of the applied field, and ϵ_0 the dielectric constant of

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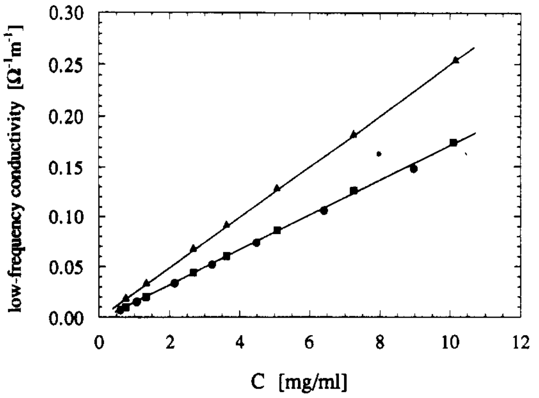

Figure 1. Electrical conductivities of PGA solutions of different molecular weights, measured at a frequency of 1 MHz, as a function of the polymer concentration: (▲) MW 13 × 10^3; (●) MW 36 × 10^3; (■) MW 74 × 10^3.

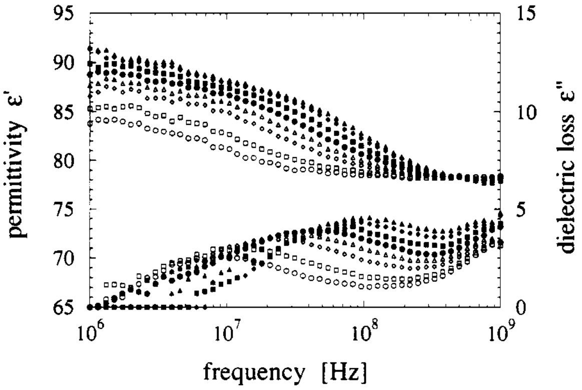

Figure 2. Permittivity ϵ' and dielectric loss ϵ'' of poly(L-glutamic acid) aqueous solutions in the frequency range from 1 MHz to 1 GHz, at different polymer concentrations: (▲) 11.2, (◆) 8.98, (■) 6.41, (●) 4.49, (△) 3.21, (◇) 2.14, (□) 1.07, (○) 0.61 mg/mL. The polymer molecular weight is 36 × 10^3. The high-frequency end of the dielectric loss spectrum rises sharply, indicating the beginning of the dispersion of the bulk water.

free space. This contribution term in the low-frequency region of the dielectric spectrum investigated is generally high, ranging, for example, at the frequency of 1 MHz, from 2 × 10^2 to 5 × 10^3, because of the high dc conductivity of the samples.

The electrical conductivities of the PGA solutions of three different molecular weights, measured at a frequency of 1 MHz, are shown as a function of the polymer concentration in Figure 1.

The overall accuracy of the experimental setup is within 2–3% for the permittivity ϵ' and within 5% for the dielectric loss ϵ''. At lower frequencies, the uncertainty on ϵ'' reaches a value of about 10% owing to the high dc electrical conductivity of the samples investigated.

Results and Discussion

Typical dielectric spectra (permittivity ϵ' and dielectric loss ϵ'' in the frequency range 1 MHz to 1 GHz) are shown in Figure 2 for PGA aqueous solutions at a temperature of 25 °C. The polyion molecular weight is 36 × 10^3, and the pH of the solvent is 8.5. The polymer concentration was varied from 0.6 to 11 mg/mL. Similar results, showing the appearance of well-defined dielectric dispersions, have been obtained for all the different molecular weight polymers investigated.

Since the dielectric loss clearly evidences that, at frequencies higher than 1 GHz, a further dielectric dispersion occurs, attributable to the polarization of the water molecules, the relaxation data have been analyzed on the basis of a Cole–Cole function, to which a term due to the loss of the pure solvent has been added, according to the expressions

