Measurement of Mesh Sizes in Concentrated Rigid and Flexible Polyelectrolyte Solutions by an Electron Spin Resonance Technique

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ABSTRACT: Mesh sizes in concentrated aqueous solutions of collagen and hyaluronic acid were measured using an electron spin resonance (ESR) based technique. The technique involved spin labeling poly(acrylic acid) probe molecules of various sizes to serve as sensors which were mixed into the concentrated polyelectrolyte solution. When probe molecules were smaller than the mesh size of the polyelectrolyte solution, the local rotational correlation time (RCT) of the spin label was essentially unchanged from free solution. When the mesh size approached or was smaller than the probe size, the RCT was altered due to close-range interactions between the probe molecule and neighboring polymer chains. The smallest size probe that interacted with a polymer matrix was used as the basis for quantifying mesh sizes. There was reasonable agreement between theoretical estimates of mesh sizes in collagen solutions and estimates from the experimental technique. Measured mesh sizes in hyaluronic acid solutions show good quantitative agreement with reported mesh size calculations from measured probe diffusion rates in similar matrices.

Introduction

Polyelectrolyte hydrogels and concentrated polyelectrolyte solutions are an important class of polymeric materials which are widely used in medical and other applications.[1–4] Medical applications range from drug delivery to soft tissue augmentation. These hydrogels and concentrated polyelectrolyte solutions typically contain over 90% water. In drug delivery applications, the mesh size, which is the average spacing between neighboring polymer chains, is a critical parameter in defining diffusional hindrance.[5] Mesh sizes are also important in defining other physical properties of these materials. Experimental determination of mesh sizes has not always been possible. Mesh sizes in low concentration polymer gels (mesh size 50–100 nm) can be measured by light scattering.[6] However, the small mesh sizes (mesh size < 50 nm) in concentrated gels cannot be resolved by light scattering. Most attempts to quantify mesh sizes in more concentrated polymer matrices (less than 50 nm range) have been based on theoretical interpretations of macroscopically measured physical properties like mechanical moduli,[7] equilibrium swelling,[8] or diffusion measurements.[9] A molecular-scale technique which directly measures mesh size, at a molecular scale, eliminates the implicit assumptions required in theoretical interpretations. This study reports on an electron spin resonance (ESR) based technique for the direct experimental measurement of mesh sizes and on results of its application to collagen and hyaluronic acid matrices.

The ESR-based technique involves labeling probe molecules of various sizes with a paramagnetic spin label. Care was taken to select probes with no physical binding affinity to the polymer molecules comprising the concentrated matrix. The probes were incorporated into the polymer matrices, and the local dynamics of the probe were inferred from the ESR spectrum of the paramagnetic spin label. Analysis of the ESR spectra of spin-labeled polymers has shown that the ESR spectrum is dominated by the local segmental motion of the polymer chain, and the contribution of the overall tumbling of the polymer is negligible.[10] When the probe size was much smaller than the matrix mesh size, there was no interaction between the probe and the neighboring matrix polymer chains. Hence, the local dynamics of the probe were unchanged from that in free solution. However, when the size of the probe approached the matrix mesh size, due to close-range interactions between the matrix polymer chains and the probe, the local dynamics of the probe were altered (from free solution behavior). The mesh size was therefore inferred from the size of the smallest probe which interacted with the matrix. This molecular method for measuring mesh size is shown schematically in Figure 1. In essence, the probe molecule is a molecular sensor which undergoes conformational and dynamic rearrangement when the surrounding matrix polymer chains become concentrated enough to impinge on it. The change in the local dynamics of the probe molecule was detected by ESR. This technique was applied in collagen and hyaluronic acid (HA) matrices. Since collagen molecules have a well-defined solution geometry (rigid rods), mesh sizes in collagen matrices were estimated theoretically.[11] A comparison between the estimated mesh size and the measured mesh size served as the

