Highly Cross-Linked Azlactone Functional Supports of Tailorable Polarity

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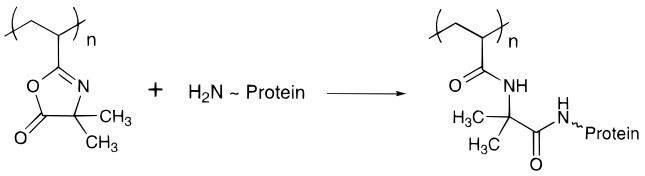
ABSTRACT: Highly cross-linked azlactone functional supports were prepared by dispersion polymerization of 2-vinyl-4,4-dimethylazlactone (VDM), 2-hydroxyethyl methacrylate (HEMA), and trimethylolpropane trimethacrylate (TMPTMA). The supports were recovered in high yield and possessed high surface areas. Individual particles, which ranged from 10 to 300 μm in diameter, were irregularly shaped and comprised of filamentous strands. Support polarity was tailored through increases in the HEMA level in the polymer formulations and was quantified by a lipophilicity index derived from solvent partition data collected on the constituent monomers. Support reactivity, as determined by azlactone aminolysis and measured by IR, was found to increase linearly with increasing support polarity. This effect was shown to be independent of the support cross-link density.

Introduction

Azlactone functional polymers are particularly well-suited reactive base materials for covalently attaching performance-specific functionality. The electrophilic azlactone ring undergoes facile addition reaction with alcohol, thiol, and especially amine nucleophiles,[1−3] providing a four-atom spacer between the polymer backbone and bound ligand. Reaction with primary amines is particularly rapid, occurring in preference to hydrolysis—even in dilute aqueous solutions.[4] This feature enables azlactone functional supports to bind proteins efficiently in aqueous media, through reaction with the ϵ-amino group of lysine residues.

High densities of Protein A have been immobilized, in high yield and with rapid binding kinetics, on hydrophilic, azlactone functional acrylic beads developed for protein affinity chromatography separations.[5] Hydrophilic supports are desired for such applications in order to minimize any subsequent nonspecific (hydrophobic) interactions between solute proteins and support, which would restrict the desired protein affinity separation.



Hydrophilic supports also are considered important for maintaining the catalytic activity of immobilized enzymes,[6,7] but efficient immobilization of enzymes may require supports with a specifically tailored hydrophilic−hydrophobic balance.[8] Moreover, support polarity has been shown to impact the catalytic activity[9−11] (perhaps by influencing the diffusion of substrates and products[12,13]) and even enantioselectivity[14] of immobilized enzymes. Such observations have prompted the conclusion that there is no universal support for enzyme immobilization.[15,16] In order to immobilize enzymes efficiently and to utilize the resulting catalysts effectively, therefore, a series of supports encompassing a range of polarities is needed.[17]

The polarity of azlactone functional supports can, in principle, be adjusted through reaction with less than stoichiometric amounts of hydrophilic or hydrophobic nucleophiles. In designing supports for subsequent enzyme binding, however, such an approach might result in the annihilation of azlactone residues most accessible to large nucleophiles such as proteins. Alternatively, because of the facile copolymerizability of vinyl azlactones, and 2-vinyl-4,4-dimethylazlactone in particular,[2] polarity of azlactone functional acrylic supports can be tailored through incorporation of either hydrophobic or hydrophilic comonomers in the support polymer formulation.

In this paper, we describe the preparation and characterization of a series of highly cross-linked, azlactone functional acrylic supports with tailored polarities and address the impact of overall support polarity on pendant azlactone reactivity.

