# Preparation of Poly(dimethylsiloxane)–Polypeptide Block Copolymers

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## Synopsis

Block copolymers composed of poly(dimethylsiloxane) segments linked to polypeptide have been synthesized by reacting primary amine-terminated silicone with *N*-carboxy anhydrides of amino acids. The amine-terminated silicone was prepared by reacting "living" polysilanolate with γ-aminopropyltriethoxysilane. The block copolymers prepared consisted of segments of poly(DL-phenylalanine) or poly(γ-benzyl-L-glutamate). The block copolymers were purified by extraction with solvents which are selective for the homopolymers. Analysis of the products was accomplished by hydrolysis of the polypeptide segments, infrared spectra, gel permeation chromatography, and elemental analysis. The content of the copolymers ranged from 50 to 87% polypeptide.

## INTRODUCTION

There have been numerous successful attempts at blocking silicones to a variety of condensation and addition polymers.[1,2] Also, there have been successful preparations of block copolymers of polypeptides with other polymeric units.[3–6] The combination of silicones and polypeptides to form block copolymers has been described in a patent nearly 10 years ago.[7] In this disclosure, Chow and Byck described methods for preparing silicone–polyamide compositions by end-to-end coupling of suitably functionally terminated homopolymers. There have been no subsequent descriptions for preparation of these unique polymers. The potential utility of silicone–polypeptide block copolymers in medicine and other areas has prompted us to develop a facile route to their preparation.

Primary amines initiate the polymerization of *N*-carboxy-α-amino acid anhydrides (NCAs) to give polypeptides possessing an end group derived from the initiating amine.[8,9] A block copolymer such as 1 could be produced by employing a primary amine-terminated silicone, **2**, as an initiator for the polymerization of an NCA, **3**. In the present work, the amine-terminated silicone was prepared by reacting a "living" polysilanolate with γ-aminopropyltriethoxysilane

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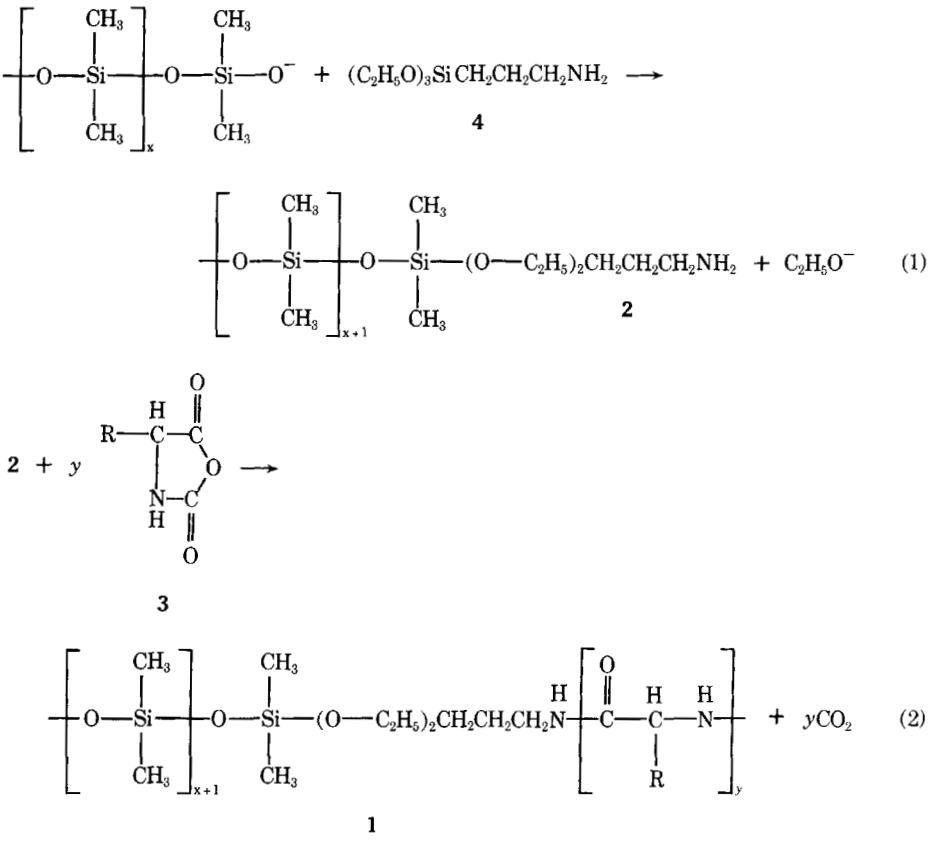
(GAPTS), **4**, as shown in eq. (1):