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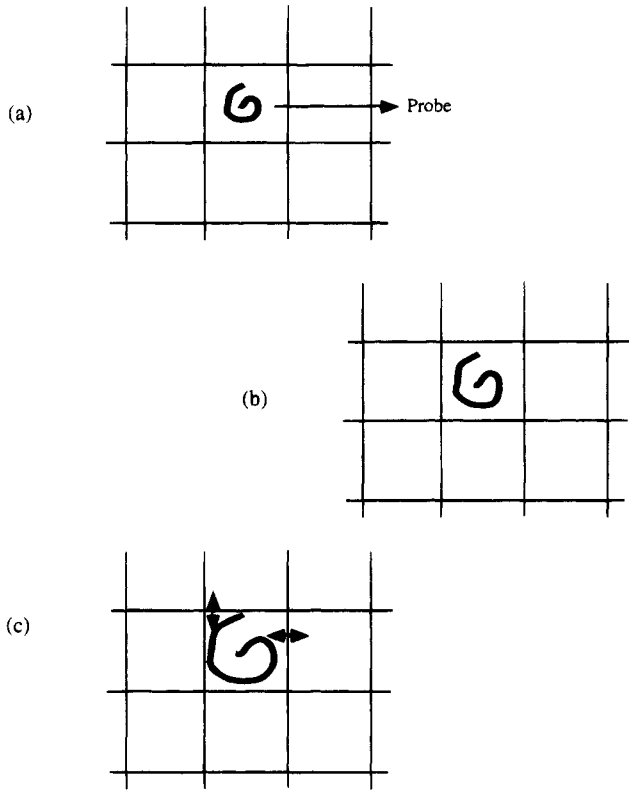

Figure 1. Schematic representation of the ESR-based mesh size measurement technique: (a) Probe is much smaller than the mesh size and there is no interaction. (b) Probe is still smaller than the mesh size and there is still no interaction. (c) Probe size is equal to the mesh size, and due to the interactions, the local dynamics of the probe changes. This change in the segmental dynamics of the probe can be detected by ESR (as described in the text).

basis for confirming the validity of this technique in collagen matrices. Measured mesh sizes in the HA matrices were also compared with mesh size estimates based on dextran probe diffusion measurements.[9]

Materials and Methods

Matrix Preparation. Collagen is a naturally occurring structural protein (MW 300 000). The tightly wound triple helical structure of collagen[12] gives rise to rigid-rod behavior in solution which distinguishes it from most other polymers which are flexible. Collagen (Collagen Corp., Palo Alto, CA) was succinylated by the procedure of Miyata et al.[13] [Certain commercial equipment, instruments, and materials are identified in this paper in order to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the material or equipment is the best available for the purpose.] Purified acidic collagen (pH 2.0) was adjusted to pH 9.0 with 1.0 M NaOH. Succinic anhydride (0.2 mg/mg of collagen) dissolved in acetone (1/20 volume of collagen solution) was added to the collagen solution with stirring. The pH during the addition of succinic anhydride was maintained at 9.0. Stirring was continued for 30 min until the solution turned clear. The pH of the solution was then adjusted to 4.5 with 1.0 M HCl. The succinylated collagen (pI ~ 4.5) precipitate was then centrifuged and washed repeatedly to remove any unreacted succinic anhydride and acetone. The concentrated precipitate was then resuspended in a 20 mM sodium phosphate−130 mM sodium chloride solution buffered to pH 7.4 (PBS). Succinylated collagen, due to its net negative charge at neutral pH, does not form fibrils. The collagen concentration was then determined by the biuret method.[14] Succinylated collagen at high concentrations (>20 mg/mL) forms a very viscous translucent solution.

Hyaluronic acid (HA), a natural muco-polysaccharide, is a linear flexible polymer which is present in the connective tissues of vertebrates, the synovial fluid of joints, and the vitreous humor of the eye.[15] Hyaluronic acid matrices were prepared by dissolving Rooster Comb hyaluronic acid (Sigma Chemical Co., St. Louis, MO) in PBS. The molecular weight determination of the hyaluronic acid was attempted using gel permeation chromatography (GPC). The GPC setup included a Water (Millipore Inc., Milford, MA) Model 501 pump and two Waters Ultrahydrogel columns (2000 and 500) in series preceding a differential refractive index detector. Since hyaluronic acid molecular weight standards were not available, a precise determination of the HA molecular weight was not possible. However, molecular weight standards of a similar polysaccharide, dextran (Polysciences, Inc., Warrington, PA), were also eluted on the same GPC instrument. The hyaluronic acid sample (11.5 min) eluted earlier than the 2×10^6 dextran standard (16.2 min). Dextran, being a more flexible polysaccharide,[16] is expected to elute later than hyaluronic acid[17] of similar molecular weight. The hydrodynamic radius (R_h) of the hyaluronic acid was determined by dynamic light scattering. The dynamic light scattering measurements were performed at a scattering angle of 90° with a 4-W argon ion laser (λ = 514 nm) and a Langley Ford Model 1096 correlator. The free solution diffusion coefficient D_0 was determined by analyzing the measured autocorrelation function using the standard method of cumulants. A single-exponential (i.e., n = 1) function was found to be sufficient to fit the data. The hydrodynamic radius of the hyaluronic acid was then calculated from the Stokes−Einstein equation:[18]