Experimental Section

Materials. Pluronic L-31 was obtained from BASF, trimethylolpropane trimethacrylate (TMPTMA) was obtained from Sartomer Co. (Exton, PA), 2-vinyl-4,4-dimethylazlactone (VDM) was obtained from SNPE (Princeton, NJ), and 2-hydroxyethyl methacrylate (HEMA), butyl methacrylate (BMA), lauryl methacrylate (LMA), 1,4-dimethylnaphthalene, 1,8-diazabicyclo[5.4.0]undec-7-ene, 2,6-di-*tert*-butyl-4-methylphenol, azobis(isobutyronitrile) (AIBN), methylamine, and butylamine were obtained from Aldrich Chemical Co. Isopar G, a synthetic isoparaffinic hydrocarbon of bp 160−177 °C, was obtained from Exxon (Houston, TX). All monomers and reagents were used as received. Hexane and acetonitrile used in the partition experiments were high-purity, UV grade obtained from Baxter. All other solvents were of reagent grade and were used without further purification.

Measurements. Support surface areas and pore sizes were measured by nitrogen gas adsorption using a Micrometrics ASAP 2400 instrument and analyzed by the BET method.[18] Gas chromatography was performed on a Hewlett-Packard 5890 Series II gas chromatograph with an HP 3396A integrator. IR analysis was performed on a Perkin-Elmer Model 1760 FTIR, and peak heights were analyzed by LabCalc software. Samples, as a 10% Nujol mull, were placed between polished sodium chloride crystals with a 13 μm spacer.

Support Polymerization Procedure. The following procedure for preparation of TMPTMA:VDM:HEMA 60:20:20 support is representative: A 3 L, three-neck, round-bottom flask equipped with a mechanical stirrer, thermometer, gas inlet, dropping funnel, and condenser was charged with a solution of TMPTMA (60.0 g), VDM (20.0 g), HEMA (20.0 g), and a particle stabilizer (1.67 g of a 33% Isopar G solution of

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Table 1. Support Formulation and Characterization

support	monomer formulation[a]	yield (%)	VDM wt %[b]	surface area (m^2/g)	particle bed volume (mL/g)	% swell (in THF)	LI[c]
1	TMPTA:VDM (80:20)	99	18	112	17.0	2.0	32.2
2	TMPTA:VDM:HEMA (70:20:10)	98	18	152	16.6	1.5	28.7
3	TMPTA:VDM:HEMA (60:20:20)	96	18	97	14.2	0	25.1
4	TMPTA:VDM:HEMA (50:20:30)	98	18	93	12.8	9.0	21.6
5	TMPTA:VDM:HEMA (40:20:40)	91	17	76	11.2	16	18.0
6	TMPTA:VDM:HEMA (30:20:50)	97	16	36	8.8	14	14.5
7	TMPTA:VDM:HEMA (20:20:60)	96	16	28	6.6	40	10.9

[a] TMPTMA is trimethylolpropane trimethacrylate, VDM is 2-vinyl-4,4-dimethylazlactone, and HEMA is 2-hydroxyethyl methacrylate. [b] Determined by combustion analysis. [c] Lipophilicity index (LI) computed for each support using a weighted sum of constituent monomer lipophilicities.

lauryl methacrylate:VDM copolymer pretreated with HEMA) in heptane (800 mL). The solution was stirred (500 rpm) and sparged with nitrogen. After 10 min, the solution was warmed to 70 °C and AIBN (2.5 g) was added. Within a few minutes, particles were visible in the reaction vessel. As the polymerization proceeded, ten 100 mL portions of deoxygenated heptane were added to facilitate stirring. After 2 h from the detection of particles, heating was discontinued and the reaction mixture, a flocculant polymer mass, was allowed to cool. The solid was filtered, washed with heptane, and then immersed in an ethyl acetate solution (1000 mL) containing 0.1% Pluronic L-31 surfactant. After 1 h, the mixture was filtered and the filter cake rinsed with fresh ethyl acetate. The recovered polymer was dried under nitrogen for 12 h at 40 °C and then at 0.2 kPa for 12 h.

Support 1: yield, 99%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{1.64}(C_7H_9NO_2)]_n$: C, 63.2; H, 7.5; N, 2.0. Found: C, 62.2; H, 7.4; N, 1.8.

Support 2: yield, 98%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{1.44}(C_7H_9NO_2)(C_6H_{10}O_3)_{0.53}]_n$: C, 62.3; H, 7.5; N, 2.0. Found: C, 61.4; H, 7.4; N, 1.8.

