Table II
Electrophoretic Mobilities in Native and Denatured Calf Thymus DNA

	$u_{native}(0°) \times 10^4$ cm^2 sec^{-1} V^{-1}	$u_{denatured}(0°) \times 10^4$ cm^2 sec^{-1} V^{-1}
This work	2.8	2.35
Ref 11	2.3	2.1
Ref 10	3.2	
Ref 12	2.17	1.90

used electrophoretic light scattering cells that have scattering regions with 0.2 and 0.4 cm^2 cross-sections and we have directed the laser light beam into different regions of the cross-sections, and in all cases we observe no changes in the frequency shifts (electrophoretic mobility). In addition, the frequency shifts are independent of preconditioning the cell with protein. Our consistent results agree with earlier conclusions on the negligible effects of electroosmosis.[7,21]

Conclusion

Our measurements demonstrate that electrophoretic light scattering is well suited for measuring the electrophoretic mobilities of nucleic acids and viruses. This work and previous works[3-5] demonstrate that electrophoretic light scattering is a reliable way to measure electrophoretic mobilities from small proteins up to large bacteria. The technique has the following advantages over the conventional moving boundary technique: the technique is much faster; the experiment is done at equilibrium and does not require the build-up of unstable concentration gradients; instantaneous velocities are measured instead of long time migration of the molecules; the measurements can readily be done at higher temperatures, and measurements of lower ionic strengths can be made.

Acknowledgment. We acknowledge the work of I. Chabay in modifying the electrophoretic light-scattering cell. We would also like to thank Drs. Russel Steere and Arthur Knight for their generous samples of tobacco mosaic virus.

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Reaction of Vinylidene Cyanide with Styrene. Structure of the Cycloadduct and Copolymer

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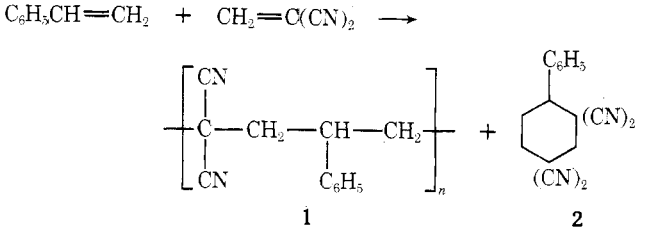
The reaction of vinylidene cyanide with styrene spontaneously yields an alternating copolymer and a product composed of one molecule of styrene and two molecules of vinylidene cyanide.[1-5] It has been suggested that the products arise from two competing reactions, although it was reported[1] that the trimer was formed only in the presence of large amounts of a free radical inhibitor, *tert*-butylhydroquinone. Oxidation of this trimer was also reported to yield phthalic acid, and 1-(2,2-dicyanoethyl)-4,4-dicyano-1,2,3,4-tetrahydronaphthalene was suggested as the structure of the product.

The reaction of styrene and vinylidene cyanide in benzene at 25° spontaneously yields an alternating copolymer (1) and the cycloadduct, 1,1,3,3-tetracyano-4-phenylcyclohexane (2) (Table I). The cycloadduct had the correct elemental analysis and showed a parent ion in the mass spectrum, *m/e* 260. Both the ^{1}H and ^{13}C nmr spectra were consistent with the assigned structure (Table II).

Table I
Reaction of Vinylidene Cyanide (VC) with Styrene (ST) in Benzene at 25°

Molar ratio (VC/ST)	% yield[a] Cycloadduct	% yield[a] Copolymer	η_{sp}/c of copolymer[b]
2.17	20	78	0.45
1.45	23	53	0.65
0.73	33	49	0.57
0.47	30	53	0.69

[a] % yields were calculated with respect to the reactant present in the smaller molar amount. [b] Reduced viscosities were taken in dimethylformamide solutions, concentrations varying from 0.35 to 0.45 g/100 ml at 30°.