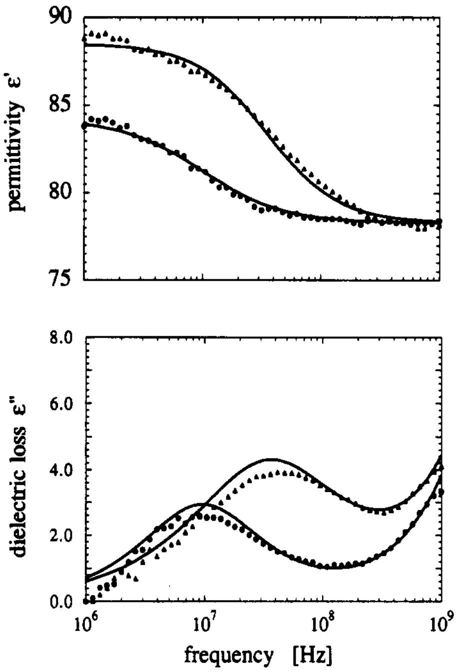

Figure 3. Permittivity ϵ' and dielectric loss ϵ'' of PGA solutions as a function of the frequency, at two different polymer concentrations: (▲) 4.49 and (●) 0.61 mg/mL. The full lines are the calculated values on the basis of eqs 1 and 2. The dielectric parameters have been estimated by the simultaneous fit of the real and imaginary parts of the complex dielectric constant, employing the Marquardt method for complex functions.

$$\epsilon'(\omega) = \epsilon_{\mathrm{inf}} + \frac{(\epsilon_s - \epsilon_{\mathrm{inf}})(1 + (\omega\tau)^{(1-\alpha)} \sin(\pi\alpha/2))}{1 + (\omega\tau)^{2(1-\alpha)} + 2(\omega\tau)^{(1-\alpha)} \sin(\pi\alpha/2)} \qquad (1)$$

$$\epsilon''(\omega) = \frac{(\epsilon_s - \epsilon_{\mathrm{inf}})((\omega\tau)^{(1-\alpha)} \cos(\pi\alpha/2))}{1 + (\omega\tau)^{2(1-\alpha)} + 2(\omega\tau)^{(1-\alpha)} \sin(\pi\alpha/2)} + \frac{(\epsilon_{sw} - \epsilon_{\infty w})\omega\tau_w}{1 + (\omega\tau_w)^2} \qquad (2)$$

where ϵ_s and ϵ_{inf} are the limiting values of the permittivity at low and high frequency, respectively, τ is the relaxation time, and α is a parameter governing the spread of the relaxation time around the mean value. The dielectric parameters of the pure water, at a temperature of 25 °C, assume the values[12] $\epsilon_{sw} = 78.5$, $\epsilon_{\infty w} = 4.5$, and $\tau_w = 8.25$ ps.

The parameters of the dielectric dispersions, $\Delta\epsilon = (\epsilon_s - \epsilon_{\mathrm{inf}})$, $\nu = 1/2\pi\tau$, and α have been determined by means of a nonlinear least-squares fitting procedure employing the Marquardt algorithm for complex functions.[13] The method gives parameter estimates based on the simultaneous fit of the real part, the permittivity ϵ', and the imaginary part, the dielectric loss ϵ'', of the complex dielectric constant $\epsilon^*(\omega)$.

As an example of the overall consistency of our dielectric data and the goodness of the fitting procedure, typical results are shown in Figure 3, for two different polymer concentrations (molecular weight 36 × 10^3). As can be seen, the agreement between the calculated and the experimental values both for ϵ' and for ϵ'' is quite good in the whole frequency range.

The dielectric parameters of the PGA dielectric dispersions obtained from the above-stated fitting procedure are shown in Figures 4–6 for solutions of different polymer contents and polymers of different molecular weights.

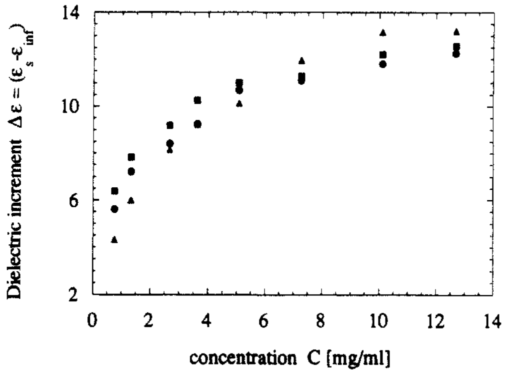

Figure 4. Dielectric increment $\Delta\epsilon$ as a function of the polymer concentration for three different molecular weights: (▲) 13 × 10^3; (●) 36 × 10^3; (■) 74 × 10^3. These parameters have been derived by fitting eq 1 to the experimental data.