Support 3: yield, 96%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{1.23}(C_7H_9NO_2)(C_6H_{10}O_3)_{1.07}]_n$: C, 61.5; H, 7.5; N, 2.0. Found: C, 60.9; H, 7.5; N, 1.8.

Support 4: yield, 98%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{1.03}(C_7H_9NO_2)(C_6H_{10}O_3)_{1.60}]_n$: C, 60.6; H, 7.5; N, 2.0. Found: C, 59.5; H, 7.6; N, 1.8.

Support 5: yield, 91%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{0.82}(C_7H_9NO_2)(C_6H_{10}O_3)_{2.14}]_n$: C, 59.8; H, 7.5; N, 2.0. Found: C, 58.3; H, 7.3; N, 1.7.

Support 6: yield, 97%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{0.62}(C_7H_9NO_2)(C_6H_{10}O_3)_{2.67}]_n$: C, 58.9; H, 7.5; N, 2.0. Found: C, 56.8; H, 7.2; N, 1.6.

Support 7: yield, 96%. Anal. Calcd for $[(C_{18}H_{26}O_6)_{0.41}(C_7H_9NO_2)(C_6H_{10}O_3)_{3.21}]_n$: C, 58.1; H, 7.5; N, 2.0. Found: C, 56.4; H, 7.4; N, 1.6.

Preparation of a Dispersion Polymerization Stabilizer: Reaction Product of 2-Hydroxyethyl Methacrylate and Copoly(lauryl methacrylate−2-vinyl-4,4-dimethylazlactone [94:6]). A 2 L, three-neck, round-bottom flask fitted with a mechanical stirrer, condenser, gas inlet, and thermometer was charged with lauryl methacrylate (188 g), VDM (12.0 g, 86 mmol), Isopar G solvent (400 g), and AIBN (2.0 g). The solution was sparged with nitrogen and heated at 70 °C. After 24 h, HEMA (11.2 g, 86 mmol), 1,8-diazabicyclo-[5.4.0]undec-7-ene (2.0 g, 13 mmol), and 2,6-di-*tert*-butyl-4-methylphenol (35 mg) were added to the colorless, thickened solution. Within 90 min, infrared analysis indicated that the reaction was complete by the virtual disappearance of the azlactone carbonyl absorption at 1810 cm^{-1}. The percent solids of the solution was determined to be 32.0 wt % by heating a weighed aliquot for 2 h at 125 °C.

Monomer Lipophilicity Determination. Monomers (500 mg each) were added to a 6 dram vial and the resulting mixture was diluted to 15 mL with hexane. Similarly, an internal standard solution was prepared by diluting 1,4-dimethylnaphthalene (500 mg) to 15 mL with hexane. A reference solution (to compensate for GC detector response variation) was prepared by mixing 1 mL of monomer solution with 1 mL of the internal standard solution and then adding 4 mL of hexane. Standard areas for each monomer were computed from the ratio of monomer peak area to 1,4-dimethylnaphthalene peak area on the gas chromatogram [30 m × 0.53 mm HP-5 column; 50 °C (2 min) to 250 °C (5 min) at 15 °C/min; flame ionization detector].

The partition mixture was prepared by adding 1 mL of the monomer solution to a mixture of 4 mL of hexane and 5 mL of 75/25 acetonitrile−water (prepared by mixing 75 mL of acetonitrile with 25 mL of water). The resulting biphasic mixture was shaken for 5 min. After stratification, the lower phase was removed carefully by pipet, and 1 mL of internal standard solution was added to the hexane phase. From the GC chromatogram of the partitioned hexane phase, monomer area ratios were again computed and then related back to the standard areas. Monomer lipophilicity is defined as the weight percent of monomer remaining in the hexane phase following partition.

Support Reactivity with Methylamine. Each support was suspended in a pH 10 solution of methylamine (0.35 M, 8 mL, 2.8 mmol, 19.4 equiv; containing 0.2 wt % Pluronic L-31 surfactant), and the resulting slurries were tumbled at room temperature. After 1 h, the slurries were filtered and the treated supports washed with water (3 × 10 mL) prior to being dried at 0.7 kPa overnight. The extent of aminolysis for each support was determined by IR, through comparison of support azlactone (1823 cm^{-1})/acrylate (1734 cm^{-1}) carbonyl absorbance ratios before and after exposure to methylamine.

