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Reactions on Polymer Supports¹

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The preparation of "popcorn" copolymers of styrene and divinylbenzene which contain carboxy, carbomethoxy, and hydroxymethyl side chains is described. Procedures are reported for effecting chemical transformations on the functional groups joined to polymers and for utilizing these materials as supports in chemical synthesis.

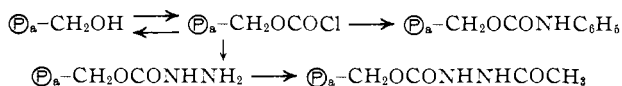
The concept of utilizing polymer supports in synthetic work has been discussed by Merrifield² and in a communication from this laboratory.³ The basic idea is that a substance to be subjected to a series of reactions be joined by a covalent bond to a polymer which is insoluble in the solvents and inert to the reagents employed in the synthetic sequence. Reactions are carried out simply by suspending the polymer-substrate mixture in a solvent containing the reagent and subsequently separating the insoluble polymer from solvent by filtration. In the final step the bond holding the synthesized material to the polymer is severed and the product eluted or filtered from the supporting matrix. This technique appears particularly promising for synthesis of polypeptides and oligonucleotides where repetitive synthetic steps are employed and the isolation of intermediates by conventional procedures is difficult or time consuming. The present paper reports the preparation of several polymers which may be used as supports, an investigation of the reactions of functional groups on the polymers, and an example demonstrating the feasibility of using these polymers as supports in synthetic work.

Styrene-divinylbenzene "popcorn" polymer was selected as the structural base for the support. This material, obtained by copolymerization of the components in absence of added radical or ionic initiators, differs from the "glassy" type copolymer in that highly insoluble materials with a low swelling capacity can be obtained with very low divinylbenzene-styrene ratios.⁴ Most of our work has been done with polymer about 0.2 and 0.5% in divinylbenzene.⁵ Initially, carboxyl groups were introduced into preformed polymer by acylation followed by oxidation with hypobromite or by reaction of the polymer with diphenylcarbonyl chloride followed by hydrolysis. It was later found that functionalized "popcorn" polymer could be obtained directly by copoly-

merizing styrene and divinylbenzene with *p*-vinylbenzyl alcohol, *p*-vinylbenzoic acid, or ethyl *p*-vinylbenzoate. These polymers were cleaner than those obtained from the Friedel-Crafts reactions and have been used routinely in recent work.

A question of prime importance involved the extent to which functional groups on the polymers could be transformed by reagents in solvents surrounding the support. This problem was investigated by carrying out series of reactions in which the carbonyl-bearing functional group was altered and observing the shift in position and relative intensity of the carbonyl stretching band in the infrared spectrum. It was found in these studies that functional groups on the "popcorn" polymers were generally available for normal chemical reactions. Complete conversion could usually be realized in solvents such as benzene, pyridine, and dimethylformamide, which penetrate the polymers effectively. With hydroxylic solvents such as water and methanol, regions of the polymer appeared to be inaccessible and transformations were often incomplete even when long reaction times were employed. Specific examples supporting these conclusions follow. In this discussion P-X refers to a polymer in which a portion of the aromatic rings contain substituent X, and the origin of the polymer is indicated by a subscript. Thus, P_s refers to a support prepared by introducing substituents into preformed styrene-divinylbenzene polymer and P_c , P_e , and P_a refer to copolymers prepared with *p*-vinylbenzoic acid, methyl *p*-vinylbenzoate, and *p*-vinylbenzyl alcohol, respectively.

Conversion of the hydroxymethyl polymer obtained by copolymerization to some amide derivatives is outlined below, and pertinent infrared spectral regions for these materials are indicated in Fig. 1.



The hydroxymethyl polymer exhibited bands at 2.77 and 2.90 μ , indicative of both free and hydrogen-bonded hydroxyl groups. When the polymer was treated with phosgene in benzene these bands disappeared entirely and a strong band developed at 5.62 μ . The chloroformyl derivative was transformed cleanly to the hydrazide (λ 5.84 μ) and the anilide (λ 5.86 μ) on treatment with hydrazine or aniline in benzene, and the hydrazide afforded an acetylhydrazine derivative (λ 5.78, 5.94 μ) on reaction with acetic anhydride or *p*-nitrophenyl acetate.