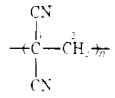
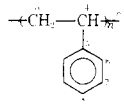
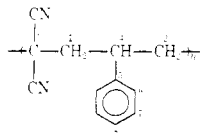
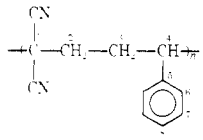
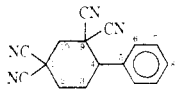


1 2

The copolymer was soluble in acetonitrile but insoluble in toluene. The composition of the copolymer was consistently 1:1, regardless of the feed ratio (Table III). The absence of ^{13}C absorption in the copolymer at 66.02 ppm corresponding to the C-2 of poly(vinylidene cyanide) and at 46 ppm corresponding to C-4 of polystyrene established the absence of blocks of polystyrene or poly(vinylidene cyanide) in the copolymer. The absence of absorption near 17.5 ppm excludes a head-to-head structure and supports the head-to-tail copolymer.

Thus, the closest intermediate, to both the cycloadduct and the copolymer, is perhaps a donor–acceptor complex since the cycloadduct is a product of a head-to-head addition of styrene and vinylidene cyanide, while the copolymer is head-to-tail.

Table II
^{13}C Nmr Spectra

Compd	Solvent	Chemical shift, obsd (calcd)[a] C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	CN
	DMSO-d_6	30.2 (28.2)	66.0 (50.3)									113.6
	$CDCl_3$			40.8 (34.6)	46.0 (35.4)	145 (137)	128 (C6, C7) (129)	(128)	126 (126)			
	DMSO-d_6	33.6 (31.0)	43.0 (41.7)		34.0 (27.2)	138 (137)	128 (C6, C7, and C8) (129)	(128)	(126)			113.6
	DMSO-d_6	33.6 (32.4)	34.0 (35.8)	(17.5)	43.0 (45.6)	138 (137)	128 (C6, C7, and C8) (129)	(128)	(126)			113.6
	$CDCl_3$	30.3 (29.0)	37.1 (40.2)	21.0 (17.3)	36.1 (34.0)	136 (137)	131 (129)	130 (128)	127 (126)	30.5 (30.7)	42.3 (47.1)	115.2 115.3

^1H nmr (CD_3CN) δ 7.58 (m, 5, $5H_{6-8}$), 4.20 (q, 1, H_4), 3.63 (m, 1, H_{10}), 2.20 (m, 1, H_{10}), 2.50 (m, 4, $2H_3 + 2H_4$).

[a] See ref 6 for method of calculation. [b] ^{13}C nmr spectrum of poly(styrene) has been reported in ref 7.

Table III
Elemental Analyses of the Copolymer of Vinylidene Cyanide and Styrene

Molar ratio (VC/ST)	Elemental analysis: Calcd C	H	N	Found C	H	N
0.47	79.09	5.53	15.38	79.32	5.26	15.58
0.73				79.43	5.19	15.07
1.45				79.12	5.29	15.28
2.17				79.32	5.41	15.16

Experimental Section

Vinylidene cyanide and benzene were synthesized and purified as previously described.[8] Styrene was dried over calcium hydride and fractionally distilled. The center cut of the distillate was stored over sodium metal in an ampoule under reduced pressure.

The reactions of vinylidene cyanide and styrene were carried out in benzene with different molar ratios under reduced pressure (10^{-4} mm), inside the reaction vessel, which has been previously described.[8] The following reaction procedure is illustrative.