(1)



(2)

## EXPERIMENTAL

### Materials

Hexamethylcyclotrisiloxane, $D_3$ (PCR, Incorporated), was used without purification, but its solutions were stored over calcium hydride. $\gamma$-Aminopropyltriethoxysilane, GAPTS (PCR, Incorporated), was dried several days in a heated desiccator containing NaOH pellets. Fluorescamine, 4-phenylspirofuran-2(3*H*)-1′-phthalan-3,3′-dione (Roche Diagnostics), needed no purification. Solvents were distilled and, when necessary, drying was done over calcium hydride. Normal-butyllithium was used as supplied (Foote Mineral Co.) as a 15.08% solution in heptane. The concentration of the organolithium was determined by titration.[10]

## Procedures

**Preparation of the *N*-Carboxyanhydride of Phenylalanine and γ-Benzylglutamic Acid.** Standard published procedures were followed in the synthesis.[11,12] *dl*-Phenylalanine was suspended in dry 1,4-dioxane, and phosgene gas was allowed to rapidly bubble through the mixture at 40° until the suspension turned clear. The dioxane was removed by vacuum, and the solid residue was recrystallized five times with ethyl acetate–petroleum ether in a nitrogen dry box. The solid dry product melted at 126–127° (lit. 127°). The γ-benzyl glutamate was prepared from L-glutamic acid by the method of Blout and Karlson[13] and then treated in dioxane with phosgene. The carboxy anhydride was recrystallized from chloroform–*n*-hexane and dried (mp 91–92°, lit. 93–94°).

**Preparation of Primary Amine-Terminated Poly(dimethylsiloxane).** The "living" anionically polymerized silicone was prepared by initiating $D_3$ at room temperature using *n*-butyllithium as initiator. The following is a typical procedure. A dry, round-bottom flask was flushed with nitrogen, and a solution containing 21.7 g (0.098 mol) $D_3$ in 30 mL toluene was introduced. This was followed by 5 ml of a 30% solution of dimethylsulfoxide in toluene. (The DMSO served as a promoter for the polymerization.[14]) The appropriate amount of *n*-butyllithium ($10^{-4}$ mol) was introduced by syringe injection. Mixing was accomplished with a Teflon-coated stirring bar. A slight positive pressure of nitrogen was maintained throughout the reaction. The reaction mixture increased in viscosity noticeably within 12 h, and it was usually necessary to introduce additional dry toluene in order to facilitate stirring. After 70 h at room temperature, 9.96 g (0.045 mol) γ-aminopropyltriethoxysilane (GAPTS) was added by syringe injection and stirred with the polymer for 16 h at room temperature. The apparatus was opened, and the polymer was given one or more precipitations using chloroform/methanol as the solvent/nonsolvent pair. After vacuum drying for several days, 12 g of white semisolid was isolated.

**Primary Amine End Group Analysis of Poly(dimethylsiloxane).** Primary amine analysis was accomplished by a fluorometric assay using fluorescamine as the fluorogenic reagent. The procedure for using this reagent in a nonaqueous system was similar to that developed previously.[15] The amine-terminated silicone polymer which had been given several (six to ten) cyclohexane–methanol precipitations was dissolved in chloroform ($2.00 \times 10^{-3}$ g/mL), and 2 ml of this solution was combined with 3 ml fluorescamine in chloroform ($5 \times 10^{-5}$ g/mL) and 1 ml triethylamine in chloroform ($10^{-5}M$). Fluorescence intensity was measured at 480–495 nm with excitation at 390 nm. Fluorescence intensities of known GAPTS solutions in chloroform were used as standards.

**Preparation and Isolation of Block Copolymer.** A dry, three-necked flask equipped with nitrogen inlet, magnetic stirring bar, condenser, and drying tube was charged with 210 ml dry chloroform and 2 to 10 g of the NCA. While stirring, 2 to 10 g of the amine-terminated silicone polymer was then added. The mixture was allowed to stir at room temperature under nitrogen for 100–200 h. The polymer was then isolated by precipitation in excess methanol. The filtered polymer (occasionally suspended particles had to be isolated by centrifugation) was then treated with selective solvents as described below.