Analogous transformations have been carried out on the other polymer systems. Details are given in the

(1) This research was supported by the National Science Foundation, Grant G25069, and by the Division of General Medical Sciences of the National Institutes of Health, Grant GM 10265-01. It also benefitted from a Public Health Service Training Grant 5T1-626, from the National Institute of General Medical Science, Public Health Service.

(2) R. B. Merrifield, *Federation Proc.*, **24**, 412 (1962); *J. Am. Chem. Soc.*, **85**, 2149 (1963); **86**, 304 (1964). Particularly noteworthy is the synthesis of bradykinin on polymer beads (last reference).

(3) R. L. Letsinger and M. J. Kornet, *ibid.*, **85**, 3045 (1963). Experimental details for the reactions described are included in the present paper.

(4) J. L. Amos, K. E. Coulter, and F. M. Tennant in "Styrene," R. H. Boundy and R. F. Boyer, Eds., Reinhold Publishing Corp., New York, N. Y., 1952, p. 729. See E. H. Immergut, *Makromol. Chem.*, **10**, 93 (1953), and R. L. Letsinger and S. B. Hamilton, *J. Am. Chem. Soc.*, **81**, 3009 (1959), for copolymers of the "popcorn" type.

(5) Polymer with 0.1% cross-linking has been used satisfactorily for a number of reactions. It is insoluble in benzene, pyridine, and other common solvents; however, its use is limited by the fact that some solubilization occurs when the polymer is subjected to the action of strongly alkaline aqueous pyridine solutions.