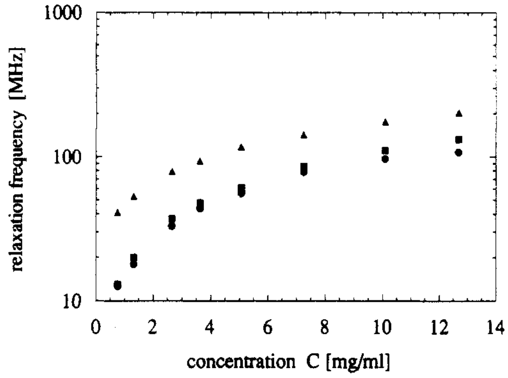

Figure 5. Relaxation frequency as a function of the polymer concentration for three different molecular weights: (▲) 13 × 10^3; (●) 36 × 10^3; (■) 74 × 103. These parameters have been derived by fitting eq 1 to the experimental data.

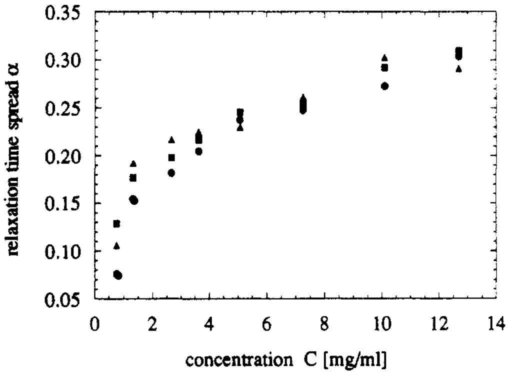

Figure 6. Parameter indicating the spread of the relaxation times as a function of the polymer concentration for three different molecular weights: (▲) 13 × 10^3; (●) 36 × 10^3; (■) 74 × 10^3. These parameters have been derived by fitting eq 1 to the experimental data.

It must be noted, however, that, if ϵ' and ϵ'' are fitted separately, each fit gives slightly different values of the parameters, in particular the spread of the relaxation times α and the relaxation frequency ν. This circumstance is attributable to the moderate accuracy of the dielectric loss spectrum in the low-frequency region due to the dc ionic conductivity contribution, and ϵ'' curves appear more sharp than the corresponding curves of ϵ'. However, the difference in the values of the fitting parameters is confined, in the most cases, within 10–20%.

Finally, to verify if the maximum in the dielectric loss or the whole shape of the curves could depend on the value of the dc conductivity σ_0, we have further analyzed our data considering σ_0 as a free parameter. Its estimate is in excellent agreement with the value measured at the frequency of 1 MHz.

As pointed out by Mashimo et al.,[5] the probably origin of the observed dielectric dispersions is the presence of an electric dipole fluctuation due to micro-Brownian motion of the chain which is cooperative with the motion of the side chains of the polymer extending into the solvent. The effective dipole moment μ_0 of each monomer can be estimated from the statistical average $\langle\mu^2\rangle^{1/2}$ of the dipole moment of the whole chain, using the usual Onsager equation[14]

$$\langle\mu^2\rangle = ng\mu_0^{\,2} = \frac{9K_BT(\epsilon_s - \epsilon_{inf})(2\epsilon_s + \epsilon_{inf})}{4\pi N\epsilon_s(\epsilon_{inf} + 2)^2}$$

where N is the numeric polymer concentration, K_BT the thermal energy, n the number of polar groups in the chain, and g a parameter, related to the Kirkwood parameter, depending on the degree of flexibility of the chain. Using $g = 0.8$ for a randomly coiled polymer, μ_0 assumes values ranging from 6 to 2.5 D as the polymer concentration is increased, roughly independent of the molecular weight.

However, a different contribution to the overall observed dielectric behavior in this frequency range, arising from the counterion fluctuation effect, should be taken into account. In fact, the typical dielectric spectrum of polyelectrolyte solutions generally displays two different dispersions, the high frequency of which occurs at frequencies of about 1–10 MHz. This "high-frequency" dielectric dispersion is attributed either to a diffusion process of counterions along subunits of the polymer[15] or to the Maxwell–Wagner effect.[1] Thus the dielectric spectra we report here can be considered as the overlap of two different relaxation mechanisms occurring in a narrow range of frequencies. This circumstance, when the spectra are analyzed in terms of a single relaxation process, might justify the observed increase of the relaxation frequency with the polymer concentration.