**Purification and Analysis of Block Copolymer.** Purification of the block copolymer was based upon the difference in solubility behavior of the dimethylsiloxane polymer and the polypeptide homopolymers. The silicone is soluble

in cyclohexane or benzene and insoluble in dioxane,[16] while the reverse is true for the poly(phenylalanine).[17] Both polymers are soluble in chloroform. Poly($\gamma$-benzyl-L-glutamate) is soluble in most of the common solvents including benzene, dioxane, and chloroform but is insoluble in methanol and alkanes.[18] The filtered polymer was stirred 15–25 h with excess benzene or chloroform. Insoluble materials, if observed, were filtered and dried *in vacuo*. The polymer solutions were mixed with excess methanol to give a precipitate which after filtration was given three additional solvent–methanol (either chloroform, benzene, dioxane, or cyclohexane) treatments. This gave 2–5 g block copolymer as identified by its infrared spectrum. The infrared spectrum of the original solvent-insoluble material and residues after methanol evaporation indicated the presence of polypeptide and amino acid but no silicone polymer.

**Separation of Physical Mixture.** An intimate mixture of poly(dimethylsiloxane) and poly(phenylalanine) was made by dissolving 1 g of each polymer in 35 mL chloroform and then precipitating the mixture in 200 ml methanol. The dried solid was divided into two equal weight (0.5 g) portions. One portion was stirred 6 h at room temperature with 30 mL benzene, the other portion was refluxed for 6 h with 50 mL cyclohexane. The benzene-insoluble solid showed strong IR absorptions characteristic of poly(phenylalanine) and very weak absorptions of silicone. The cyclohexane solution was poured into 150 mL methanol to give a solid which exhibited only silicone IR absorptions.

**Infrared Spectra of Polymers.** Homopolypeptides used as infrared standards were prepared by NCA polymerization in chloroform with initiation by *n*-butylamine. The infrared spectra were obtained using a model 727B Perkin–Elmer infrared spectrometer. The polymer samples were deposited on KBr plates as chloroform solutions which were then allowed to air dry under a heat lamp to yield a polymeric film. Block copolymers were similarly examined as films or as KBr pellets.

**Gel Permeation Chromatograms.** GPC analyses were performed on a Waters Associates instrument using du Pont Bimodal-S columns. Polymer solutions were prepared in dimethylformamide containing 0.05*M* LiBr and filtered to remove any insoluble material. The eluting solvent was tetrahydrofuran, and a differential refractometer detector was used.

**Molecular Weight Determination of Silicones.** Viscosity-average molecular weights for the amine-terminated silicone polymers were obtained by intrinsic viscosity determination of toluene solutions. Flow times were measured at 30 ± 0.1°C for a series of successively more dilute solutions using a Cannon–Ubbelhode dilution-type viscometer. The intrinsic viscosity was converted to molecular weight by use of the Mark–Houwink equation and published constants. Molecular weight of some silicone samples was determined by vapor pressure osmometry using a Mechrolab VPO instrument with chloroform as solvent and cholesterol as the calibration compound.

## RESULTS AND DISCUSSION

### Silicone Preparation

The silicones were obtained as viscous oils or semisolid pastes. The molecular weights ranged from 5,400 to 59,000 as determined by intrinsic viscosity. The polymers were obtained at a yield of 50 to 75%, based on the mass of polymer

precipitated in methanol after the reaction with γ-aminopropyltriethoxysilane (GAPTS).

The type of substitution reaction illustrated in eq. (1) between silanolate and GAPTS has been utilized in a number of applications. Merker and co-workers[19] prepared amine-substituted silicone rubber by an exchange reaction with GAPTS. Bailey[20] described a patented method for producing amine-terminated silicones by concomitant anionic reaction of cyclic silioxane monomers with cyclic 3-aminopropylmethoxysilanes. Amine functionality has been imparted to glass by reacting its surface with GAPTS.[21] The exchange reaction of various hydroxyl-substituted compounds, including glass, with alkoxy-substituted silanes has been studied quantitatively.[22,23]

Early in the present study, the reaction between GAPTS and negative silanolate prepared by butyllithium initiation was found to be much more efficient than reactions using other silicones. Thus, silicone prepared by KOH initiation and then reacted with GAPTS gave polymer with no detectable amine end groups. Even the addition of potassium metal to the silicone to ensure the presence of silanolate did not produce significant end capping. Using GAPTS in more than 100-fold excess relative to the *n*-butyllithium ensured that only one ethoxide would be replaced per GAPTS molecule.