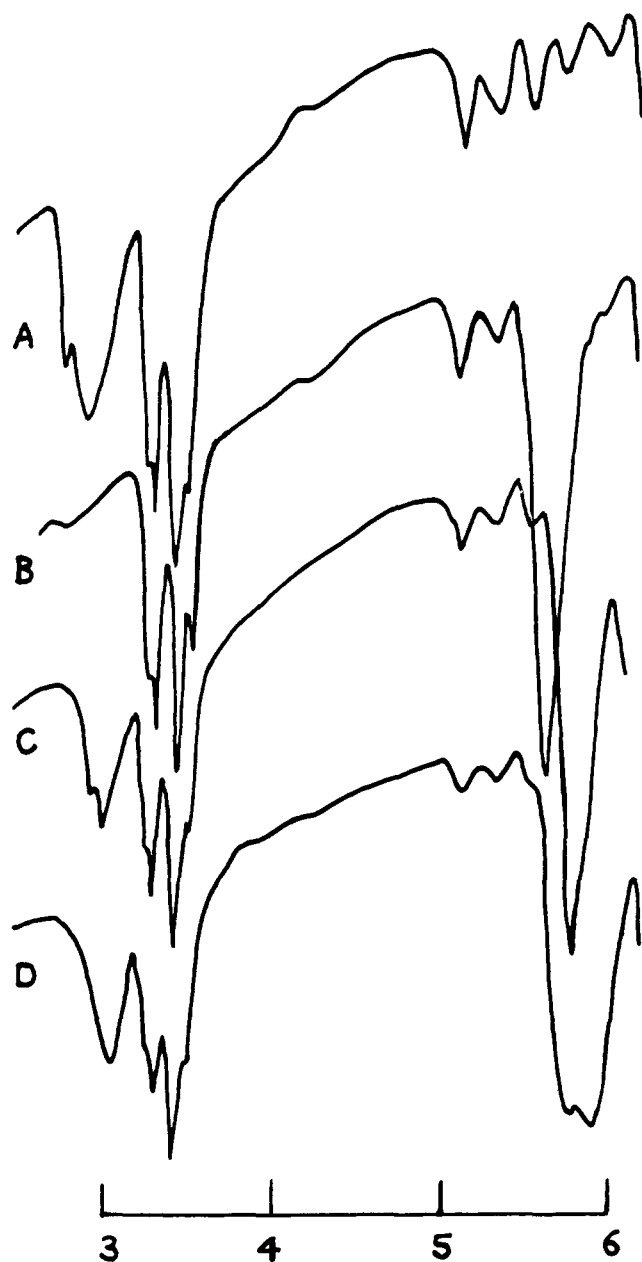


Fig. 1.—Infrared spectra: A, $\text{P}_a\text{-CH}_2\text{OH}$; B, $\text{P}_a\text{-CH}_2\text{OCOC}_2\text{H}_5$; C, $\text{P}_a\text{-CH}_2\text{OCONHNH}_2$; D, $\text{P}_a\text{-CH}_2\text{OCONHNHCOCH}_3$.

Experimental section. An interesting spectral feature is that all samples of P-COOH showed two bands in the carbonyl region, a strong one at 5.90 and a weaker one at 5.79μ . The former may be attributed to hydrogen-bonded carboxyl groups and the latter to free carboxyl groups.⁶ Generally hydrogen bonding is so extensive in solid samples of carboxylic acids that only the longer wave length band is observed. Absorption at 5.79μ indicates that a substantial proportion of the carboxyl groups on the polymer are in an isolated position with respect to the other carboxyl groups.

The effect of the solvent medium upon the availability of the functional groups on the polymer is typified by the behavior of P-COCl with methanol. Approximately 43% of the acid chloride groups were converted to ester groups when a sample of the polymer was stirred with pure methanol for 3 hr. at room temperature. Under the same conditions the conversion

was 55% when a 40% solution of methanol in benzene (v./v.) was employed, and the conversion was complete when a 10% solution of methanol in pyridine was used. $\text{P}_c\text{-COOH}$ and $\text{P}_s\text{-COOH}$ could be reduced satisfactorily with lithium aluminum hydride in ether. Surprisingly, however, $\text{P}_s\text{-COC}_6\text{H}_5$ failed to react appreciably with lithium aluminum hydride in ether or with phenylmagnesium bromide or phenyllithium in ether or ether-benzene mixtures.

As a test of the possibility of synthesizing peptides on the "popcorn" polymer supports, leucylglycine was constructed on a polymer. First, the ethyl ester of L-leucine was coupled to $\text{P}_s\text{-CH}_2\text{OCOC}_2\text{H}_5$. Following hydrolysis of the ester function, the product was condensed with glycine benzyl ester *via* a mixed anhydride. Cleavage of the dipeptide from the support with hydrogen bromide in acetic acid, filtration, and addition of ether to the filtrate afforded crystalline leucylglycine hydrobromide.

Experimental

Infrared spectra were determined with a Baird recording spectrophotometer with the sample in potassium bromide. Microanalyses were performed by the Micro-Tech Laboratories, Skokie, Ill.

Monomers.—Commercial styrene and *p*-divinylbenzene were distilled immediately before use. *p*-Vinylbenzoic acid was obtained from *p*-chlorostyrene by the method of Leebrick and Ramsden.⁷

Methyl *p*-vinylbenzoate was prepared by addition of sufficient ethereal diazomethane to 32.5 g. (0.22 mole) of *p*-vinylbenzoic acid in ether to give a yellow color which persisted. After acidification with acetic acid the solution was washed with aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated *in vacuo*. On recrystallization of the residual solid from ether-pentane, 34.0 g. (95%) of the crystalline ester was obtained; m.p. $33\text{--}34^\circ$, lit.⁸ m.p. $34\text{--}35^\circ$.

***p*-Vinylbenzyl alcohol** was made by reduction of 29.4 g. (0.20 mole) of *p*-vinylbenzoic acid, dissolved in 500 ml. of ether, with 15.4 g. (0.40 mole) of lithium aluminum hydride in 1 l. of ether. Following addition of the hydride solution (30 min.) the mixture was stirred an hour at reflux, cooled, and treated with 30 ml. of water and 260 ml. of 10% aqueous sodium hydroxide. Insoluble inorganic salts were separated by filtration and washed with ether. The combined ether layers were then dried with magnesium sulfate and stripped *in vacuo* to a clear oil; weight 26.0 g. (97%), n_D^{25} 1.5711. For analysis a small portion was purified by evaporative distillation; n_D^{25} 1.5725, m.p. $23\text{--}24^\circ$.