$$R_h = k_B T/(6\pi\eta D_0) \quad (1)$$

where k_B is the Boltzmann constant, η is the solvent viscosity, and T is the sample temperature. The R_h of hyaluronic acid was calculated to be 65 nm (i.e., $D_0 = 3.3 \times 10^{-8}$ cm^2/s). An estimate of the molecular weight can be made from data in the literature relating the M_w of HA to its R_g. For HA, which is a long wormlike polymer chain, in the limit of infinite chain length, $R_g = 1.5R_h$.[19] This result is identical to the Rouse−Zimm model for a neutral flexible polymer chain of infinite length. Comparing the calculated value of R_g to those reported by Fouissac et al.[20] gives a molecular weight of approximately 800 000. Like succinylated collagen, concentrated hyaluronic acid (>20 mg/mL) forms a translucent viscous solution.

Probe Preparation and Characterization. Poly(acrylic acid) (PAA) of various molecular weights (Polysciences, Inc., Warrington, PA) were covalently labeled with the spin label, 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (4-amino-TEMPO), by a dicyclohexylcarbodiimide (DCC) condensation reaction.[21] 4-Amino-TEMPO and DCC were added to a solution of PAA in anhydrous dimethylformamide (DMF). PAA samples which were available in powder form were directly dissolved in DMF. PAA samples which were only available in concentrated solution were further concentrated or dried and then added to DMF. After 3 days, the labeled polymer was precipitated in ether and subsequently hydrolyzed in deionized water at 50 °C. The *N,N*-dicyclohexylurea which precipitated was filtered off, and the pH of the solution was adjusted to 11.0 with 1.0 M NaOH. The solution at pH 11.0 was dialyzed against deionized water until the signal from the free (unreacted) spin label was completely absent from the ESR spectrum. The pH of the dialyzed solution was then adjusted to 2.0 with 1.0 M HCl. The pH 2.0 solution was then dialyzed exhaustively against deionized water. The successive dialyses of acidic and basic pH presumably eliminated any spin label that might have been electrostatically bound to the PAA. The labeled probe was then dialyzed against PBS.

The label concentration in the probe solution was determined by double integrating the ESR spectra and comparing the area to areas of spectra from standard solutions of known 4-amino-TEMPO concentration. The PAA concentration was determined by differential refractometry. The labeling density for all probes prepared (ratio of the label concentration to the PAA concentration) was approximately 1 in all preparations. The labeling density was kept low to prevent dipole interac-

Table 1. Characterization of the Labeled Poly(acrylic acid) Probes[a]

mol wt M_w	polydispersity M_w/M_n	rotational corr. time τ_R (ns)	hydrodynamic radius (nm)
6 000	2–3	0.52 ± 0.03	4 ± 0.5
15 000	2–3	0.57 ± 0.03	6 ± 0.5
31 000	2–3	0.63 ± 0.03	11 ± 0.5
38 000	~6	0.67 ± 0.03	30 ± 0.5

[a] M_w is the weight-average molecular weight, and M_n is the number-average molecular weight. The uncertainties in the hydrodynamic radius and the rotational correlation time were determined by the maximum variation between three measurements for all the samples.

tions between different spin labels on the probe molecules.[22] The molecular weight and polydispersity of the labeled probe were determined by gel permeation chromatography (Table 1) using PAA standards (Polysciences, Inc., Warrington, PA). GPC was performed on the system described previously.

The hydrodynamic radii (R_h) of the labeled PAA probes were determined by dynamic light scattering using the same setup described earlier. The PAA molecules tend to form aggregates even at the lowest concentrations studied. The presence of even a small amount of aggregates complicates the interpretation of the low-angle static light scattering measurements. Dynamic light scattering discriminates between monomers and aggregates and permits an unambiguous determination of the hydrodynamic radius of the monomer molecules. A high-power laser was necessary for these measurements since the PAA molecules in aqueous solution are relatively isorefractive (i.e., low scattering efficiency).[23-25] The free solution diffusion coefficient D_0 was determined by analyzing the autocorrelation spectra using the standard method of cumulants. A single-exponential (i.e., $n = 1$) function was found to be sufficient to fit the data. The hydrodynamic radii of the probes were then calculated using eq 1. The measured hydrodynamic radii for the different size PAA probes are also listed in Table 1.

