CCl_4 (66.6 wt %) results in a trifluoromethyl macroester absorption at −75.7 ppm, while in polar medium (66.6 wt % in CH_3NO_2) a signal at −78.7 ppm due to triflate anion appears (Table I). These examples are further illustrated in Figure 2.

The macroester ⇄ macroion equilibrium in the polymerization of tetrahydrofuran catalyzed by "superacid" anhydrides and esters, proposed by Smith and Hubin[7] and Penczek,[5] as well as the dependence of such an equilibrium on solvent polarity can therefore be directly observed by ^{19}F-nmr. The polymerization of THF catalyzed by proton acids such as CF_3SO_3H and HSO_3F reported in our previous paper[3] is complicated by the possibility of secondary reactions.[4] A detailed analysis of this system has been made using fluorine nmr spectroscopy, and the results will be published elsewhere.

References and Notes

(1) After this manuscript had been submitted for approval by our company, a paper on a similar subject appeared: S. Kobayashi, H. Danda, and T. Saegusa, *Macromolecules*, **7**, 415 (1974). The authors reported ^{19}F and ^{1}H-nmr studies of THF polymerizations initiated by ethyl triflate.

(2) (a) Plastics Department; (b) Industrial Chemicals Department.

(3) G. Pruckmayr and T. K. Wu, *Macromolecules*, **6**, 33 (1973).

(4) S. Kobayashi, H. Danda, and T. Saegusa, *Bull. Chem. Soc. Jap.*, **46**, 3214 (1973).

(5) K. Matyjaszewski, P. Kubisa, and S. Penczek, *J. Polym, Sci.*, **12**, 1333 (1974).

(6) K. Matyjaszewski and S. Penczek, *Macromolecules*, **7**, 137 (1974).

(7) S. Smith and A. J. Hubin, *J. Macromol. Sci., Chem.*, **7**, 1399 (1973).

(8) The peak positions were identified by adding more ethyl fluosulfate to the nmr sample.

Microstructure of Poly(2,3-dimethyl-1,3-butadiene) Prepared by Butyllithium in Polar Solvent

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Recently, Yuki, *et al.*,[1] reported an extensive structural study on poly(2,3-dimethyl-1,3-butadiene) (PDMB) prepared by anionic polymerization under various conditions. Using 100-MHz nmr spectroscopy they showed that the 1,2 content of PDMB prepared by *n*-butyllithium in a polar solvent (tetrahydrofuran) depends strongly upon the polymerization temperature, behavior of which contrasts with both butadiene and isoprene polymerization. In fact, when the polymerization temperature was increased from −78 to 50° the microstructure varied gradually from 86 to 42 mol % of 1,2 units. They attributed this behavior to the steric hindrance involved in the 1,2 structure propagation. On the same basis they suggested that the polymers prepared above −30°, which contain nearly 50 mol % of 1,2 units, have alternating arrangements of 1,2 and 1,4 structures.

In a previous paper[2] devoted to the nmr analysis of highly 1,4-PDMP prepared in cyclohexane we have reported that the 220-MHz spectra of PDMB show features in both the methylene and methyl proton regions that are not apparent in spectra recorded at lower fields. In fact, from these spectra it has been possible to determine the distribution of the dyads and triads of the cis-1,4 and trans-1,4 units. With the hope that 220-MHz spectroscopy might also reveal interesting features for PDMB prepared in polar solvents, we have investigated the nmr spectra of PDMB prepared with *n*-butyllithium in pure tetrahydrofuran at −45 and 25° as well as those of PDMB prepared in cyclohexane in the presence of a small amount of tetrahydrofuran at 25°.