Anal. Calcd. for $\text{C}_9\text{H}_{10}\text{O}$: C, 80.56; H, 7.51. Found: C, 80.12; H, 7.65.

Polymerization Procedure.—Freshly distilled styrene (59.9 g., 0.575 mole) and divinylbenzene (0.120 g., 0.92 mmole) were placed with a few "seeds" of "popcorn" polymer in a 1-l. erlenmeyer flask which had been well purged with nitrogen. After the mixture had stood at $50\text{--}55^\circ$ for a few days (in different runs the time varied from 1 to 5 days) polymerization became evident by appearance of a white, insoluble polymer which protruded above the surface of the liquid. Thereafter the process proceeded to completion within a day, at which time the flask was over half filled with the white polymer. The product was washed with ether, ground in a Waring Blendor, again washed with ether, and dried. The yield was essentially quantitative, and the infrared spectrum was that of polystyrene.

Copolymers of styrene and *p*-substituted styrenes were prepared in the same manner by including an additional vinyl component in the polymerizing system. Quantities of reagents are given in Table I. These polymers were pure white.

Preparation of $\text{P}_s\text{-COOH}$ (Method A).—Diphenylcarbonyl chloride (18.5 g., 0.080 mole) in 60 ml. of nitrobenzene was added over a period of 15 min. to a well stirred mixture of 41.6 g. (0.40 mole) of the styrene-divinylbenzene "popcorn" polymer (0.5% divinylbenzene) and 15.0 g. (0.113 mole) of aluminum chloride in 360 ml. of dry nitrobenzene. The dark mixture was then

(6) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 142.

(7) J. R. Leebrick and H. E. Ramsden, *J. Org. Chem.*, **23**, 935 (1958).

(8) L. A. Brooks, *J. Am. Chem. Soc.*, **66**, 1295 (1944).

warmed at 80° for 2.5 hr., cooled, and treated with 200 ml. of water. The insoluble polymer was separated by filtration and washed in succession with diluted hydrochloric acid, methanol,

TABLE I
REAGENTS FOR PREPARATION OF "POPCORN" COPOLYMERS

Polymer	Substituted	<i>p</i> -Subst. styrene		Styrene		Divinyl- benzene	
		G.	Mole	G.	Mole	G.	Mole
⊕ _s -COOH	-COOH	6.5	0.04	26	0.25	0.06	0.00046
⊕ _s -COOCH ₃	-COOCH ₃	16.2	.10	52	.50	.12	.00092
⊕ _s -CH ₂ OH	-CH ₂ OH	13.4	.10	52	.50	.12	.00092

and ether. On drying, the pale tan product weighed 42 g. and exhibited a sharp, strong band at 6.02 μ. Attempts to carry out Friedel-Crafts reactions in carbon disulfide were not satisfactory since in this medium degradation occurred and a partially soluble, glassy type polymer was obtained.

For hydrolysis, the carboxamido polymer was heated at 130–135° for 32 hr. with a mixture of 335 ml. of acetic acid, 250 ml. of sulfuric acid, and 150 ml. of water. Filtration and washing with water and ether afforded ⊕_s-COOH as a pale greenish material showing the characteristic carboxyl absorption in the infrared and a very weak band at 6.02 μ attributed to a little of the unreacted carboxamido polymer.⁹

Titration was effected by suspending 0.500 g. of polymer in 25 ml. of 95% ethanol, adding 25 ml. of 0.1 N sodium hydroxide, warming the mixture to reflux, cooling, and back titrating with hydrochloric acid; 0.33 mequiv. of carboxyl per gram of polymer was found. With diazomethane in ether ⊕_s-COOH was converted to ⊕_s-COOCH₃.