ESR Measurements. The PAA probes were loaded into the matrices by syringe-to-syringe exchange. The ESR spectra for the labeled PAA molecules in the collagen and hyaluronic acid matrices were recorded using a Varian E-4 X band spectrometer at room temperature (Figure 2) presents typical spectra). Since the line shape of the ESR spectrum is sensitive to the scanning parameters[26] (i.e., microwave power, modulation amplitude, scan time, and time constant), operating parameters were selected such that they did not affect the accuracy of the line shapes. The effect of tumbling of the spin probe on the line shape has been studied extensively[27-30] and quantified in terms of a rotational correlation time. In cases where the molecular motion is by Brownian motion, the rotational correlation time is an approximate measure of time required for the probe molecule to tumble through 1 rad about its principal axis.[31] Hence, the greater the rotational correlation time, the slower the local segmental reorientation of the polymer chain. For nitroxide radicals, in general, apparent rotational correlation times, assuming quasi-isotropic motion, have been calculated using the motional narrowing formalism[32,33] to be

$$\tau_R = (3^{0.5}\pi\Delta\nu(0)/2C)((h_0/h_{-1})^{0.5} + (h_0/h_{+1})^{0.5} - 2) \quad (2)$$

where h_0, h_{-1}, and h_{+1} are the peak-to-peak heights of the mid-, low-, and high-field lines in an absorption first-derivative spectrum, $\Delta\nu(0)$ is the peak-to-peak width of the mid-field line, and C is a constant based on the hyperfine interaction tensors of the spin label. For 4-amino-TEMPO, the rotational correlation times were calculated from:[21]

$$\tau_R = 6.5 \times 10^{-10} w_0((h_0/h_{-1})^{0.5} + (h_0/h_{+1})^{0.5} - 2) \quad (3)$$

where w_0 is the peak-to-peak line width of the midfield line (in gauss). Although the ESR spectrum directly indicates the dynamics of the spin label, it is a reasonable assumption for the chemistry employed here that it closely reflects the dynamics of the polymer backbone itself.[34] The free solution τ_R values computed from the ESR spectra of the various PAA probes are listed in Table 1.

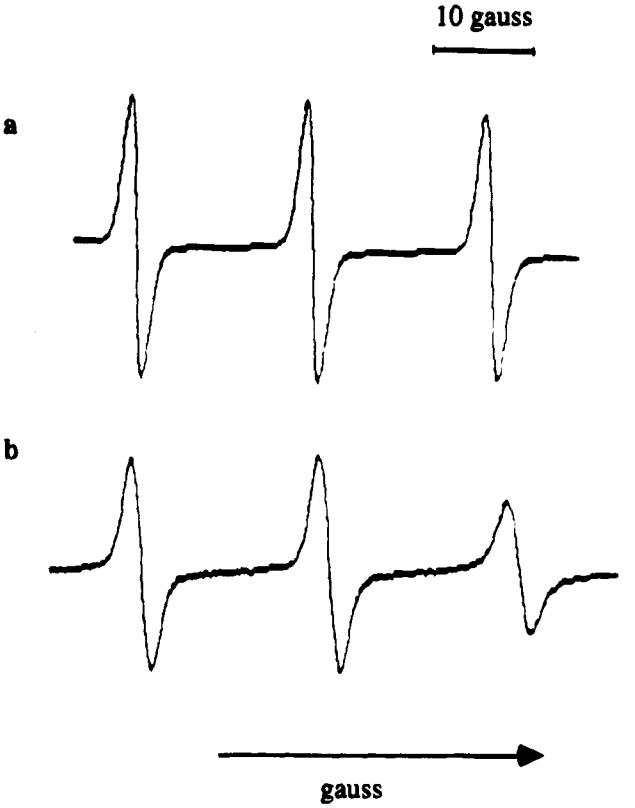

Figure 2. First-derivative ESR spectra recorded on the Varian E-4 X band spectrometer at 9.54 GHz. The midfield line is at 3400 G. (a) 4-Amino-TEMPO in water; the rotational correlation time calculated from this signal was 0.03 ns. (b) 4-Amino-TEMPO covalently bound to poly(acrylic acid) (M_w = 31 000); the rotational correlation time calculated from this signal was 0.63 ns.

