

the same factor,  $\exp(-E_s/RT)$ . However, if the stabilization energy is different for intrachain reaction and for intermolecular reaction, its effect is not canceled and values of  $k_1/k_2$  are considerably affected. An increase in the intrachain stabilization energy as small as 0.6 kcal/mol makes  $k_1/k_2$  about 2.7 times larger than its original value. At present this is the most likely reason for the discrepancy, although the origin of the energy difference is not known.

The experimental value of the cyclization constant did not show a clear maximum in the short-chain-length region. To discuss this point in more detail, however, studies on the shorter chains having definite chain lengths are needed. It can be mentioned here that in the polymerization of DL-phenylalanine *N*-carboxyanhydride, initiated by poly(sarcosine)dimethylamide<sup>29</sup> and other poly(*N*-alkylglycine)dimethylamides,<sup>30</sup> the intrachain reaction rate constant showed a distinct maximum at  $n = 7-9$ . This is in agreement with the present calculation.

To conclude, the calculated dependence of the cyclization constant on the chain length was consistent with the experimental one, but the agreement of the absolute values between the calculation and the experiment was not perfect.

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## Densities of Polymer Solutions to 1 kbar

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Received November 3, 1975

**ABSTRACT:** Volumetric measurements are reported for mixtures of polyisobutylene with benzene and with cyclohexane and for mixtures of polydimethylsiloxane with hexamethyldisiloxane and with cyclohexane. Measurements from 25 to 65 °C extend to 1 kbar. The experimental results show that the pure liquids, as well as their mixtures, are more compressible than predicted by Flory's equation of state.

During the last 10 years, the theory of polymer solutions, as developed by Flory, Patterson, and others, has given primary attention to "equation-of-state" effects, i.e., to that contribution to the solution's thermodynamic excess functions which can be ascribed to free-volume differences between polymer and solvent. At atmospheric pressure and at temperatures near the normal boiling point of the solvent, the free volume of the solvent is generally larger than that of the polymer. This difference in free volume produces significant contributions to both the excess entropy and the excess enthalpy. At higher temperatures, when the solvent is already well expanded, these free-volume differences become a dominant influence on the solution's properties, often leading to phase instability with a lower critical solution temperature.<sup>1</sup> To calculate the effect of free-volume differences, we require an equation of state and it has become common practice to use an equation of the van der Waals type, proposed by Flory.<sup>2</sup> In this work we study the applicability of Flory's equation to volumetric properties of some polymer solutions. Toward that end, we have ob-

tained experimental data for the densities of four, binary polymer-solvent systems at pressures to 1 kbar.

## Experimental Section

For high-pressure studies, the experimental equipment is the same as that used by Beret;<sup>3</sup> the central component is a high-pressure cell with a flexible bellows to contain the sample and to isolate it from the pressure-transmitting fluid.<sup>4</sup> The apparatus is calibrated with highly accurate PVT data for mercury and for heptane.<sup>3</sup>

Pressures are measured with a precision Heise gauge whose accuracy is 0.1% of full scale ( $\pm 1$  bar).

The high-pressure cell is located in a constant-temperature bath using silicone oil as heating fluid. The bath is controlled to  $\pm 0.01$  °C by a Hallikainen proportional temperature controller. Calibrated thermometers, used for temperature measurements, are accurate to  $\pm 0.1$  °C.

Taking into consideration errors in temperature and pressure measurements as well as errors in calibration, the overall uncertainty of our relative volume measurements is 0.04%.

For measuring specific volumes at atmospheric pressure, a conventional glass dilatometer was used. The dilatometer was cali-

Table I  
Specific Volumes of Mixtures at Low Pressure<sup>a</sup>

System	Vol fraction of polymer	$A^{(1)}$	$A^{(2)} \times 10^3$	$A^{(3)} \times 10^6$	Stand dev $\times 10^4$
PIB–B	0.1989	1.101 02	1.109 65	1.562 20	1.03
	0.4355	1.094 12	1.056 58	–0.600 33	1.74
	0.5923	1.090 88	0.902 93	0.152 81	0.46
PIB–C	0.1971	1.195 08	1.211 36	2.156 55	0.49
	0.3731	1.170 33	1.079 70	0.925 79	1.15
	0.5445	1.146 72	0.911 38	0.812 16	1.41
PDMS–C	0.3530	1.077 07	3.347 60	–0.019 03	2.22
	0.5017	1.106 46	1.131 89	1.246 03	1.12
	0.4370	1.125 18	1.382 01	0.729 86	0.84
PDMS–HMDS	0.6497	1.049 32	1.809 18	–3.752 08	2.50

<sup>a</sup> Coefficients in eq 1.