Vinylidene cyanide (0.99 g, 1.0 ml, 12.7 mmol) and 20 ml of degassed benzene were distilled into ampoule A by trap-to-trap distillation and the ampoule was sealed at constriction C under reduced pressure. Into ampoule B, 0.91 g (1.0 ml, 8.7 mmol) of styrene and 20 ml of degassed benzene were transferred by trap-to-trap distillation. The ampoule was sealed at constriction D under reduced pressure. The reaction solution was allowed to mix inside one ampoule *via* the breakable seal E. Upon mixing of the reactants, no color developed but a white precipitate gradually formed. After 3 days, the solvent was transferred to the empty ampoule which had been cooled in liquid nitrogen. The ampoule was then opened. The residue was fractionated into two fractions by extraction with toluene. The toluene soluble fraction (0.30 g) was a white crystalline solid, which was identified as 1,1,3,3-tetracyano-4-phenylcyclohexane: mp 136–138°; ^1H nmr (CD_3CN) δ 7.58 (m, 5 H), 4.20 (q, 1 H), 3.63 (m, 1 H), 2.20 (m, 1 H), and 2.50 ppm (m, 4 H). ^{13}C nmr data are reported in Table II. The mass spectrum (70 eV) showed a parent peak at *m/e* 260. *Anal.* Calcd for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.53. Found: C, 73.61; H, 4.66; N, 21.57.

The toluene insoluble fraction was dissolved in acetonitrile. Addition of toluene into the acetonitrile solution afforded a white precipitate (0.84 g). The compound was identified as an alternating copolymer of vinylidene cyanide and styrene. ^{13}C nmr data are reported in Table II. *Anal.* Calcd for $C_{12}H_{10}N_2$: C, 79.09; H, 5.53; N, 15.38. Found: C, 79.12; H, 5.29; N, 15.28.

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The Morphology of Polystyrene–Polybutadiene–Polystyrene Three-Block Copolymers

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It has been reported[1] recently that polystyrene–polybutadiene–polystyrene (SBS) three-block copolymers with constant composition (≃30% by weight of polystyrene) and overall molecular weights M_n ranging from 4.9×10^4 to 9.0×10^5 g mol^{-1} show morphologies characterized by spherical domains of polystyrene arranged on a simple face centered cubic lattice. The study involved low-angle X-ray scattering using films cast from a 10:90 mixture of methyl ethyl ketone and tetrahydrofuran. The conclusions were found to be in good agreement with the theory of Meier.[2]

We have some experimental results which are in disagreement with these conclusions.

A well-characterized SBS copolymer,[3] having a polystyrene content of about 33% by weight, molecular weight M_w of 1.02×10^5, and a rather low heterogeneity ($M_w/M_n \simeq 1.2$) was used to prepare thin films by casting dilute solutions (≃1% w/v). The evaporation rate was controlled and was rather slow (0.5 cm^3/hr) at the temperature of 50 ± 0.5°; this operation was performed on a mercury surface in order to obtain uniformly thin films (0.2–0.4 mm) suitable for testing in a stress–strain dynamometer. The films were completely dried under vacuum at 50°. The solvents used to prepare the solutions were different mixtures of methyl ethyl ketone and tetrahydrofuran (90:10, 50:50, and 10:90).

The morphological study was performed on ultrathin sections (about 500 Å thick) obtained by low-temperature microtomy[4] after inclusion of the specimens in an epoxy resin.[5] The sections were cut perpendicular to the surface of the films and were stained with osmium tetroxide.[5] Therefore, in the following pictures the polystyrene phase appears bright and the rubbery matrix dark.

Figure 1 shows the electron micrographs of samples obtained both from pure methyl ethyl ketone and from its mixtures with tetrahydrofuran. Clearly, the morphology of our specimens is always characterized by lamellae, roughly parallel to the surface of the films. The thickness of the polystyrene lamellae does not seem to change systematically with the solvent composition and it is about 120–150 Å. The distance between two consecutive lamellae is about 300 Å. These values correspond to the regions where the lamellae seem to be perpendicular to the section plane. We have not been able to find regions where the observed structure could be correlated with a face centered cubic arrangement of spherical polystyrene domains.[6]

Obviously our results are completely in disagreement with earlier studies by Campos-Lopez and coworkers.[1] We do not have any definitive explanation for this.

Actually small differences in the polystyrene content of the copolymer cannot produce such different morphologies. By solution casting from methyl ethyl ketone we obtained a lamellar structure like that of Figure 1a using a copolymer having a lower polystyrene content, *i.e.*, 26% by weight.[7]

The molecular weight brings us to the same conclusions. Campos-Lopez and coworkers[1] found the same structure for very different M_n values, as stated at the outset.