Results and Discussion

Rotational correlation times were calculated from the ESR spectra recorded for PAA probes loaded in succinylated collagen and hyaluronic acid matrices. Figure 3 presents τ_R as a function of collagen concentration for the PAA probes. The maximum concentration of collagen matrices studied was limited by handling problems at very high concentrations. The PAA probes displayed a drop in τ_R at a critical collagen concentration as shown in Figure 3. The critical collagen concentration at which τ_R drops becomes progressively smaller with increasing probe molecular weight. The drop in τ_R, which reflects faster segmental motion of the PAA probe, presumably is due to short-range repulsive interactions between the negatively charged PAA and the negatively charged succinylated collagen. This hypothesis was tested by measuring τ_R of the 38 000 PAA probe at different ionic strengths. In a 250 mM NaCl solution the PAA probe had a τ_R of 0.80 ns, while at 130 mM the τ_R decreased to 0.65 ns. This confirms the sensitivity of the polymer chain dynamics (as detected using ESR) to local repulsive forces. The drop in τ_R for PAA in the collagen matrices is presumed to occur at the concentration where neighboring collagen rods just begin to impinge on the PAA molecule. The results for the succinylated collagen matrices are summarized in Table 2.

Succinylated collagen molecules maintain a triple helical rigid-rod molecular conformation yet do not

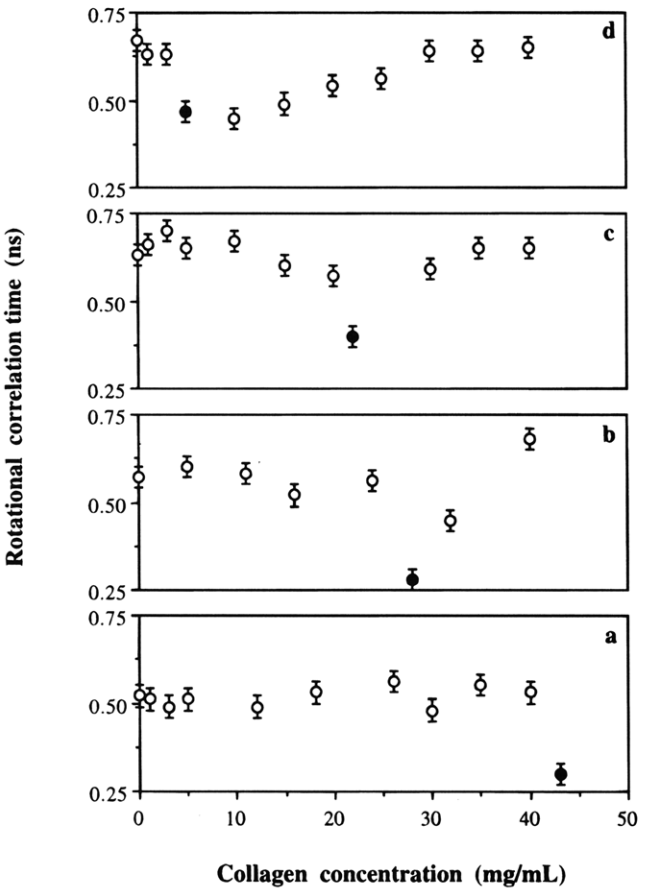

Figure 3. Rotational correlation time of the PAA probe in succinylated collagen matrices. The error bars specified were determined by the maximum variation between three measurements for all the samples. (a) M_w = 6000. (b) M_w = 15 000. (c) M_w = 31 000. (d) M_w = 38 000.