(Method B).¹⁰—Acetylation of 50 g. of 0.2% cross-linked styrene-divinylbenzene "popcorn" polymer was achieved by stirring 15 g. of aluminum chloride and 8 g. of acetyl chloride with 50 g. of polymer in 100 ml. of nitrobenzene for an hour, warming the mixture with stirring at 70° for an hour, and allowing the mixture to stand at room temperature overnight. Hydrolysis with water and washing yielded ⊕_s-COCH₃, which had a strong band in the infrared at 5.95 μ.

An oxidizing medium was prepared by adding 100 g. of bromine to 115 g. of potassium hydroxide and 180 ml. of water in 900 ml. of dioxane while maintaining the temperature below 10°. The acetyl polymer was added to this solution and the mixture was stirred 1 hr. at room temperature, 1 hr. at 40°, and 1 hr. at 70°. The resulting carboxy polymer, isolated by filtration, was near white and had the same absorption bands as that prepared by direct copolymerization. Titration indicated 0.74 mequiv. of carboxyl per gram.

Preparation of ⊕_s-CH₂OCOCl.—A mixture of 45 g. of ⊕_s-COOH (prepared by method A) and 4 g. of lithium aluminum hydride in 600 ml. of ether was heated at reflux for 18 hr. Hydrolysis and filtration afforded ⊕_s-CH₂OH (λ 2.9 μ, no absorption in the carbonyl region) quantitatively. A portion (20 g.) was converted to the chloroformate (λ 5.62 μ, 0.27 mequiv. of chloride per gram of polymer by Volhard titration) by stirring in 175 ml. of 9% phosgene in benzene for 2.5 hr., filtering, and washing with ether.

Leucylglycine Hydrobromide.—At the end of each of the following reactions the polymer was separated from reactants by filtration and washed thoroughly with solvents. The sample was then dried and an infrared spectrum taken.

Leucine ethyl ester was joined to the support by stirring 2.48 g. (12.7 mmoles) of L-leucine ethyl ester hydrochloride with 20 g. of ⊕_s-CH₂OCOCl and 4.8 ml. of triethylamine in 174 ml. of dry dimethylformamide at room temperature for 2.5 hr. The product (λ 5.78 μ) was hydrolyzed with 70 ml. of 0.8 N sodium hydroxide in 100 ml. of methanol and 100 ml. of acetone at room temperature over a 3-hr. period; then it was acidified with hydrochloric acid. Titration indicated 0.22 mequiv. of carboxyl per gram of polymer. To a portion (5 g.) of this N-blocked leucine derivative and 10.2 g. of triethylamine in 50 ml. of toluene was added 10 g. of

isobutyl chloroformate. After an hour of stirring, the polymer was filtered, washed with toluene, and added to a cold solution of 10 g. (30 mmoles) of glycine benzyl ester *p*-toluenesulfonate and 4.1 ml. (30 mmoles) of triethylamine in 50 ml. of dimethylformamide. The mixture was stored at room temperature for 2.5 days, although cessation of evolution of carbon dioxide after several hours indicated that the reaction was probably complete in a much shorter time. It was then cooled, mixed with 100 ml. of water, and filtered. The polymer was washed thoroughly, and after drying it was added to 45 ml. of 7% hydrogen bromide in acetic acid. The next day the mixture was filtered and the polymer washed with 10 ml. of acetic acid. On addition of 2 l. of ether to the combined filtrate and washings and standing at 0°, leucylglycine hydrobromide (91 mg.) precipitated. The product was characterized by chromatographic analysis at 50° on an Amberlite 150 A column on a Beckman/Spinco Model 120 B amino acid analyzer according to the method of Moore, Spackman, and Stein.¹¹ The retention volumes and peak areas showed the molar percentages of amino acids and peptides to be: leucylglycine 89.2%, glycine 7.2%, and leucine 3.6%. Paper chromatography of the product, followed by hydrolysis of the dipeptide portion and chromatography of the resulting amino acids, confirmed the identity of the dipeptide.