Only the evaporation rate of the solvent and the thermal

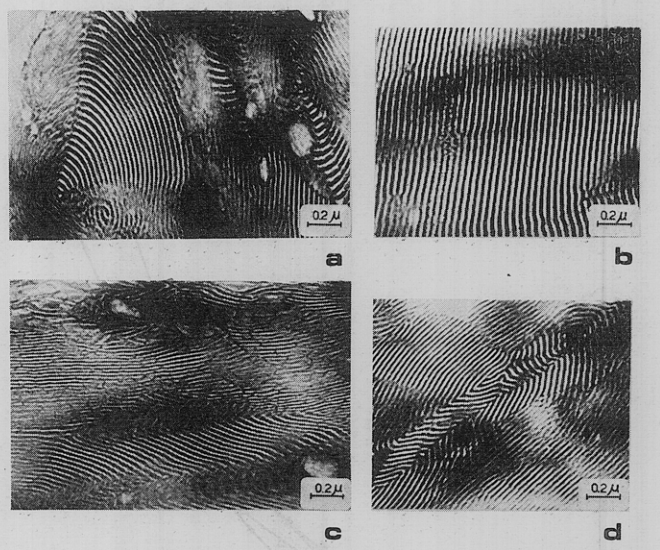

Figure 1. Electron micrograph of osmium-stained ultrathin sections of SBS copolymer films cast from dilute solutions: (a) methyl ethyl ketone; (b) methyl ethyl ketone–tetrahydrofuran mixture, 90:10; (c) methyl ethyl ketone–tetrahydrofuran mixture, 50:50; (d) methyl ethyl ketone–tetrahydrofuran mixture, 10:90

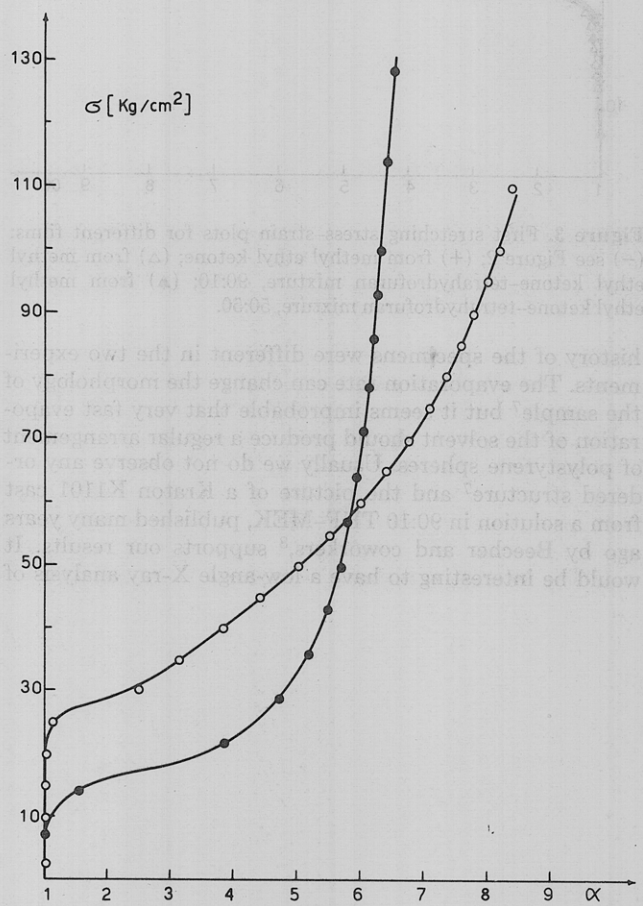

Figure 2. Stress–strain plot for the 10:90 methyl ethyl ketone–tetrahydrofuran film: (○) first stretching; (●) second stretching after relaxation of 10 min at zero load.