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Hydrophilic polymer supports grafted by poly(ethylene glycol) derivatives via atom transfer radical polymerization

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Abstract

In this study, a newly structured hydrophilic polymer supports are prepared by the chemical modification (grafting) of the polystyrene-based preformed beads via atom transfer radical polymerization (ATRP) employing the CuBr/PMDETA catalyst system. Hydrophilic monomers including *N*,*N*-dimethylacrylamide (DMA) and poly(ethylene glycol) (PEG) macromonomers were grafted onto Merrifield type resin. Based on optical microscopic data, the particle sizes of the resins were increased in the order of 10 μm after the polymerization. This increment indicated that monomers are grafted not only to the particle surface but also to within a polymer matrix. These resins demonstrate well-swellability in polar solvent, and they enable high functional loadings up to 0.7–1.8 mmol g^{-1}. The high loading capacity in polar solvents and favorable mechanical properties of resins render them to have great potential applications in peptide and oligonucletide syntheses.
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Keywords: Merrifield resin; ATRP; Poly[poly(ethylene glycol)]

1. Introduction

Polymer supports have a wide range of applications in organic synthesis, from acting as a catalyst to biotechnology and combinatorial syntheses [1–3]. The most commonly used polymer supports in solid-phase synthesis are derived from ''Merrifield resin'' [1], which is a chloromethylbenzene-incorporated cross-linked polystyrene resin. Such resins can be further converted to have particular functional groups for various applications and the added physical and chemical characteristics of functionalized resins can broaden their uses. One of the crucial issues while serving as an effective polymer support in solid-phase synthesis is the accessibility of the reagent to the reactive sites in the polymer matrix under specific conditions. Merrifield resins are typical hydrophobic materials and swell only in nonpolar organic solvents. When Merrifield resins were used in polar organic solvents, the reactive sites in the unswollen beads remained ''hidden''; and this hydrophobicity renders these types of resins inefficient for reaction in polar media [4,5]. An effective approach to solve this problem is to introduce hydrophilic units into the polymer matrix. For instance, TentaGel resin is reasonably compatible with polar solvents because its cross-linked polystyrene core is grafted with a large number of poly(ethylene glycol) (PEG) chain fragments [6]. Consequently, it can be swollen in various polar solvents due to this PEG linkage. However, only a limited number of functional groups can be introduced at the end of each PEG chain if the PEG chain is grown onto the core by anionic grafting polymerization and thus give a poor loading capacity in use. Therefore, a resin that can simultaneously swell in polar media and exhibit a high loading capacity is ardently being sought. Herein, we report a plausible approach to obtain a high swelling factor resin along with high functional capacity by grafting monomers with functional groups onto a supporting polymer matrix. Accordingly, two types of hydrophilic monomers are incorporated into the traditional Merrifield resin by living free radical polymerization.

Atom transfer radical polymerization (ATRP), a controlled/living polymerization method, has been developed recently

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[7,8]. The high tolerance for water and some functional groups while polymerizing monomers in a controlled fashion are the favorable characteristics of this process [9]. Typically, ATRP initiators are halide compounds, including α-halo carboxylates and benzyl halides. The growth of polymer chains follows the activation of the initiator by a metal complex. A series of surface modifications of polystyrene-based spheres have been demonstrated by this method, including modifications by poly(methyl methacrylate), poly(benzyl methacrylate) and poly(*N*,*N*-dimethylacrylamide) (DMA) [10–12]. Ayres et al. demonstrated the copolymerization of DMA with *N*-acryoyl sarcosine methyl ester which is very compatible with hydrophilic solvents [5]. However, DMA-based resins exhibit unfavorable mechanical characteristics while mixing is applied, which minimizes its practical usefulness. In this study, hydrophilic monomers (DMA and PEG macromonomers) were incorporated onto polystyrene beads to mitigate this problem. The loading capacity can be enhanced via the brush feature of this newly designed structure while the swellability in polar solvents can be further enhanced via the incorporation of DMA (Fig. 1). Highly flexible and bio-compatible PEG-based resins are well-known as superior polymers to support the peptide synthesis and subsequent bio-assays using resin-bound peptides [13,14]. The combination of high loading capacity, high swellability and bio-compatible properties of the resins designed in this study enables them to have potential applications in peptide and oligonucletide syntheses.