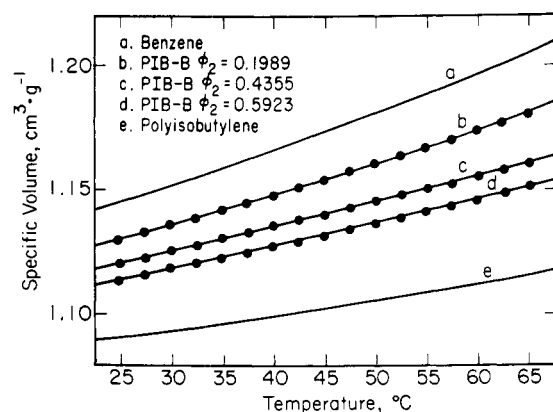


Figure 1. Specific volumes at atmospheric pressure for the system polyisobutylene–benzene.

brated with mercury at 22 and 70 °C. Readings of the meniscus in the capillary were made with a cathetometer to  $\pm 0.5$  mm. The calibrated dilatometer was tested with carefully degassed *n*-dodecane. In the temperature range 25 to 72.5 °C, measured results agreed with those reported by Rossini<sup>5</sup> to within better than  $\pm 0.01\%$ . The maximum deviation was  $3 \times 10^{-4}$  cm<sup>3</sup>/g.

Polymer solutions were prepared gravimetrically from thoroughly degassed polymer and solvent.

Experimental measurements were made for mixtures of polyisobutylene (PIB) with benzene (B) and with cyclohexane (C), and for mixtures of polydimethylsiloxane (PDMS) with cyclohexane and with hexamethyldisiloxane (HMDS).

## Results

For each PIB solvent system, measurements were made at three compositions. For each PDMS–solvent system,

measurements were made at two compositions. These are shown in the second column of Table I.

For the region 25–65 °C, measurements at low pressure are represented by the empirical equation

$$v_{sp}^0 = A^{(1)} + A^{(2)}t + A^{(3)}t^2 \quad (1)$$

where  $v_{sp}^0$  is in cm<sup>3</sup>/g and  $t$  is in degrees Centigrade. As indicated in Table I, coefficients  $A^{(1)}$ ,  $A^{(2)}$ , and  $A^{(3)}$  are functions of composition. The final column of Table I shows that eq 1 reproduces the experimental data well within experimental uncertainty.

Figure 1 shows experimental results at atmospheric pressure for the PIB–B system. Also shown are results for pure PIB<sup>6</sup> and for pure benzene.<sup>7</sup>

Measurements at high pressure are represented by Beret's form of the Tait equation:<sup>3</sup>

$$v_{sp}/v_{sp}^0 = 1 - c \ln \left[ 1 + \frac{P}{b^{(1)} \exp(-b^{(2)}t)} \right] \quad (2)$$

where  $v_{sp}$  is the specific volume at pressure  $P$  and  $v_{sp}^0$  is the specific volume at zero pressure. Temperature  $t$  is in °C and the pressure is in bars.

For the temperature range 25–65 °C and for pressures to 1 kbar, Tait coefficients are given in Table II. The last column of that table shows that the Tait equation represents the experimental data well within experimental uncertainty.

Tables given in the supplementary material report original data at 25, 35, 45, 55, and 65 °C.

Figure 2 shows relative volumes at 65 °C for the systems PIB–B and PIB–C. For completeness, Figure 2 also shows new results for pure benzene and cyclohexane and pre-

Table II  
Specific Volumes at High Pressure<sup>a</sup>

System	Vol fraction of polymer	$c \times 10^2$	$b^{(1)} \times 10^{-3}$ , bar	$b^{(2)} \times 10^3$ , °C <sup>-1</sup>	Stand dev $\times 10^4$
PIB–B	0	9.732 970	1.210 153	8.044 481	4.81
	0.1989	8.966 583	1.251 642	7.851 594	4.97
	0.4355	8.911 763	1.376 756	6.862 239	3.15
	0.5923	8.211 724	1.381 603	6.473 317	3.74
PIB–C	0	8.967 567	0.982 769	8.461 492	6.44
	0.1971	9.125 086	1.114 994	7.853 660	5.26
	0.3731	8.066 102	1.134 639	7.460 571	5.06
	0.5445	8.122 835	1.281 160	6.457 454	3.36
PDMS–C	0.3530	9.136 689	0.939 672	7.512 650	7.66
	0.5017	9.061 028	0.962 103	7.913 653	7.07
PDMS–HMDS	0	9.496 673	0.613 347	9.840 095	17.25
	0.4370	9.227 156	0.701 099	7.974 029	12.83
	0.6497	8.568 493	0.681 781	7.013 003	11.34

<sup>a</sup> Coefficients in eq 2.