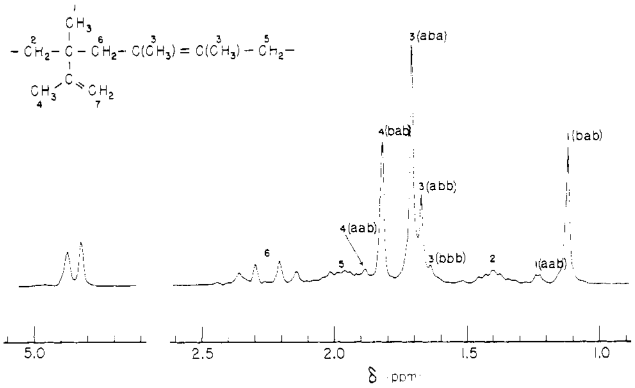


Figure 1. Nmr spectrum (220 MHz) of poly (2,3-dimethyl 1,3-butadiene) prepared at 25° in cyclohexane containing 1% v/v of tetrahydrofuran. Chlorobenzene solution at 100° with tetramethylsilane as reference.

Experimental Section

All preparations were carried out in sealed high-vacuum systems. 2,3-Dimethyl-1,3-butadiene of a purity of 99.9% (vapor-phase chromatography) was degassed on the vacuum line, submitted to a partial prepolymerization with *n*-BuLi, and distilled into a flask where it was stored over calcium hydride. Tetrahydrofuran was refluxed over sodium, fractionally distilled, degassed, and stored in a flask containing sodium–potassium alloy. Cyclohexane was purified by the usual method and distilled over *n*-BuLi before use. Polymerizations were carried out as follows.

Solvent (100 ml) and 15 ml of monomer were transferred to a flask which had been evacuated, degassed, and placed in a Dry Ice–acetone bath. Then 0.2 ml of a 1.6 *M* solution of *n*-BuLi in hexane (as received from Foote Mineral Co.) was added by syringe through a serum cap and the flask was sealed and immediately placed in a thermostat where the reaction was allowed to proceed for 7 days. The reaction mixture was killed by adding a small amount of methanol and the polymer was isolated by pouring the solution into methanol containing 0.03% of 2,6-di-*tert*-butyl-4-methoxyphenol.

The 220-MHz nmr spectra were measured at 100° with a Varian HR 220 spectrometer using chlorobenzene as solvent and tetramethylsilane as internal reference. Sample concentrations were close to 10%. The individual peak outlines were constructed by hand on expanded scale spectra. Peak areas were determined with a planimeter.

Results and Discussion

Figure 1 shows the spectrum of PDMB prepared at 25° in cyclohexane containing 1% v/v of tetrahydrofuran. A quite similar spectrum was obtained for PDMB prepared at the same temperature in pure tetrahydrofuran. Figure 2 shows the spectrum of PDMB prepared at −45° in pure tetrahydrofuran. As expected these two spectra reveal much more detail than those measured at 100 MHz.[1] The resonances of almost all groups of protons are strongly affected by the variation of the microstructure of the polymer. This makes the spectra capable of yielding useful information concerning the triad distribution of the 1,2 and 1,4 units. Evaluation of the 1,2 content from the olefinic proton intensities at 4.8–4.9 ppm yields 45 and 75% of 1,2 units in polymers prepared at 25 and −45°, respectively. The first result is in good agreement with that published by Yuki, *et al.*, but the later is slightly higher than that reported by these authors for the same system.

Peak assignments given in Figures 1 and 2 as well as the discrimination of triad sequences of the 1,2 and 1,4 units are based on the relative 1,2 content of the two polymers. For the sake of simplicity, the 1,2 and 1,4 structures are designated by the letters a and b, respectively. No other specification is given concerning the 1,4 structure because it is believed that one of the isomeric cis or trans forms is dominant in these polymers. This is evidenced by the sin-