Reactions of ⊕_s-CH₂OH.—Hydroxymethyl polymer (5 g.) prepared from *p*-vinylbenzyl alcohol was stirred with 100 ml. of 12% phosgene in benzene for several hours. A portion (1 g.) of the resulting chloride (⊕_s-CH₂OCOCl) was converted to the anilide (*Anal.* Found: N, 2.11) by stirring with 3 ml. of aniline in 10 ml. of benzene for 1.5 hr. while another portion (1 g.) was converted to the hydrazide (*Anal.* Found: N, 3.97) by stirring with 6 ml. of hydrazine in 20 ml. of benzene for 12 hr. Acetylation of the hydrazide was effected on a 0.20-g. portion with either 0.5 ml. of acetic anhydride in 10 ml. of pyridine for 3 hr. or with 0.2 g. of *p*-nitrophenyl acetate in 10 ml. of pyridine for 20 hr. Cleavage from the polymer with 6 ml. of 32% hydrogen bromide in acetic acid over a 12-hr. period afforded acetylhydrazine, which was recovered by filtering off the polymer, evaporating the solution to dryness at low temperature, and neutralization. The product was characterized by thin layer chromatography on silica plates (a single spot was obtained in 5:2:4 butanol-ethanol-water at *R_f* 0.23 and in acetone at *R_f* 0.64, corresponding to the behavior of a known sample of acetylhydrazine). Infrared spectra for the polymer derivatives are given in Fig. 1.

Reactions on ⊕_s-COOCH₃.—The carboxymethyl polymer (1.0 g., λ 5.81 μ) was heated on a steam bath for 48 hr. with 5 ml. of water, 5 g. of sodium hydroxide, and 50 ml. of dimethyl sulfoxide. After washing with water, ethanol, and ether the carboxylate derivative (λ 6.44 and 7.18 μ; these bands are characteristic for the carboxylate anion; no band attributable to a normal carboxyl was found) was converted to ⊕_s-COOH (λ 5.79 and 5.92 μ) by stirring with 10 ml. of hydrochloric acid in 10 ml. of water and 20 ml. of dioxane for 6 hr. Warming a portion of this material (0.5 g.) with thionyl chloride in 10 ml. of benzene for 5 hr. afforded the acid chloride, ⊕_s-COCl (λ 5.65 (s), 5.75 (w) μ), which yielded ⊕_s-CONHC₆H₅ (a single, sharp band in the carbonyl region at 6.00 μ. *Anal.* Found: N, 1.91) and ⊕_s-CONHNH₂ (a single, sharp band in the carbonyl region at 6.04 μ. *Anal.* Found: N, 3.81) on reaction with aniline and hydrazine in benzene.¹²

Reduction of ⊕_s-COOH with excess lithium aluminum hydride in ether (2 hr. at reflux) gave ⊕_s-CH₂OH (λ 2.90 μ) in good yield, though a very weak band remaining at 5.90 μ indicated that a small amount (~5%) of the carboxypolymer had failed to react.

⊕_s-COOH was indistinguishable in the reactions investigated (formation of the acid chloride and anilide, reduction with lithium aluminum hydride) from ⊕_s-COOH.

(11) S. Moore, D. H. Spackman, and W. H. Stein, *Anal. Chem.*, **30**, 1185, 1190 (1958). The analysis was performed by Mr. K. Thompson.

(12) Formation of hydrazide and anilide derivatives whose spectra were free of absorption bands attributed to carboxyl or carboxylate anion indicates that the weak band at 5.75 μ for ⊕_s-COCl arises from the acid chloride and not from anhydride or carboxyl groups which could contaminate the product. It may be noted that double peaks are frequently observed in the carbonyl region for acid chlorides: K. Nakanishi, "Infrared Absorption Spectroscopy," Holden-Day, San Francisco, Calif., 1962.

(9) With the exception of the small band at 6.02 μ, the bands were the same throughout the 2–15 μ region as those for ⊕_s-COOH, ⊕_s-COOH, and ⊕_s-COOH obtained from ⊕_s-COCH₃.

(10) These experiments were carried out by E. N. Oftedahl and M. J. Guinard.