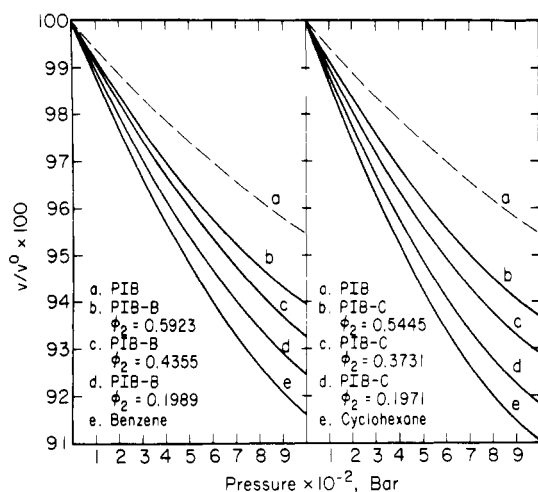


Figure 2. Effect of pressure on volume. Experimental results at 65 °C.

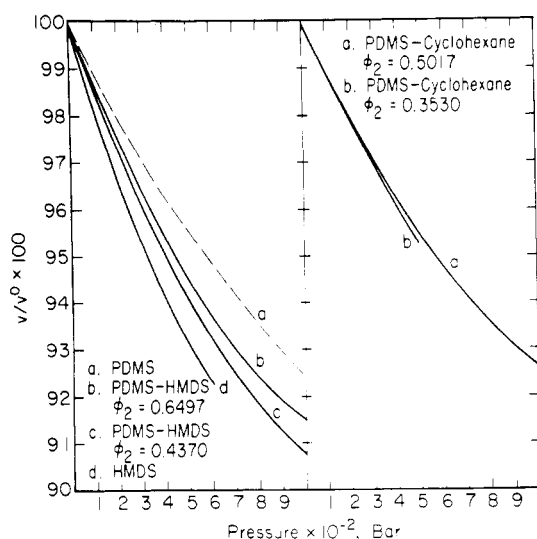


Figure 3. Effect of pressure on volume. Experimental results at 25 °C.

viously published data for pure PIB.<sup>3</sup> Figure 3 gives similar results at 25 °C for the systems PDMS-HMDS and PDMS-C. Figures 2 and 3 indicate once again that the compressibility of a liquid solvent is appreciably larger than that of a polymer.

In Figure 3, left side, line d terminates at 600 bars because for highly compressible HMDS, the limit of the bellows compression was reached at that pressure. In Figure 3, right side, line b terminates at 500 bars because of indica-

tions that a phase change (freezing?) may be occurring at higher pressures. Benzene at 25 and 35 °C, cyclohexane at 25, 35, and 45 °C, and dilute polymer solutions using these solvents also appear to undergo a phase change at high pressures as indicated in the table of original data in the supplementary material.

### Comparison with Flory's Equation

As discussed elsewhere,<sup>8</sup> Flory's equation relates reduced pressure  $\tilde{P}$  to reduced volume  $\tilde{v}$  and reduced temperature  $\tilde{T}$  by

$$\frac{\tilde{P}\tilde{v}}{\tilde{T}} = \frac{\tilde{v}^{1/3}}{\tilde{v}^{1/3} - 1} - \frac{1}{\tilde{v}\tilde{T}} \quad (3)$$

where  $\tilde{P} = P/P^*$ ,  $\tilde{v} = v/v^*$ , and  $\tilde{T} = T/T^*$ . For a pure fluid, characteristic parameters  $P^*$ ,  $v^*$ , and  $T^*$  are evaluated from low-pressure density data. The data required are the specific volume  $v_{sp}$  at some temperature, the thermal expansion coefficient  $\alpha$ , and the thermal pressure coefficient  $\gamma$  [ $\alpha = 1/v(\partial v/\partial T)_P$  and  $\gamma = (\partial P/\partial T)_v$ ].

Characteristic parameters for the pure fluids are given in Table III, which also indicates sources of experimental data used in their determination.