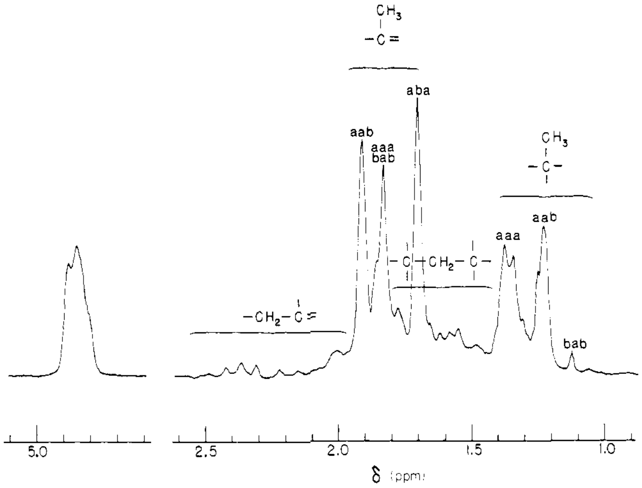
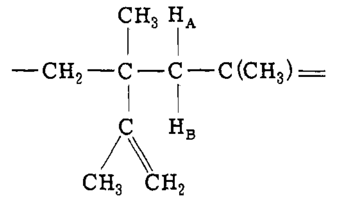


Figure 2. Nmr spectrum (220 MHz) of poly(2,3-dimethyl-1,3-butadiene) prepared at −45° in pure tetrahydrofuran. Chlorobenzene solution at 100° with tetramethylsilane as reference.

gle sharp peak observed for the CH_3-C– resonance at 1.12 ppm and for the CH_3—C= resonance at 1.82 ppm; both are attributed to bab triads. Moreover on the basis of the previous work done on highly 1,4-PDMB, the chemical shift of the 1,4-methyl resonance, which appears close to 1.70 ppm in the present case, would suggest the predominancy of the cis form for the 1,4 structure.

Analysis of the spectrum in Figure 1 confirms remarkably the previous interpretation of Yuki, *et al.* The polymers prepared at 25° are nearly alternating copolymers of 1,2 and 1,4 monomer units. This can be concluded first from the predominancy of the methyl resonance observed at 1.12 ppm characteristic of the bab triads and second from the intensity of the methylene resonance appearing as an AB quartet (J = |13.5| Hz, $\Delta\nu$ = 33.4 Hz) centered at 2.24 ppm characteristic of the 1,4–1,2 linkages.



According to the measured triad intensities which are given in Table I, the polymer of Figure 1 conforms to a first-order Markov model[3] characterized by the following probabilities

$$P_{a/a} = 0.054$$

$$P_{a/b} = (1 - P_{a/a}) = 0.946$$

$$P_{b/b} = 0.160$$

$$P_{b/a} = (1 - P_{b/b}) = 0.840$$

where $P_{i/j}$ means the probability that the monomer adds in j fashion to an i chain end. Thus $P_{a/b}$ and $P_{b/a}$ probabilities are close to unity as expected for alternating placements of a and b units. Dyad frequencies are not shown in Table I since the methylene resonances lead to overlapping multiplets. Nevertheless, the intensity of the AB quartet centered at 2.24 ppm is also in good agreement with the ab dyad frequency of 0.89 one can predict from the postulated first-order Markov model. Here the notation ab is used for both the ba and ab dyads which are equally numerous. The former are characterized by the AB quartet and the latter by the multiplet centered near 1.97 ppm.

Table I
First-Order Markov Model Fitting for PDMB Prepared at 25°

Triad	Obsd intensities	Predicted $P_{a/a}$ = 0.054 $P_{b/b}$ = 0.160
aaa		0.001
aab	0.05	0.048
bab	0.42	0.420
bbb	0.01	0.014
abb	0.15	0.142
aba	0.37	0.374

Table II
Bernoullian Model Fitting for PDMB Prepared at −45°

Triad	Obsd intensities	Predicted P_a = 0.76
aaa	0.44	0.44
aab	0.30	0.28
bab	0.02	0.04
bbb	0.24	0.01
abb		0.08
aba		0.14

Analysis of the spectrum in Figure 2 shows that the monomer units in the polymer prepared at −45° are considerably more randomly distributed. In fact, as shown in Table II, a fairly close agreement is obtained between the measured aaa, aab, and bab triad intensities and the predicted frequencies based on a Bernoullian model[3] assuming a value of 0.76 for the probability of 1,2 placements. This value is close to the 1,2 content (0.75) measured from the vinyl resonance. Unfortunately, it is impossible to determine the aba, abb, and bbb triad intensities in the present case since the 1,4-methyl resonance appears as a broad peak indicating that the aba and abb triads are no longer resolved for this polymer rich in 1,2 units. On the other hand, another complication arises from the fact that a part of the aa dyad methylene resonance lies under the 1,4-methyl resonance.