Acetylation of Support 5. A suspension of support **5** (2.00 g) and acetic anhydride (100 mL), under an inert atmosphere, was heated on a steam bath for 1 h prior to addition of pyridine (1 drop). After a total of 3 h, the mixture was allowed to cool to room temperature overnight. The acetylated support was collected by filtration and then extracted with methylene chloride in a Soxhlet apparatus for 6 h. Drying overnight at 0.2 kPa afforded 2.26 g (96.5% yield) of white solid. IR data on the acetylated support revealed very little OH stretch remaining and an expected reduction in the azlactone to ester carbonyl absorbance ratio.

Comparison of Support 5 (HEMA-40) and HEMAc-40 in Reaction with Butylamine in Organic Solvent. The supports (100 mg each, 0.14 mequiv azlactone) were treated with a 36 mM solution of butylamine in heptane (10 mL, 0.36 mmol, 2.5 equiv), and the resulting slurries were rocked gently at room temperature. Similarly, each support was treated with a 36 mM solution of butylamine in THF. After 4 h, the slurries were filtered and the collected supports were rinsed with fresh solvent and then dried overnight at 0.2 kPa. The extent of aminolysis for each support was determined by IR, as described above.

Results and Discussion

A homologous series of highly cross-linked, azlactone functional supports was prepared by dispersion polymerization[19,20] of trimethylolpropane trimethacrylate (TMPTMA), 2-vinyl-4,4-dimethylazlactone (VDM), and increasing levels of a hydrophilic comonomer, 2-hydroxyethyl methacrylate (HEMA)[14,21] (which was shown in controlled experiments not to undergo ring-opening reaction with VDM under the polymerization conditions).[22] The level of VDM was kept constant at 20 wt % in order to facilitate subsequent comparisons of support reactivity and enzyme binding capacity. Polymerizations were conducted in heptane, using a copolymerizable particle stabilizer, and initiated at 70 °C with AIBN (2 wt %). The polymers (Table 1) were

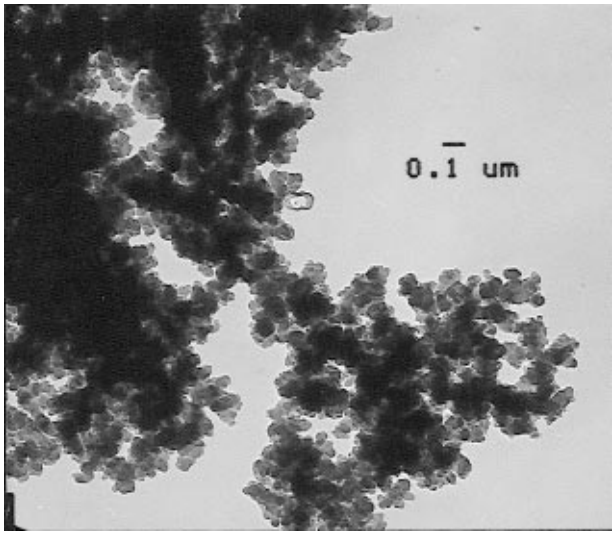

Figure 1. Transmission electron micrograph of support **2** at 33×10^3 magnification.

recovered in high yield, indicating high levels of incorporation of formulated monomers; levels of incorporated azlactone functionality were verified independently by combustion analysis. Incorporation of lipophilic comonomers such as butyl methacrylate (BMA), cyclohexyl methacrylate (CyMA), and lauryl methacrylate (LMA) also was attempted; however, yields of the resulting polymers were only 85−90%, resulting in supports of inadequately defined composition and suggesting a lower degree of copolymerizability for these monomers.