For a mixture, eq 3 is used in conjunction with the one-fluid theory which assumes that the volumetric properties of the liquid mixture are identical with those of a hypothetical pure fluid whose characteristic parameters are simple functions of the composition; these functions, often called "mixing rules", lead to a reduced temperature for the mixture

$$\tilde{T} = \frac{1}{P^*} (\phi_1 P_1^* \tilde{T}_1 + \phi_2 P_2^* \tilde{T}_2) \quad (4)$$

where

$$P^* = \phi_1 P_1^* + \phi_2 P_2^* - \phi_1 \theta_2 X_{12} \quad (5)$$

In these equations, subscript 1 refers to solvent and subscript 2 to polymer;  $\phi$  is the segment fraction and  $\theta$  is the surface fraction:

$$\phi_1 = m_1 v_{sp1}^* [m_1 v_{sp1}^* + m_2 v_{sp2}^*]^{-1} \quad (6)$$

and

$$\phi_2 = 1 - \phi_1 \quad (7)$$

$$\theta_2 = (s_2/s_1)\phi_2[\phi_1 + \phi_2(s_2/s_1)]^{-1} \quad (8)$$

and

$$\theta_1 = 1 - \theta_2 \quad (9)$$

In these equations,  $m$  stands for mass and  $s$  stands for the external surface of a segment. Parameter  $X_{12}$  is characteristic of the interaction between unlike segments.

Table III  
Pure-Component Parameters in Flory's Equation of State

Fluid	$v_{sp}^*$ , cm <sup>3</sup> /g		$T^*$ , °K		$P^*$ , bars	
	25 °C	65 °C	25 °C	65 °C	25 °C	65 °C
Benzene <sup>a</sup>	0.8850	0.8989	4691	4875	6203	6008
Cyclohexane <sup>b</sup>	1.0023	1.0086	4742	4813	5340	5198
HMDS <sup>c</sup>	0.9946	1.0047	4420	5422	3579	3309
PIB <sup>d</sup>	0.9493	0.9545	7575	7814	4469	4406
PDMS <sup>e</sup>	0.8395	0.8424	5528	5806	3409	3209

<sup>a</sup> Calculated from densities<sup>7</sup> and thermal expansion coefficients and thermal pressure coefficients from L. A. K. Staveley, W. I. Tupman, and K. R. Hart, *Trans. Faraday Soc.*, 51, 323 (1955). <sup>b</sup> J. M. Scigliano, Ph.D. Thesis, University of Washington, St. Louis, 1972. <sup>c</sup> R. N. Lichtenthaler, D. D. Liu, and J. M. Prausnitz, *Ber. Bunsenges. Phys. Chem.*, 78, 470 (1974). <sup>d</sup> B. E. Eichinger and P. J. Flory, *Macromolecules*, 1, 285 (1968). <sup>e</sup> H. Shih and P. J. Flory, *ibid.*, 5, 758 (1972).

Table IV  
Mixture Parameter  $X_{12}$  (cal/cm<sup>3</sup>) for Four Polymer-Solvent Systems from Density Data at Low Pressure

System	65 °C	45 °C	25 °C	25 °C <sup>e</sup>
PIB-B	1.1	2.4	4.4	10.0 <sup>a</sup>
PIB-C	1.4	1.6	5.5	1.4 <sup>b</sup>
PDMS-C	2.2	3.8	4.9	5.5 <sup>c</sup>
PDMS-HMDS	1.1	1.2	1.4	0.1 <sup>d</sup>

<sup>a</sup> B. E. Eichinger and P. J. Flory, *Trans. Faraday Soc.*, **64**, 2053 (1968). <sup>b</sup> B. E. Eichinger and P. J. Flory, *ibid.*, **64**, 2061 (1968). <sup>c</sup> P. J. Flory and H. Shih, *Macromolecules*, **5**, 761 (1972). <sup>d</sup> R. S. Chahal, W. -P. Kao, and D. Patterson, *J. Chem Soc., Faraday Trans 1*, **69**, 1834 (1973). <sup>e</sup> From enthalpy-of-mixing data.