2. Experimental section

2.1. Measurements and reagents

Chemicals were purchased from Aldrich and used as received unless otherwise specified. *N*,*N*,*N*′,*N*′,*N*″-Pentamethyldiethylenetriamine (PMDETA) and triethylamine (TEA) were used as received. *N*,*N*′-Dimethylacrylamide (DMA) (Aldrich, 99%) and poly(ethylene glycol) macromolecules, including poly(ethylene glycol) acrylate (PEG-375) ($M_n = 375$), poly(ethylene glycol) methacrylate (PEG-526) ($M_n = 526$) and poly(ethylene glycol) methyl ether methacrylate (PEG-1100) ($M_n = 1100$) (Aldrich, 99%) were passed through an alumina column immediately before use in order to remove inhibitors and impurities. Copper(I) bromide (CuBr) (Aldrich, 98%) was stirred overnight in acetic acid, and then filtrated and washed with absolute ethanol. Merrifield resin (chloromethylpolystyrene) was used as received from Aldrich. The particle sizes of beads are ~80 μm and are 1% cross-linked with a 2–3 mmol g^{-1} loading capacity. FT-IR measurements were performed on a Perkin–Elmer Spectrum One FT-IR spectrometer. Bead's particle sizes were measured using Olympus CH30 microscope. The UV spectrum was recorded with a Hitachi U-2010 UV–vis spectrophotometer.

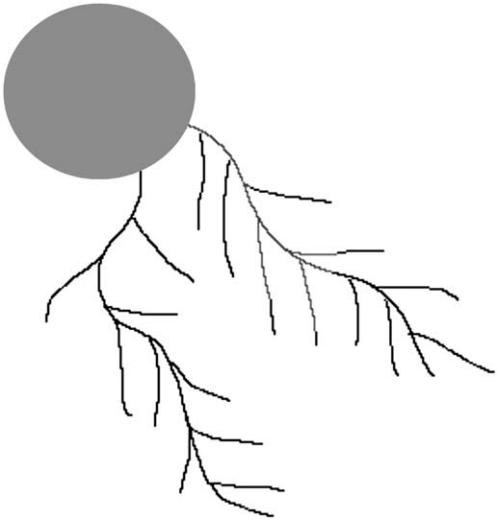


Fig. 1. Illustration of brush-structure.

2.2. Synthesis of hydrophilic polymer supports

Merrifield resin (0.4 g, 2.5 mmol g^{-1} loading) and CuBr (143 mg, 1 mmol) were placed in a round-bottom flask filled with N_2. A Schlenk tube was charged with poly(ethylene glycol) derivatives, PMDETA (346 mg, 2 mmol) and 10 mL DMF. The mixture was degassed using three freeze/pump/thaw cycles and injected using a degassed syringe into the round-bottom flask containing the Merrifield resin. The reaction mixture was heated to 80 °C stirred overnight. After the reaction had attained completion, the liquid was removed by filtration and the resin was washed thrice with aliquots of CH_2Cl_2, MeOH, THF and DMF sequentially. The product was recovered as a green solid. Table 1 presents the yields of this study. The polymerization of DMA and copolymerization of DMA/PEG macromonomers adopted the same synthesis process; the feed ratio of monomers was indicated in Table 1 (Scheme 1).