The polymeric supports were obtained as white, irregularly shaped particles, ranging from 10 to 300 μm in diameter. Under magnification, individual particles exhibit a filamentous structure appearing as a tangled network of strands, each strand comprised of nodules of 70−100 nm in diameter (Figure 1). The filamentous particle morphology probably arose as a result of the high levels (20−80 wt %) of cross-linking monomer employed coupled with the insolvency of the medium (heptane) for the incipient polymer particles. This combination would promote early precipitation of primary particles and perhaps agglomeration of those particles to generate the tangled, nodular strands exhibited in the micrograph. The observed tortuous microscopic structure of these materials is consistent with their high surface areas, measured at 30−150 m^2/g, and their lack of any definitive pore structure, as determined by nitrogen adsorption−desorption.

Despite the rather fragile appearance of the particles, the supports were found to be quite durable: particle size distributions were reduced only marginally after aqueous support slurries had been stirred with a magnetic stir bar (0.1% w/v slurry, 65 h, ca. 350 rpm). The nonuniform size and shape of the particles also need not preclude use of these supports in packed columns: a reasonable gravity flow rate of 3.5 mL/min was measured using water as eluent for a 3.4 × 11.6 cm column bed.[23]

The densities of these support polymers were measured (by gas intrusion) at ca. 1.3 g/mL but, because of the open architecture of the particles, support bed volumes were found to be quite large. Particle bed volume was determined by measuring the volume of 1 g of support immersed and equilibrated in a nonswelling medium (heptane). As indicated in Table 1, particle bed volume decreases, almost linearly, with decreasing cross-linker level. Although particle size certainly would impact packing of the particle bed, no such trend was observed in the particle size distributions of supports **1**−**7**. Swelling behavior of the polymeric supports was assessed according to a similar procedure, by measuring volume increases following immersion in tetrahydrofuran (THF) versus heptane. As indicated in Table 1, only when the level of cross-linker was decreased below 30 wt % in this series of polymers was significant swelling observed.

Table 2. Monomer Lipophilicities

HEMA	1.0	BMA	73.2
VDM	15.2	LMA	96.7
TMPTMA	36.5		

[a] Determined by partitioning monomers in 1:1 hexane/75:25 acetonitrile−water; monomer lipophilicity is weight percent remaining in hexane phase following partition.

LI is an abbreviation for lipophilicity index, an empirically derived measure of relative support polarity. Polymer lipophilicities were not measured directly but rather were derived from lipophilicity data collected on the constituent monomers. Monomers were partitioned between equal volumes of a polar/nonpolar solvent combination, and then monomer concentrations in the nonpolar phase were measured by capillary GC. The lipophilicity for each monomer was defined as the weight percent of the monomer remaining in the nonpolar phase following partition. For the monomers listed below, the standard octanol/water partition system[24] proved inadequate so resort was made to a hexane/75:25 acetonitrile−water system which allowed measurement of a wide range of monomer lipophilicities without causing appreciable azlactone hydrolysis. Support lipophilicities were computed using a weighted sum of constituent monomer lipophilicities. Although support aquaphilicity determination, based on water affinity in organic solvent as developed by Reslow et al.,[9] may provide a more universal characterization of relative support polarity, the present method, based on monomer lipophilicity measurement, allows greater polarity discrimination in a closely related series of synthetic supports.

It has been reported that the polarity of a polymer backbone can influence the reactivity of attached substituents.[25] We were interested to learn whether relatively minor graduations in support polarity, induced by increases in polymer HEMA content, would be reflected in the reactivity of pendant azlactone functionality in this series of supports. Toward that end, supports **1**−**6** were compared in reaction with aqueous methylamine. Reactions of azlactone functional polymers can be monitored conveniently by IR, by following the disappearance of the azlactone carbonyl absorption band at 1820 cm^{-1}. Conversions were determined after a 1 h exposure to ca. 20 equiv of methylamine at pH 10 and then plotted versus support lipophilicity. As shown in Figure 2, reaction conversions decreased *linearly* with increasing support lipophilicity.