The presence of primary amine end groups can be demonstrated by fluorescence analysis using fluorescamine. This reagent was developed for analysis of picomole quantities of primary amines in aqueous systems.[24] Its use was extended by Tan and Patsiga[15] to nonaqueous systems. A similar determination was later developed by Eckstein and Dreyfuss.[25]

Fluorescence analysis of silicones isolated after reaction with excess GAPTS indicated that primary amine end groups were indeed present. The polymers were given frequent solvent–nonsolvent precipitations in order to remove unreacted GAPTS, and Figure 1 illustrates the efficacy of each precipitation in its removal. The fluorescence intensity of the polymer decreased with each precipitation until it reached a minimum, constant value after about eight precipitations. The precipitations evidently also removed low-molecular-weight silicones since the polymer became noticeably more viscous with each purification step. This also accounts for the higher molecular weights calculated from the fluorescence data (see Table I).

## Block Copolymer Preparation

Several poly(dimethylsiloxane)-polypeptide block copolymer preparations are summarized in Table I. Because of their solubility in common solvents, the DL-phenylalanine and γ-benzyl-L-glutamate polymers were most easily handled in the laboratory. Preparations of a silicone–poly-*DL*-valine block copolymer could not be substantiated because of the extreme insolubility of the material. Polyvaline is reported to be soluble only in such solvents as dichloroacetic acid and polyphosphoric acid,[26] although Coleman and Farthing claimed it to be soluble in chloroform.[17]

The block copolymers could be isolated from the homopolymers (if present) by using selective solvents as described in the experimental section. The identification of polymer type was accomplished by noting prominent absorptions in the infrared spectra of the isolated solids. Comparison was made with spectra

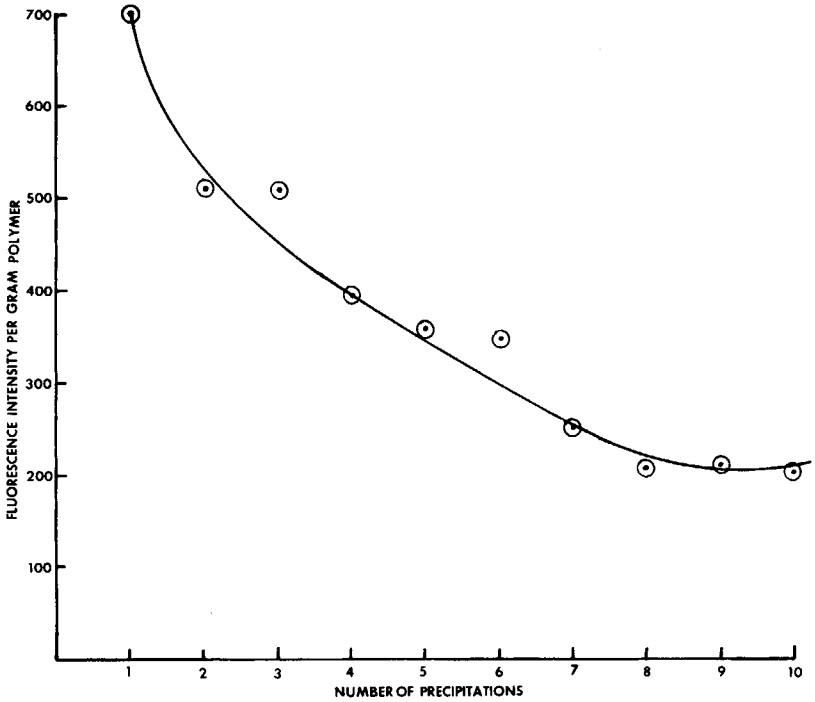



Fig. 1. Fluorescence determination of primary amine end groups in poly(dimethylsiloxane) as function of purification by precipitations from chloroform/methanol.

of synthesized homopolymers as well as referring to published information about silicones[27] and polypeptides.[28] The polypeptides can be readily identified by their N—H stretch absorption at 3300 $cm^{-1}$, C=O stretch at 1630 $cm^{-1}$, and N—H deformation at 1530 $cm^{-1}$. These absorptions could be distinguished from silicone absorptions which occurred at 2960 $cm^{-1}$ due to methyl C—H stretch and at 1100–1000 $cm^{-1}$ (very strong and broad) due to Si—O stretch. Figures 2 and 3 show IR spectra of the silicone–poly(phenylalanine) and silicone–poly(benzylglutamate) block copolymers.