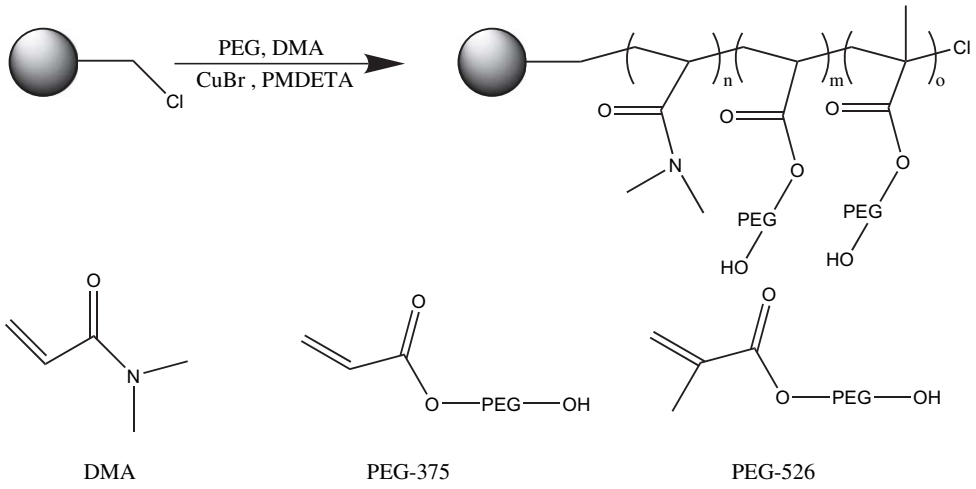
2.3. Swelling studies on synthesized resin [15]

Dry resins were weighed and placed in vials to which the appropriate solvent was added in excess. The solvents used for the swelling studies were water, THF and methanol. The vials were sealed, and the samples were allowed to swell for 3 days at room temperature. Extra solvent was removed by

Table 1
Grafting of hydrophilic monomers onto polystyrene-based resin[a]

Sample	Monomer	[M]:[I]	Loading capacity (mmol/g)	Yield (g)
P1	PEG-375	10:1	0.8	0.76
P2	PEG-526	5:1	1.3	1.32
P3	PEG-526	10:1	1.8	2.35
P4	PEG-1100	10:1	N/A	2.12
D1	DMA	30:1	N/A	0.49
D1-N[b]	DMA	30:1	N/A	0.5
DP1	PEG-375/DMA = 1:6	5:1	N/A	0.65
DP1-N[b]	PEG-375/DMA = 1:6	5:1	0.7	0.75
DP2	PEG-526/DMA = 1:6	5:1	1.3	1.24
DP3	PEG-526/DMA = 1:3	10:1	1.8	2.21

[a] Polymerization from resin, copper(I) bromide was used as the catalyst with PMDETA as the ligand. All reactions were carried out in DMF at 80 °C for 20 h.
[b] Without agitation.



Scheme 1. Graft-polymerization of hydrophilic monomers onto Merrifield resin.

filtration, the residual solvent on the surface was dried with filter paper, and the weight of the swollen beads was recorded. The swellability was calculated as the volume of solvent (mL)/weight of the dry resin (g). This value was calculated as

$$S(\mathrm{mL/g}) = (W_s - W_d)/(d \times W_d)$$

where W_s is the weight of the swollen resin, W_d is the weight of the dry resin, and d is the density of the chosen solvent.

2.4. Determination of loading capacity [4]

A mixture of 100–200 mg of beads and 4 mL DMF was placed in a 10 mL glass vessel. The dye molecule, *p*-nitrophenyl chloroformate, was added to the vial and the amount used was 10 times the theoretical content of the hydroxyl groups. The mixture was stirred for 1 h in an atmosphere of nitrogen. After the *p*-nitrophenyl chloroformate had dissolved, the TEA was added. The reaction was stirred overnight at 30 °C in an atmosphere of nitrogen. The resulting solution was filtered and washed thrice in each of the solutions such as acetone, THF, methanol and DMF. Afterwards, the modified resin was dried at reduced pressure at 70 °C for 12 h to yield a yellow bead. The modified bead (50 mg) was then placed in a 10 mL vial, and 0.5 mL of aqueous 1 M NaOH solution and 2 mL of DMF were added. The hydrolytic reaction proceeded overnight. Five microliters of the solution in the vial were diluted to 10 mL by adding DMF. The absorbance of UV (435 nm) due to the presence of nitrophenol in each sample was recorded. The loading capacity of the beads was then determined from the calibration curve.