This reactivity comparison excluded, however, any potential effect of support cross-link density on pendant azlactone reactivity. Because the level of VDM was kept constant at 20 wt % throughout the series of support formulations, incorporation of comonomers (i.e., HEMA) necessarily was done at the expense of cross-linker (TMPTMA) level. As a consequence, the most polar (and most reactive) supports also contained lower cross-linker levels. Even though essentially no swelling was observed (in water or THF) for supports containing as low as 30 wt % TMPTMA, the possibility of cross-link density influencing observed azlactone reactivity called for investigation.

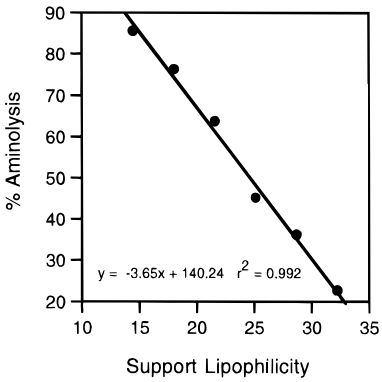

Figure 2. Aminolysis of support azlactone functionality as a function of support lipophilicity: extent of reaction measured by IR following exposure to excess methylamine for 1 h at pH 10.

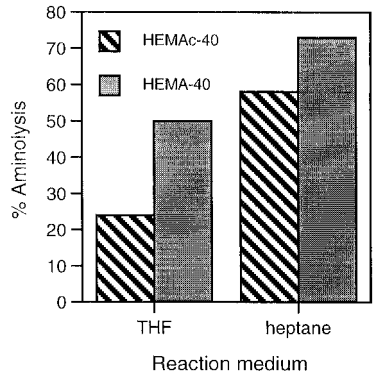

Figure 3. Examination of support reactivity at constant cross-linker level: comparison of support **5** (HEMA-40) and its acetylated derivative (HEMAc-40) in reaction with butylamine in organic solvent.

Comparison of pendant azlactone reactivity became necessary, in essence, between supports with different lipophilicities, yet identical cross-link densities. Surface areas and azlactone distributions for these supports also should be identical. These requirements would be best satisfied not through reformulation but rather through lipophilicity modification of an existing support, most easily accomplished by esterification of HEMA residues. Such an approach would generate a more lipophilic support for reactivity comparison without disturbing azlactone functionality or surface characteristics.

To maximize the lipophilicity change resulting from esterification, a support containing a high level of HEMA was needed. Accordingly, support **5** containing 40 wt % HEMA (HEMA-40) was treated with acetic anhydride/pyridine to generate the more lipophilic, acetylated derivative (HEMAc-40). The lipophilicity for the corresponding acetylated monomer (2-acetoxyethyl methacrylate[26]) was measured at 9.2, affording a support lipophilicity of 21.3 for HEMAc-40 (assuming reaction of all accessible hydroxyl groups) as opposed to 18.0 for HEMA-40.

Pendant azlactone reactivities of these supports were assessed in two organic solvents, heptane and THF. As shown in Figure 3, the more polar HEMA-40 indeed proved more reactive than HEMAc-40 toward butylamine in either heptane or THF. Small graduations in support lipophilicity, therefore, can influence azlactone reactivity in this series of nonswelling network supports.

That the azlactone reaction conversions were higher for both supports in heptane vs THF, and that the conversion differences were much narrower in heptane, could be attributed to differences in the equilibration of butylamine between support and medium. The nonpolar medium afforded higher concentrations of a polar nucleophile on the support, and hence higher reaction conversions (but also less discrimination between supports). Conversely, the more polar organic medium afforded lower concentrations of a polar nucleophile on the support, and lower reaction conversions (but more discrimination between supports).

Conclusion

Azlactone functional supports of tailorable polarity have been prepared in high yield by dispersion polymerization. Support polarity was governed by the level of comonomer (HEMA) in the support formulation and was quantified by a lipophilicity index derived from solvent partition data collected on the constituent monomers. In a series of indexed supports, pendant azlactone reactivity was found to increase linearly with increasing support polarity. The impact of graduated changes in support polarity on enzyme immobilization efficiency and immobilized enzyme activity is under investigation.