Cyclohexane is capable of dissolving silicone homopolymer or silicone-rich block copolymers, while dioxane is selective for polypeptide homopolymers or polypeptide-rich block copolymers. Generally, the use of a solvent which is specific for a given homopolymer gave a product that indicated the presence of both polymeric types. Evidently, the soluble polymer is capable of carrying its covalently bonded counterpart into solution. On the other hand, a physical mixture of the two polymer types could be cleanly separated by these extraction procedures. In those cases when free silicone could be isolated (by cyclohexane extraction), it represented 10–20% of the original material resulting from a block attempt. Trace (5%) amounts of free polypeptide and monomeric amino acid were sometimes obtained by evaporation of methanol filtrates. Thus, it may be concluded that the block reaction is on the order of 70–90% effective. One crude block mixture (H-2) which was analyzed by gel permeation chromatography showed two peaks in the GPC chromatogram. One peak corresponded

TABLE I
Block Copolymerization Data

| Run no. | Silicone, g | Mol wt silicone[a] | NCA,[b] g | Reaction time, h | Yield,[c] g | Block copolymer examination |
|---|---|---|---|---|---|---|
| K-5 | 4.96 | 147,000 Fl<br>59,000 V | 2.08 PhA | 96 | 4.9 | HCl hydrol.: 28% silicone, 72% polypeptide |
| K-6 | 9.79 | 68,000 Fl<br>33,000 V | 16.8 PhA | 120 | 16.3 | HCl hydrol.: 30% silicone, 70% polypeptide |
| H-1 | 1.81 | 24,500 V<br>17,900 VPO | 4.86 PhA | 140 | 51. | HCl hydrol.: 13% silicone, 87% polypeptide |
| H-2 | 4.78 | 6,300 V<br>7,500 VPO<br>1,000 GPC | 5.07 PhA | 171 | 6.9 | Elemental anal.: 4.15% N, 12.18% Si indicating 32% silicone and 44% polypeptide. GPC molecular weight: 4600. HCl hydrol.: 33% silicone, 67% polypeptide |
| H-6 | 5.26 | — | 2.6 BG | 205 | 6.8 | GPC mol wt: 37,400; elemental anal.: 2.19% N, 25.12% Si, indicating 66% silicone and 34% polypeptide; HCl hydrol. indicates 53% silicone and 47% polypeptide |
| H-7 | 4.8 | 5,400 V | 4.5 BG | 168 | 7.8 | HCl hydrol.: 16% silicone, 84% polypeptide |

[a] Molecular weight methods: Fl = fluorescence, V = intrinsic viscosity, VPO = vapor pressure osmometry, GPC = gel permeation chromatography.
[b] PhA = NCA of DL-phenylalanine, BG = NCA of γ-benzyl-L-glutamate.
[c] Yield based on first methanol precipitation.

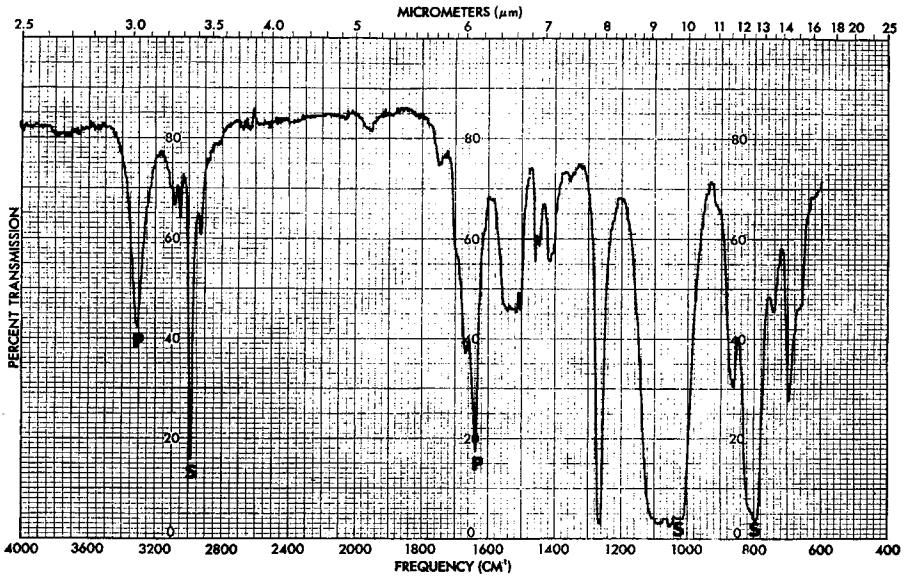



Fig. 2. Infrared spectrum of poly(dimethylsiloxane)–poly(DL-phenylalanine) block copolymer. P and S identify prominent absorptions of polypeptide and silicone, respectively.

to silicone having a number-average molecular weight of 1000 and represented 29% of the total sample. The other peak had a molecular weight of 4600. When the crude mixture was extracted with hot cyclohexane, the GPC of the cyclohexane-insoluble fraction showed a single peak with a molecular weight of 4500.