We ignored the stereochemistry of the 1,2 sequences in the above analysis. In fact, examination of the upfield methyl resonance (aaa and aab triads) as well as that of the aa dyad methylene in Figure 2 would suggest that the stereochemical m and r dyads and also the stereochemical mm, mr, and rr triads could be discriminated in the present case. Nevertheless such an interpretation should be done with care since the fine structures observed for these resonances might rather be due to longer sequence discrimination of the 1,2 and 1,4 units. It should also be mentioned that the aab triads were not distinguished from the baa triads in the above analysis. In fact, these two types of sequences are not chemically equivalent. Nevertheless, they should be observed close together with equal intensities regardless of the 1,2 content. A similar omission was done for the abb triads.

Measurements of the (1,4)/(1,2) microstructure ratio have been obtained over a 129° polymerization temperature range by Yuki, *et al.* The values ranged from 0.16 at −78° to 1.38 at 50° and the Arrhenius plot of the data showed a break at −30° indicating a change in the mechanism of the polymerization around this temperature. The results of the present nmr analysis confirm the existence of the two mechanisms proposed by these authors. At sufficiently low temperature the polymerization may lead to continuous arrangements of 1,2 units and may be described by Bernoullian statistics. At higher temperature, owing to steric hindrance, consecutive 1,2 placements are restricted ($P_{a/a} \simeq 0$) and the 1,2 units are no longer randomly distributed.

Acknowledgments. The authors thank the Canadian 220-MHz nmr Centre for making the nmr measurements. This work is supported by the National Research Council of Canada and the Quebec Ministry of Education.

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(3) This model has been discussed in detail by F. A. Bovey, "High Resolution NMR of Macromolecules," Academic Press, New York, N. Y., 1972.

Electrophoretic Light Scattering on Calf Thymus Deoxyribonucleic Acid and Tobacco Mosaic Virus

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Electrophoretic light scattering is a technique which combines electrophoresis with laser light-scattering spectroscopy.[2] In 1971, Ware and Flygare introduced electrophoretic light scattering by measuring the electrophoretic mobility of bovine serum albumin[3] and mobilities in mixtures of bovine serum albumin with fibrinogen.[4] Later, Uzgiris used this technique to measure the electrophoretic mobility of human erythrocytes, a bacterium (*Staphylococcus epidermidis*), and 0.357 μ polystyrene latex spheres.[5] Bennett and Uzgiris have also demonstrated the technique on 0.81 μ polystyrene latex spheres using a square wave AC field.[6] For further details, the reader is referred to views of the technique by Ware[7] and Flygare, Ware, and Hartford.[8]

Presently, electrophoretic light scattering has not been applied to systems of nucleic acids or viruses. We have applied this technique to the measurement of the electrophoretic mobility of calf thymus DNA and tobacco mosaic virus as a function of ionic strength. The results at high ionic strength are in agreement with measurements made using the Tiselius moving boundary electrophoresis technique.[9-13] Our measurements are reliably extended to low ionic strengths, and for TMV lower than any previous measurements. We find that the electrophoretic mobility is inversely proportional to the square root of the ionic strength at low ionic strength and approaches a constant value at higher ionic strength.