Table V  
Differences between Calculated (Flory) and Experimental Values of  $v/v^0$  at 1000 bars

System	$\phi_2$	Differences $\times 10^4$	
		25 °C	65 °C
PIB-B	0.0	33 <sup>a</sup>	67
	0.1989	35	56
	0.4355	41	69
	0.5923	41	57
PIB-C	0.0	11 <sup>b</sup>	30
	0.1971	21 <sup>c</sup>	68
	0.3731	31	48
	0.5445	43	52
PDMS-C	1.0	63	61
	0.3530	31 <sup>d</sup>	37
	0.5017	30	41
PDMS-HMDS	1.0	80	101
	0.0	43 <sup>e</sup>	24
	0.4370	89	70
	0.6497	84	74

<sup>a</sup> At 700 bars. <sup>b</sup> At 300 bars. <sup>c</sup> At 400 bars. <sup>d</sup> At 500 bars. <sup>e</sup> At 600 bars.

Table IV reports  $X_{12}$  for four binary systems. Surface area ratios  $s_2/s_1$  were taken from the literature; they are: PIB-B, 0.58;<sup>9</sup> PIB-C, 0.62;<sup>10</sup> PDMS-C, 1.57;<sup>11</sup> and PDMS-HMDS, 0.55.<sup>12</sup> Parameters  $X_{12}$  were obtained from the new binary volumetric data at 1 atm. For comparison, we also show  $X_{12}$  as obtained from calorimetric (heat-of-mixing) data.

It is unfortunate that parameters  $X_{12}$  obtained from volumetric data differ from those obtained from calorimetric measurements. This discrepancy suggests that, perhaps, the assumption of random mixing (eq 4 and 5) may be in error.

Using eq 3–9, specific volumes were calculated as a function of pressure. In this calculation we used the results shown in Tables III and IV, the values of  $s_2/s_1$  quoted above and values of  $v_{sp}^*$  for the mixture as determined from volumetric data represented by eq 1.

Calculated and experimental volumes are compared in Figures 4 and 5 and in Table V. Results are shown for the pure fluids as well as for the mixtures. In every case, the data indicate that the fluids, pure or mixed, are appreciably more compressible than predicted by Flory's equation. This conclusion is not changed when a calorimetrically determined value of  $X_{12}$  is used rather than a value (Table IV) based on volumetric data at 1 atm. For pure fluids, of course, the parameter  $X_{12}$  does not appear in the calculations.

#### Test of Empirical Relations for Mixtures

The new experimental data permit us to test some com-

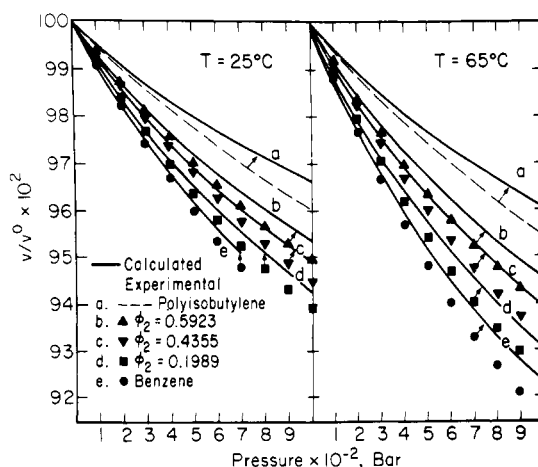


Figure 4. Effect of pressure on volume. Comparison of experimental results with those calculated with Flory's equation.

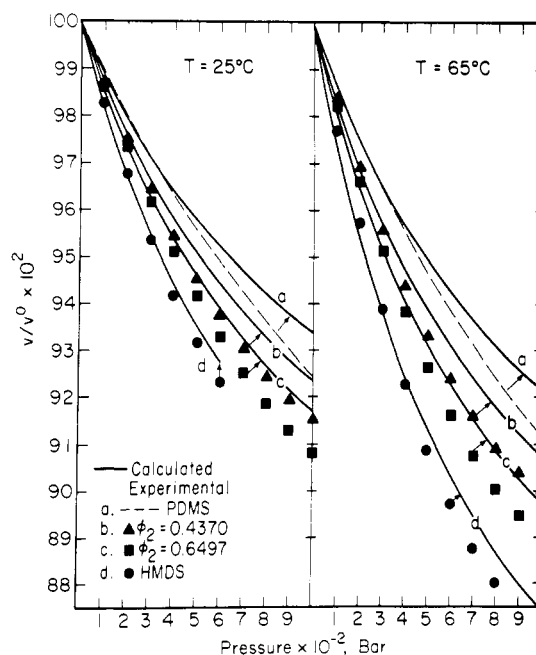


Figure 5. Effect of pressure on volume. Comparison of experimental results with those calculated with Flory's equation.