Extraction was also approached from the standpoint of utilizing a solvent that is specific for the polypeptide. For example, the crude mixture of run H-7 could

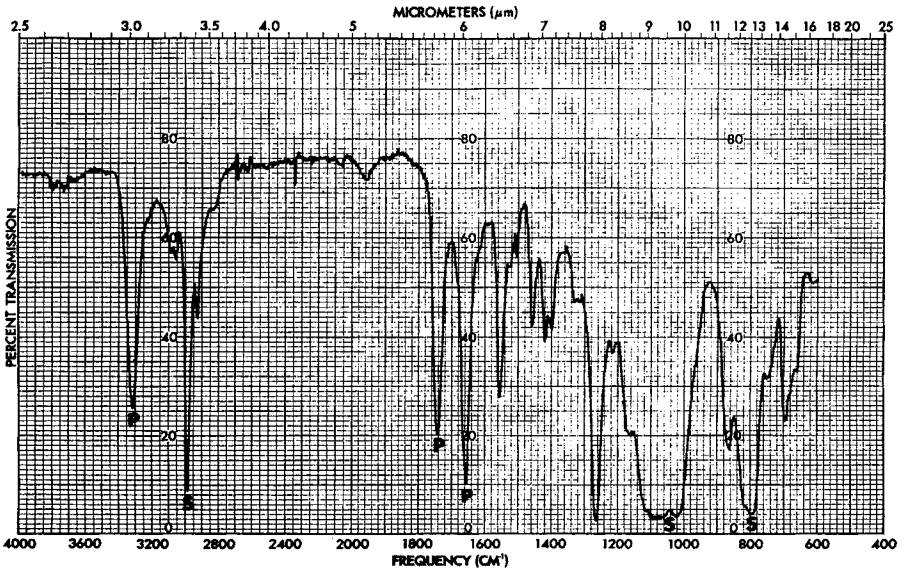



Fig. 3. Infrared spectrum of poly(dimethylsiloxane)–Poly(γ-benzyl-L-glutamate) block copolymer. P and S identify prominent absorptions of polypeptide and silicone, respectively.

be dissolved in hot dioxane [a nonsolvent for poly(dimethylsiloxane)]. Cooling the solution to room temperature resulted in a cloudy suspension. A small amount (less than 30%) solid was isolated by centrifugation. Although the IR spectrum of this material showed very strong silicone absorptions, weak polypeptide absorptions were still visible. When precipitated into methanol, the dioxane soluble portion showed strong absorptions of both polymer types. These observations indicate that the block copolymers may show a graduation in solubility characteristics depending on the relative size of each block segment. Benzene and chloroform were able to dissolve all polymer types. Samples containing poly(DL-phenylalanine), however, became insoluble after a few weeks of storage as dry solids. This observation was also made on the homopolymer and its copolypeptides by Coleman and others.[17,29]

### Analysis of Block Copolymers

In addition to the infrared procedures described above, the block copolymers were studied by methods which were able to give information regarding the relative content of the two components.

Being composed of hydrolyzable groups, the polypeptide could be eliminated from the copolymer by using hot hydrochloric acid. Thus, treatment of weighed samples with 8.5*M* hydrochloric acid at 125° and 18 psi in an autoclave for 15–20 h was sufficient to degrade the polypeptide block and leave the silicone portion uneffected. The resistance of the silicone under these conditions was determined in a separate experiment. Most of the block copolymers examined by the hydrolysis procedure indicated a composition which is approximately two thirds polypeptide and one third silicone (see Table I). The composition is somewhat dependent on the initially used ratio of silicone to NCA. For example, H-1 with a reactant ratio of 1.81/6.67 (27% silicone) gave a block copolymer containing 13% silicone. Run H-6, with a ratio of 5.26/7.86 (66% silicone), gave a block containing 50–60% silicone.

Elemental analysis for silicone and nitrogen provided another means of determining composition. There is reasonable agreement between this method and the HCl hydrolysis method (runs H-2 and H-6). The poly(DL-phenylalanine) copolymer, H-2, gave a nitrogen analysis which accounted for less than 100% material, although the silicone content determined by two different methods agree (32 and 33%).

The block copolymers were white, soft solids. The $\gamma$-benzylglutamate block copolymer exhibited cottonlike appearance, yet showed slightly waxy texture when pressed with a spatula.

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