References and Notes

(1) Taylor, L. D.; Kolesinski, H. S.; Mehta, A. C.; Locatell, L.; Larson, P. S. *Makromol. Chem., Rapid Commun.* **1982**, *3*, 779.
(2) Rasmussen, J. K.; Heilmann, S.; Krepski, L. In *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; Mark, H. F., Bikales, N., Overberger, C. G., Menges, G., Eds.; Wiley-Interscience: New York, 1988; Vol. 11, p 558.
(3) Heilmann, S. M.; Rasmussen, J. K.; Palensky, F. J.; Smith II, H. K. *J. Polym. Sci., Polym. Chem. Ed.* **1984**, *22*, 1179.
(4) Rasmussen, J. K.; Heilmann, S. M.; Krepski, L. R.; Jensen, K. M.; Mickelson, J.; Johnson, K.; Coleman, P. L.; Milbrath, D. S.; Walker, M. M. *React. Polym.* **1991/1992**, *16*, 199.
(5) Coleman, P. L.; Walker, M. M.; Milbrath, D. S.; Stauffer, D. M.; Rasmussen, J. K.; Krepski, L. R.; Heilmann, S. M. *J. Chromatogr.* **1990**, *512*, 345.
(6) Scouten, W. H. *Methods Enzymol.* **1987**, *135*, 30.
(7) Kitano, H.; Hasegawa, M.; Kaku, T.; Ise, N. *J. Appl. Polym. Sci.* **1990**, *39*, 241.
(8) Kamei, S.; Okubo, M.; Matsuda, T.; Matsumoto, T. *Colloid Polym. Sci.* **1986**, *264*, 743.
(9) Reslow, M.; Adlercreutz, P.; Mattiasson, B. *Eur. J. Biochem.* **1988**, *172*, 573.
(10) Jayakumari, V. G.; Rajasekharan Pillai, V. N. *J. Appl. Polym. Sci.* **1991**, *42*, 583.
(11) Lie, E.; Molin, G. *J. Chem. Technol. Biotechnol.* **1991**, *50*, 549.
(12) Kokufuta, E. *Prog. Polym. Sci* **1992**, *17*, 647.
(13) Johansson, A. C.; Mosbach, K. *Biochim. Biophys. Acta* **1974**, *370*, 348.
(14) Orsat, B.; Drtina, G. J.; Williams, M. G.; Klibanov, A. M. *Biotechnol. Bioeng.* **1994**, *44*, 1265.
(15) Kolarz, B. N.; Trochimczuk, A.; Bryjak, J.; Wojaczynska, M.; Dziegielewski, K.; Noworyta, A. *Angew. Makromol. Chem.* **1990**, *179*, 173.
(16) Coupek, J.; Gemeiner, P.; Jirku, V.; Kalal, J.; Kubanek, V.; Kuniak, L.; Peska, J.; Rexova, L.; Stamberg, J.; Svec, F.; Turkova, J.; Veruovic, B.; Zemek, J. *Chem. Listy* **1981**, *75*, 512.
(17) Sokolovskii, V. D.; Kovalenko, G. A. *Biotechnol. Bioeng.* **1988**, *32*, 916.
(18) Brunauer, S.; Emmet, P. H.; Teller, E. *J. Am. Chem. Soc.* **1938,** *60*, 309.
(19) Arshady, R. *J. Microencapsulation* **1988**, *5*, 101.
(20) Ober, C. K. *Makromol. Chem., Macromol. Symp.* **1990**, *35/36*, 87.
(21) Rasmussen, J. K.; Heilmann, S. M.; Krepski, L. R.; Smith II, H. K.; Walker, M. M.; Stauffer, D. M.; Milbrath, D. S.; Coleman, P. L. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **1990,** *31*, 442.
(22) Uncatalyzed reactions of alcohols and VDM are sluggish; see ref 3.
(23) Use of these supports as aqueous slurries may require pretreatment with dilute surfactant, such as 0.2 wt % Pluronic L-31.
(24) Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.
(25) Morawetz, H. *J. Polym. Sci., Polym. Symp.* **1985,** *72*, 9.
(26) Fischer, K.; Eisenbach, C. D. *Makromol. Chem., Rapid Commun.* **1988,** *9*, 503.

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