We have also measured the electrophoretic mobility of the denatured form of calf thymus DNA and have compared it to the native form. The mobilities obtained were $u_{\text{native}}(20°) = 5.0 \times 10^{-4}$ cm^2 sec^{-1} V^{-1} and $u_{\text{denatured}}(20°) = 4.2 \times 10^{-4}$ cm^2 sec^{-1} V^{-1} at 0.01 *M* NaCl and pH 7.0. These values and the approximately 15% difference between them agree with measurements made by the Tiselius technique.[11,12]

In addition, we have improved the design of the electrophoretic light-scattering cell invented by Ware and Flygare.[14] The improved design makes optical alignment of the cell much easier. Also the molecules in the scattering region are prevented from making direct contact with the electrodes.

Theory

When an electric field is applied to charged molecules, they will move in the direction of the electrode of opposite polarity. Laser light which is scattered from these molecules will be Doppler shifted. As the molecules migrate they also experience random Brownian motion which Doppler broadens the scattered light. The resulting spectrum is a Lorentzian shifted by a frequency proportional to the electrophoretic mobility with a half-width proportional to the translational diffusion coefficient. The theory has been worked out in detail by Flygare and Ware,[3] and we only summarize the result here. The heterodyne spectrum for the experimental geometry in Figure 1 is given by the equation

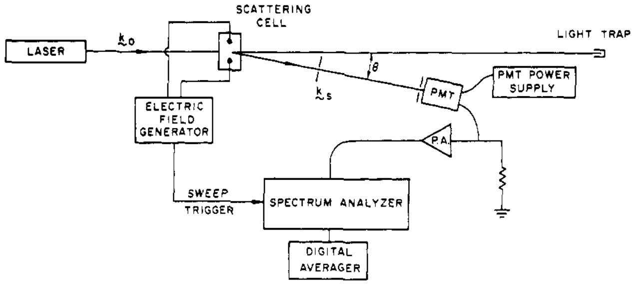

Figure 1. Block diagram of the experimental apparatus.

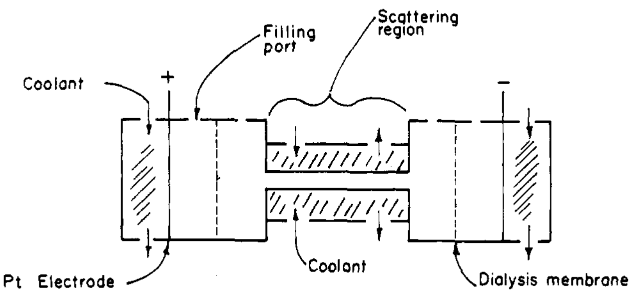

Figure 2. Cross-section of the electrophoretic light-scattering cell.

$$I(\omega) = NA^2\left[\frac{DK^2/\pi}{[\omega - \omega_0 + KuE\cos(\theta/2)]^2 + (DK^2)^2}\right]$$

where N is the number of molecules in the scattering region, A is an amplitude factor, E is the electric field (which is perpendicular to the incident radiation) applied to the molecules, D is the translational diffusion coefficient, θ is the scattering angle, u is the electrophoretic mobility, and K is the scattering vector. K is equal to $(4\pi n/\lambda_0)\sin(\theta/2)$ where n is the index of refraction of the medium and λ_0 is the vacuum wavelength of the incident light.

The half-width at half-height of the Lorentzian spectrum is equal to $DK^2/2\pi$. The magnitude of the Doppler shift is equal to $KuE\cos(\theta/2)/2\pi$. Therefore, one can simultaneously measure the translational diffusion coefficient, D, and the electrophoretic mobility, u, from the spectrum.

Experimental Section

Figure 1 is a diagram of the experimental apparatus. The laser source is a Spectra-Physics Model 165-03 argon-ion laser which is operated in a single mode of the 5145-Å resonance. Amplitude fluctuations in the laser are reduced by monitoring the output with a servo regulator which adjusts the power supply current to keep the intensity constant. The laser beam is focused into the electrophoretic light-scattering cell. The scattered light is collimated by pinholes and directed onto an RCA 7265 phototube. Enough light is scattered from the front window of the cell to serve as a local oscillator signal at the phototube. The signal from the phototube is preamplified by a Princeton Applied Research Model 113 preamplifier which is then the input into a Federal Scientific UA 15A