3. Results and discussion

3.1. Grafting PEG macromonomers and DMA from Merrifield resin

In this study, the CuBr/PMDETA catalyst system was employed to graft monomers from Merrifield resin (Scheme 1). The detailed polymerization reaction results with various monomers are listed in Table 1. A green solid was recovered from the polymerization of PEG macromonomers after the solvent was removed. This green tint is due to the presence of copper(II) salt coordinated by the amide or ether functional groups of the polymer chains. This Cu^{+2} salt can be removed by passing through a short resin column and rinsed with a $TBA^{+}EDTA^{-}$ ($(Bu_4N)^{+4}EDTA^{-4}$) solution according to the literature method [5]. After thorough washing, the washed liquid turned from colorless to a deep blue color and the resin became a white solid. Optical microscopy was applied to study the size differences and morphology of the resin (Fig. 2). The particle sizes of the **P2** sample of the resin (Fig. 2(b)), were larger, 40–60 μm, after polymerization than before (Fig. 2(a)). The large increase in size suggested that the polymer chain was grafted onto the polymer matrix not only on the surface of the bead, but also on the initiator sites within the bead. If polymerization occurs only on the initiator sites of the surface of the beads, then the increments will be smaller, in the range of 50–100 nm [11]. This investigation demonstrates that the ATRP of PEG-derived monomers with known molecular weights (**P1**, **P3** and **P4**) resulting from 0.4 g of Merrifield resin exhibited an increase in masses upto 0.76, 2.35 and 2.15 g, or by 90%, 488% and 438%, respectively. The mass increments are more drastic for methacrylate functionality (PEG–MA) than for PEG with acrylate functionality (PEG–A) under similar condition, suggesting that PEG–MA is more efficiently polymerized by ATRP condition than PEG–A is. This result is consistent with those reported elsewhere under the solid-phase condition as Zheng and Stover observed similar phenomenon and attributed it to the fact that the 2-bromo-2-methylpropionate end group formed in the methacrylates is easier to be activated than 2-bromopropionate formed in the corresponding acrylate polymerization [11]. Hydrophilic monomer DMA was used herein to promote the swellability of the resin. Wirth et al. reported that DMA can be polymerized from a surface-supported benzyl chloride initiator [16]. During their study, reaction agitation had to be avoided to prevent structural damaging of the resin [5,16]. Similar phenomena were observed in