Table 2. Comparison of the Measured and Estimated Mesh Sizes in Collagen Matrices[a]

mol wt M_w	hydrodynamic radius (nm)	collagen conc (mg/mL)	calcd mesh size (nm)
6 000	4 ± 0.5	43	5
15 000	6 ± 0.5	28	6
31 000	11 ± 0.5	22	7
38 000	30 ± 0.5	5	15

[a] The collagen concentration refers to the lowest concentration at which there was interaction between the matrix and the PAA probe specified by the molecular weight (M_w) and hydrodynamic radius (R_h). The calculated mesh size is the mesh size at the given collagen concentration calculated using a theoretical model as described in the text.

precipitate (as fibers) at neutral pH. Scaling models used to estimate mesh sizes in polymer matrices are based on flexible polymer chain models.[9] However, in the case of collagen matrices, estimation of mesh sizes using a rigid-rod polymer model would be more appropriate. A model to estimate mesh sizes in rigid-rod polymer matrices is described below.[11] According to the Doi and Edwards model,[35] in a system of monodisperse rods of length L, the number of rods (n) intersecting an imaginary cylinder of diameter a around a test rod of length L is:

$$n = 2acL^2\langle\sin(\mathbf{uu}')\rangle \tag{4}$$

where c is the number density of the rods and $\langle\sin(\mathbf{uu}')\rangle$ is the average value of the sine of the angle between the intersecting rods. This relationship has been extended to a system of polydisperse rods,[36] whereby n is given by:

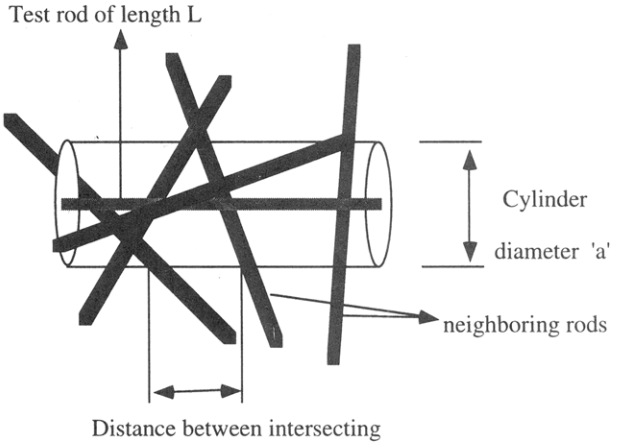

Figure 4. Depiction of the mesh confining a test rod in the matrix in terms of a tube model. The circumscribed cylinder is defined by the neighboring rods. The mesh size is defined by the distance between the neighboring rods which confine the test rod.

$$n_i = 2aL_i\sum_m c_m L_m\langle\sin(\mathbf{uu}')\rangle \tag{5}$$

The index m (in eq 5) denotes the summation over all the different rod lengths in the matrix, and the index i denotes the number of rods intersecting a cylinder of length L_i. In an isotropic matrix, the average distance between neighboring rods in any direction should be equal to the mesh size. As schematically illustrated in Figure 4, the distance between the rods in the direction perpendicular to the test rod should be equal to the distance in the direction parallel to the test rod. Hence, the mesh size which is the distance between the intersecting rods on the cylinder surface should be equal to the diameter a of the imaginary cylinder. This condition is stated mathematically as:

$$L_i/n_i = a \tag{6}$$

When eq 6 is substituted into eq 5, the resulting equation can be solved for the mesh size a:

$$a^2 = 1/2\sum_m c_m L_m\langle\sin(\mathbf{uu}')\rangle \tag{7}$$

Mesh sizes in collagen matrices were estimated by approximating the collagen molecule as a cylindrical rod of length 300 nm and diameter 1.5 nm. The polydispersity of the collagen molecules was assumed to be 85% monomers (300 nm long) and 15% dimers (600 nm long) based on previous characterization studies of similar collagen matrices.[37] The number densities of the collagen molecules were computed from:[37]

$$c_m = \{[\text{mg/mL}]/\text{MW}\}N_{AV} \tag{8}$$

where [mg/mL] is the mass/volume concentration of collagen in the matrix, MW is the molecular weight of a collagen molecule (300 000), and N_{AV} is Avogadro's number. Previous observations under a polarizing microscope of similar matrices did not show any regions of birefringence.[11] Hence, the orientation distribution was assumed to be isotropic (i.e., $\sin(\mathbf{uu}') = 45°$). The