A final comment is in order. The dielectric properties of PGA aqueous solutions in the dilute or semidilute regime have been extensively studied by different authors[10,16,17] at frequencies up to 100 MHz. These authors generally find that the high-frequency limit of the permittivity is very close to that of the pure water or to that of the solvent, indicating that no further dielectric dispersions should be expected, besides that occurring in the solvent itself at microwave frequencies (the relaxation frequency of the dipolar orientation polarization of the water molecule is about 19 GHz at 25 °C).

This is probably due to the fact that, in these investigations, the polymer concentration is too low to evidentiate the contribution of the chain dynamics. As can be seen in Figure 4, the dielectric increment decreases with the polymer concentration and moreover the relaxation shifts toward lower frequencies where, as stated, other dielectric relaxation mechanisms are present. A further support to this point of view came out from inspection of Figure 6. The spread parameter α assumes values from 0.1 to 0.35, independent of the molecular weight, as the polymer concentration is increased. These large values strongly indicate the presence of more than one dielectric relaxation process.

Finally, as can be seen in Figure 5, the relaxation times and, to a lesser extent, the dielectric increment increase with the molecular weight of the polymer. This effect, that must be further investigated in detail, may be due to interactions of different segments of the chain, reflecting an increase in the excluded volume which, in turn, may cause a hindrance of the chain motion. Polymers of lower molecular weight display higher relaxation frequencies.

Although other mechanisms responsible for the observed behavior cannot be ruled out, nevertheless, the present results corroborate the existence of dielectric dispersions in the 100-MHz region. It is therefore of interest to enquire in detail by dielectric spectroscopy, in this frequency range, about the chain dynamics in aqueous solutions of biopolymers.

References and Notes

(1) Pethig, R. *Dielectric and Electronic Properties of Biological Materials*; Wiley: Chichester, U.K., 1979.
(2) Cole, R. H.; Berberian, J. G.; Mashimo, S.; Chryssikos, G.; Burns, A.; Tombari, E. *J. Appl. Phys.* **1989**, *66*, 793.
(3) Yagihara, S.; Nozaki, R.; Mashimo, S.; Hikichi, K. *Macromolecules* **1984**, *17*, 2700.
(4) Nakamura, H.; Mashimo, S.; Wada, A. *Macromolecules* **1981**, *14*, 1698.
(5) Mashimo, S.; Ota, T.; Shinyashiki, N.; Tanaka, S.; Yagihara, S. *Macromolecules* **1989**, *22*, 1285.
(6) Mashimo, S. *J. Phys. Chem.* **1977**, *67*, 2651.
(7) Stockmayer, W. H. *Pure Appl. Phys.* **1967**, *15*, 539.
(8) Mashimo, S. *Macromolecules* **1976**, *9*, 91.
(9) Gestblom, B.; Gestblom, P. *Macromolecules* **1991**, *24*, 5823.
(10) Nakamura, H.; Wada, A. *Biopolymers* **1981**, *20*, 2567.
(11) Stogryn, A. *IEEE Trans. Biomed. Eng.* **1971**, *MTT-19*, 733.
(12) Kaatze, U. *Adv. Mol. Relax. Processes* **1975**, *7*, 71.
(13) Grant, E. H.; Sheppard, R. J.; South, G. P. *Dielectric Behaviour of Biological Molecules in Solution*; Clarendon Press: Oxford, U.K., 1978.
(14) Hill, N. E.; Vaughan, W. E.; Price, A. H.; Davies, M. *Dielectric Properties and Molecular Behaviour*; Van Nostrand Reinhold Co.: London, 1969.
(15) Mandel, M.; Odijk, T. *Annu. Rev. Phys. Chem.* **1984**, *35*, 75.
(16) Takashima, S. *Biopolymers* **1963**, *1*, 171.
(17) Muller, G.; van der Touw, F.; Swolle, S.; Mandel, M. *Biophys. Chem.* **1974**, *2*, 242.

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