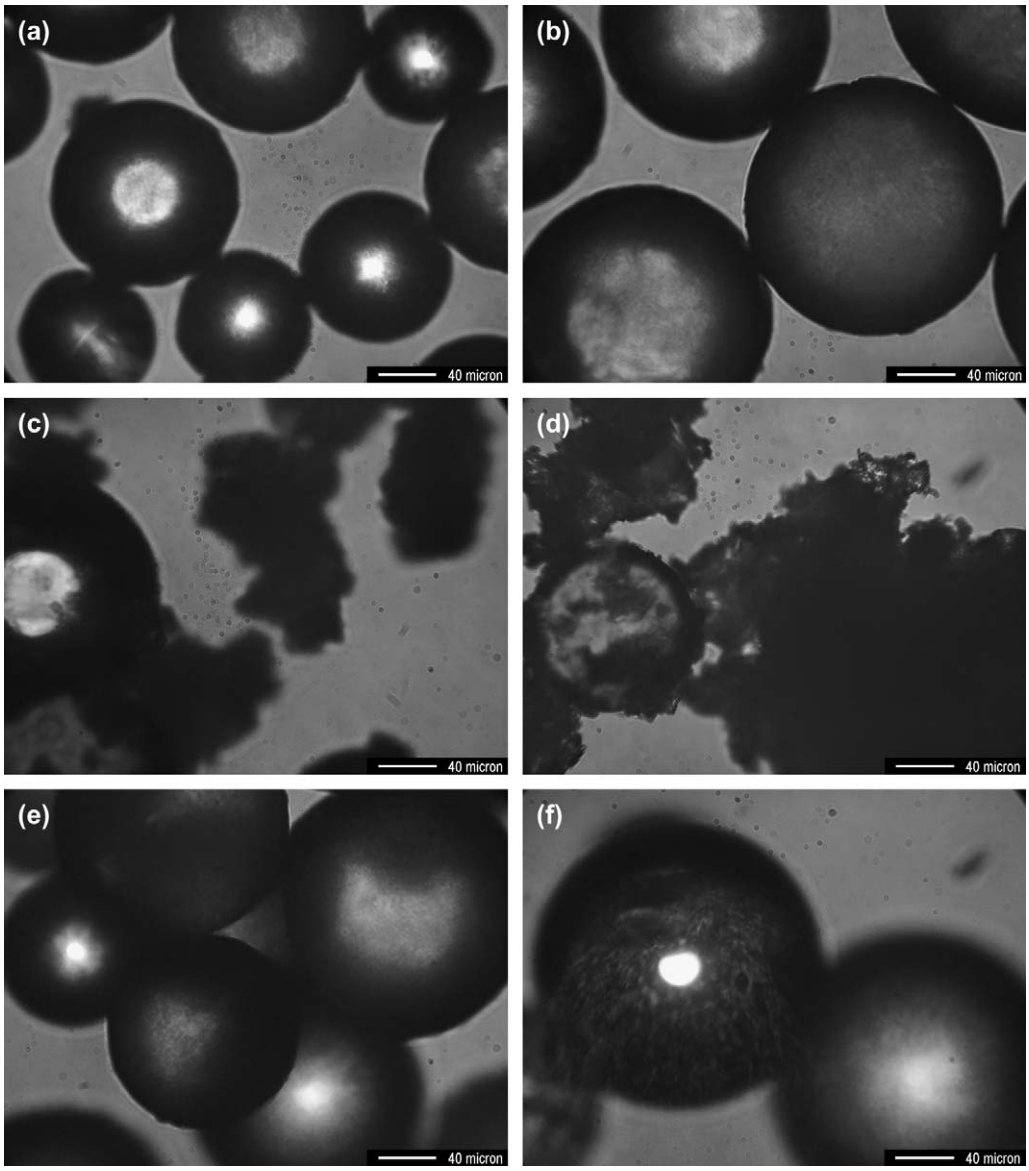


Fig. 2. Optical microscope images of ATRP beads made from Merrifield resin. (Magnified factor is 400×.) (a) Merrifield resin; (b) **P2**; (c) **D1**; (d) **DP1**; (e) **DP1-N** and (f) **DP2**.

this study. Only 35% of the weight increase after polymerization of DMA by CuBr/PMDETA system, and resins show damages when magnetic stirring is used (Fig. 2(c)). The sample of the **DP1** copolymer suffers the same problem. While no agitation was used in order to prevent this problem, the resulted beads are spherical but exhibit a large distribution of particle sizes as shown in Fig. 2(e). However, mechanical weakness still occurs with these beads when used in further applications. To solve this problem, we try to copolymerize DMA and PEG macromonomers with different PEG chain lengths. To our surprise, the **DP2** and **DP3** samples that resulted from this copolymerization showed relatively better mechanical characteristics even when magnetic stirring was used as shown in Fig. 2(f) opposed to Fig. 2(c) and (d). Fig. 2(f) demonstrates that the particles of **DP2** were of uniform diameter, ~160 μm. This result suggests that longer PEG chain may be helpful in preserving the structural integrity of beads when copolymerizing with DMA.

FT-IR spectra of the Merrifield resin onto which is grafted DMA and PEG (**P2**) macromonomers are shown in Fig. 3. The FT-IR spectra show that after grafting PEG, the carbonyl stretching vibration at 1726 cm^{-1} for **P2** resin appears, corresponding to the presence of PEG-526. Another two broad peaks at 1100 and 3460 cm^{-1} are markedly higher than those from Merrifield resin; they correspond to the C–O–C and hydroxyl groups, respectively. After DMA monomer had been copolymerized, a carbonyl stretching vibration appeared at 1640 cm^{-1}, corresponding to amide linkage (Fig. 3(c)). The emergence of these new absorption peaks verifies that polymer chain was grafted onto the polymer matrix.