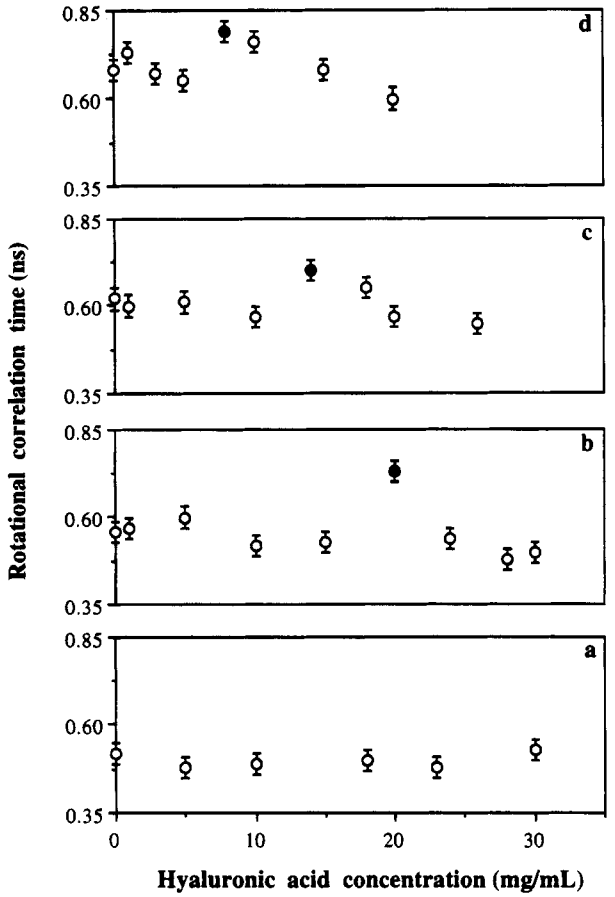

Figure 5. Rotational correlation time of a PAA probe in hyaluronic acid matrices. The error bars specified were determined by the maximum variation between three measurements for all the samples. (a) M_w = 6000. (b) M_w = 15 000. (c) M_w = 31 000. (d) M_w = 38 000.

mesh sizes calculated for the collagen matrices of various concentrations are also listed in Table 2. There is a reasonable agreement between the estimated and experimentally determined mesh sizes for the lower molecular weight PAA probes. The 38 000 PAA probe was significantly more polydisperse (M_w/M_n = 6) than the lower molecular weight probes (M_w/M_n between 2 and 3). It is possible that some covalent attachment of polymers occurred when concentrating the PAA solution before the DCC condensation reaction. This branching might also account for the apparently large measured hydrodynamic radius of this probe.

When the collagen concentration was increased beyond the critical interaction concentration, the probe appeared to become entangled with the collagen rods, thereby decreasing its segmental mobility. This entanglement or mixing of the probe is evident from the rise in τ_R as the collagen concentration is increased. It appears that the PAA probes tend to contract initially and then mix when the collagen concentration is further increased. Thermodynamically, rod–coil mixing is generally unfavorable[38] and the PAA probe would mix with the collagen rods only after it was energetically unfavorable to contract further.

Similar measurements were done with the PAA probe in hyaluronic acid matrices. The highest concentration of HA matrices studied (30 mg/mL) was limited by the equilibrium swelling concentration of HA in PBS. The τ_R of the PAA probes as a function of HA concentration is shown in Figure 5. The 6000 probe did not interact with HA in matrices up to 30 mg/mL HA concentration.

Table 3. Measured Mesh Sizes in Hyaluronic Acid Matrices

hyaluronic acid conc (mg/mL)	mesh size (nm)			
	a	*b*	*c*	*d*
30	>4 ± 0.5			
20	6 ± 0.5			
14	11 ± 0.5	13 ± 2[e]	10 ± 1[e]	20 ± 3[e]
8	30 ± 0.5	18 ± 2[e]	14 ± 2[e]	27 ± 4[e]

[a] Spin-labeled PAA probes. [b] FITC–dextran (71 200) probe. [c] FITC–dextran (148 000) probe. [d] FITC–dextran (487 000) probe. [e] Mesh sizes using FITC–dextran probes from Table 2, ref 9.