mon empirical equations for predicting volumetric properties of polymer mixtures from those of the pure components. These empirical equations are linear functions of the volume fraction  $\Phi$ :

$$v^0 = v_1^0\Phi_1 + v_2^0\Phi_2 \quad (A)$$

$$v = v_1\Phi_1 + v_2\Phi_2 \quad (B)$$

$$v/v^0 = (v/v^0)_1\Phi_1 + (v/v^0)_2\Phi_2 \quad (C)$$

$$\beta \equiv -(1/v^0)(\partial v/\partial P)_T = \beta_1\Phi_1 + \beta_2\Phi_2 \quad (D)$$

Equation A refers to low pressures while equations B, C, and D are general. We tested eq A at 1 bar and eq B, C, and D at 800 bars; results at 25 °C are given in Table VI. Similar results are obtained at 65 °C. All of the empirical equations provide good approximations for the mixtures studied here.

Table VI  
Test of Empirical Equations<sup>a</sup>

System	$\Phi$ (polymer)	Deviation, %			
		Eq A	Eq B <sup>b</sup>	Eq C <sup>b</sup>	Eq D <sup>b</sup>
PIB-B	0.1989	0.04	0.11	0.13	2.88
	0.4355	0.09	0.03	0.03	0.64
	0.5923	0.07	0.02	0.06	2.61
PIB-C	0.1971	0.81	0.79	0.06	2.30
	0.3731	1.56	1.23	0.20	3.95
	0.5445	1.03	0.71	0.19	3.41
PDMS-C	0.3530	3.35	3.53	0.16	0.93
	0.5017	6.15	6.17	0.01	1.40
PMDS-HMDS	0.4370	2.74	2.55	0.00	0.90
	0.6497	3.57	3.45	0.06	3.43

<sup>a</sup> Deviations between calculated and measured quantities at 25 °C. Deviation = 100(calcd - meas)/(meas). <sup>b</sup> At 800 bars.

## Conclusion

The volumetric measurements obtained in this work show that at high pressures, Flory's equation of state cannot correctly represent the PVT behavior of pure liquid polymers, pure liquid solvents, and their mixtures, when equation-of-state parameters are evaluated from volumetric data at 1 atm. This deficiency may be significant for polymer-solution thermodynamics since equation-of-state contributions are important in calculating excess functions of polymer mixtures. It may, therefore, be desirable to consider possible improvements in Flory's equation of state.

**Acknowledgment.** For financial support the authors are grateful to the National Science Foundation, the donors of the Petroleum Research Fund, administered by the American Chemical Society, and to the Commission of Educational Exchange between the USA and Spain.

**Supplementary Material Available:** tables for experimental relative volumes (18 pages). Ordering information is given on any current masthead page.

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## Functional Polymeric Microspheres Based on 2-Hydroxyethyl Methacrylate for Immunochemical Studies<sup>1</sup>

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**ABSTRACT:** Co  $\gamma$  irradiation of 2-hydroxyethyl methacrylate in the presence or in the absence of other acrylic monomers was found to constitute an effective technique for the synthesis of hydrophilic functional microspheres in the size range of approximately 0.3 to 3  $\mu$  in diameter. The effect of monomer concentration, steric stabilization, and electrostatic interaction on the particle size was investigated. Experimental conditions were determined to obtain desired particle sizes of relatively narrow distribution. It was shown that particles may be formed without intermediate micelles, i.e., by homogeneous nucleation, and the rate of particle formation is affected primarily by the rate of particle coalescence in the initial stages of the reaction. When covalently bound to antibodies these microspheres were successfully used to label murine and human lymphocytes.

We have recently reported<sup>3</sup> a new, generally applicable technique which has been used to label antigens on the surface of red blood cells and lymphocytes. This technique consisted of (a) the synthesis by emulsion copolymerization of spherical particles containing hydroxyl and carboxyl groups on their surface in the sizes of 30–340 nm in diameter; (b) the covalent binding of amino acids, fluorescent molecules, and antibodies to the latex spheres by means of the cyanogen bromide, carbodiimide, and glutaraldehyde

methods; (c) the interaction of the latex antibody conjugates with living cells and the examination of the labeled cells by means of scanning electron microscopy. In using transmission electron microscopy antigens on cell surfaces were previously visualized by means of ferritin,<sup>4a</sup> haemocyanin,<sup>4b</sup> enzymes,<sup>5</sup> or viruses<sup>6</sup> bound to antibodies. Haemocyanin and viruses have also served as markers for scanning electron microscopy<sup>7,8</sup> (SEM). Commercial polystyrene latex particles have been employed as immunological