Hence, the mesh size of the HA matrices at 30 mg/mL can be inferred to be greater than the size of the 6000 probe (4 nm). The results for the PAA probes in HA matrices are summarized in Table 3. Unlike the collagen matrices, at a critical HA concentration, the τ_R of the PAA probe rises above its free solution value. This implies that the probes initially mix with the hyaluronic acid, thereby decreasing their segmental mobility. This difference in response between a PAA–collagen and PAA–HA mixture is consistent with thermodynamic theories,[39] which indicate that some mixing of dissimilar flexible segments is favored by entropic forces. As the concentration of HA is increased further, however, the τ_R drops below the free solution value. This is again, presumably, due to the short-range repulsive interactions between the negatively charged HA and the negatively charged PAA molecules. It was observed (Figure 5b) in HA matrices that the τ_R of the PAA probe remains lower than its free solution value at concentrations higher than the critical interaction concentration. This behavior contrasts with the collagen matrices (Figure 3) where the τ_R of the PAA probes, after the initial drop, rises above the free solution value at higher collagen concentrations. This is likely an effect of the difference in rigidity between the collagen and HA molecules which affects the ability to mix with contracted PAA chains (i.e., whether PAA behaves as a rigid sphere when crowded by neighboring matrix polymer chains). Contraction of flexible polymers in the presence of dissimilar flexible segments at high concentrations has been reported previously. Morphology studies of dimethylstyrene in concentrated PMMA[40] by coherent neutron scattering indicate that the dimethylstyrene contracts and assumes a morphology between a true random coil and a true hard sphere in a concentrated PMMA network. Since the technique reported here only depends on the lowest limiting interaction concentration, the different responses of PAA at higher concentrations in succinylated collagen and HA solutions do not impact the mesh size measurements.

The HA mesh size measurements show very good quantitative agreement with the recently published results of De Smedt et al.[9] which compute mesh sizes of hyaluronic acid solutions based on diffusivity measurements of fluorescently labeled dextran probes in the HA matrices as well as based on rheological measurements. The HA employed in their experiments (weight-average molecular weight of 680 000) had a slightly lower molecular weight than the HA employed in these experiments. The molecular weights of the dextran probes employed in their experiments are also considerably higher than the molecular weights of the PAA probes employed in our experiments. Mesh size calculations were based on scaling laws described in ref 9, and as such the authors caution that due to assumptions

made in the calculations their reported mesh sizes are actually only estimates of the true mesh size. The HA mesh size value reported in Table 3 at 14 mg/mL quantitatively agrees with the calculated mesh sizes for the 71 000 and 148 000 dextran probes (Table 2, ref 9). At 8 mg/mL the mesh size (Table 3) is in quantitative agreement with the calculated mesh size for the 487 000 dextran probe. The weak agreement between the mesh size estimated here at 8 mg/mL and those reported for the 71 000 and 148 000 dextran probes is probably due to the high polydispersity ($M_w/M_n = 6$) of the labeled 38 000 PAA probe. Because of the high polydispersity, there is less confidence that the average measured probe size and detected interaction concentration accurately reflect the mesh size in this matrix. At higher HA concentrations mesh sizes were computed from rheological measurements (Table 2, ref 9) which were performed at 37 °C. The mesh sizes calculated from the viscoelastic measurements are somewhat higher than the mesh sizes reported in Table 3, but there is "semi-quantitative" agreement. These differences in part reflect the difference in the temperature at which measurements were performed. The quantitative agreement between the two probe methods is encouraging and suggests that the changes in the local dynamic motion within the PAA probes detected by ESR in fact do reflect very close-range interactions between the probe and neighboring polymer chains.

Conclusions

It is possible to quantify mesh sizes by measuring changes in the segmental motion of a flexible polymer when it interacts with dissimilar polymers in a concentrated system. ESR is sufficiently sensitive to detect these changes. The distinct advantage of this technique is that the measurement is independent of the complexity of the matrix being studied. This technique requires the absence of binding interactions between the probe and the matrix. Hence, the choice of the probe is crucial and should be made based on the system being studied. The results presented here indicate that the technique can be improved. One way of improving the technique would be to use more monodisperse probe molecules. The synthesis of more probes over the range 2–10 nm would help increase the resolution (i.e., the larger the number of probes in a size range, the higher the resolution of the measured mesh size). The sources of error in this technique are mainly due to uncertainties in the measured ESR spectra (i.e., τ_R), uncertainties in the probe–matrix polymer interaction length scales, and the measured hydrodynamic radii of the probes. In the case of the PAA probes, the uncertainty in the measured probe size (i.e., hydrodynamic radius) was ±0.5 nm. Also R_h is not the true coil diameter in solution and another probe coil geometry measure (such as radius of gyration) could give more accuracy in terms of measuring the absolute mesh size. This issue is partially obscured by uncertainties in the approach distances before PAA probe molecules respond to the presence of matrix polymer molecules (i.e., Debye length, excluded volume lengths, etc.). While this uncertainty in the absolute probe size scale is a source of inaccuracy in measuring the absolute mesh size, its significance is minimized when this technique is used as a relative method for comparing mesh sizes in different polymer matrices.

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