3.2. Swellability studies of beads

The swellability of a solid support is an important characteristic in solid-phase synthesis. The accessibility of reagents

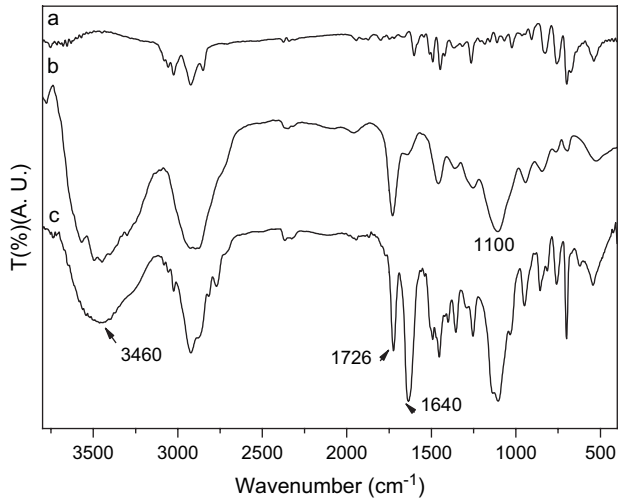


Fig. 3. FT-IR spectra of Merrifield resin grafted with DMA and PEG macromonomers (a) Merrifield resin; (b) **P2** and (c) **DP2**.

to active sites in the polymer matrix depends strongly on its swellability in certain solvents, and thus directly relates to their effectiveness as solid-phase synthesis supports. Swellability is most commonly measured by comparing the volumes of the beads in a graduated cylinder before and after swelling [5,17]. However, this approach yields inaccurate results in beads with larger particles. Therefore, the difference between the mass of dry beads and that of swollen beads was measured, and the data were transformed into the form of volume per weight [15]. In this work, data for Merrifield resin and commercially available TentaGel resin from Rapp Polymere Inc. were also compared. This comparison result is presented in Table 2.

Various PEG macromonomers were used in this study, including PEG-375, PEG-526 and PEG-1100. The swellability of polymer beads in polar solvents such as methanol increases when macromonomers with a longer PEG chain is used. For example, **P4** with higher PEG ($M_n = 1100$) gave the highest swellability, 2.5 mL/g, in MeOH. However, the use of PEG monomer with a high molecular weight limits its loading capacity in polar solvents. Hydrophilic monomer (DMA) with low molecular weight monomer was incorporated to modify the resin so as to retain this property. Consequently, the **D1** sample had excellent swellability (>4 mL/g). Graft-copolymer (**DP1**, **DP2** and **DP3**), which is similar to TentaGel in polar solvent also exhibits improved swellability, indicating that incorporating DMA monomer efficiently enhances bead's swellability without reducing its loading capacity. As expected, the swelling of resins differed greatly from that of Merrifield resin, and the polar solvents improved swelling. These resins will be expected to have potential applications in continuous solid-phase oligopeptides, as reported by Akelah et al. [18].

Table 2
Swelling properties of resins in different solvents (mL/g)

Sample	Methanol	Water	THF
Merrifield resin	1.2	0.9	4.9
TentaGel S[a] (0.4–0.6 mmol/g)	3.6	3.1	4.2
P1	1.6	1.6	2.4
P3	2.1	2.0	2.3
P4	2.5	2.4	2.5
D1-N	4.3	4.1	4.3
DP1-N	3.3	2.9	3.8
DP2	3.2	3.0	3.3
DP3	3.1	2.8	3.2

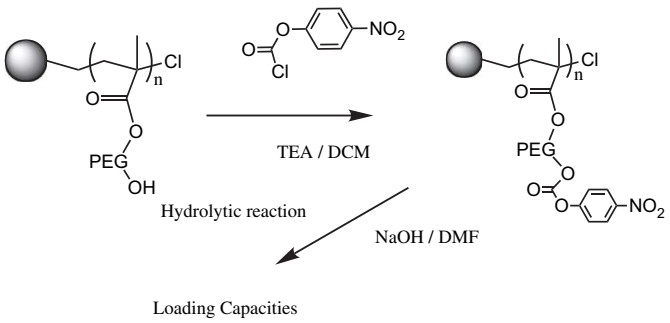
[a] Data retrieved from worldwide web on 12/14/2005 (http://www.rapp-polymere.com/).

3.3. Loading capacities

The loading capacities of the resins were determined by an initial reaction of the resin with an excess amount of dye-containing molecules, and the loading capacity of the final resin is determined by the concentration of the dye fragment after it is cleaved from the beads by UV spectrophotometry. Usually, fluorenylmethoxycarbonyl (Fmoc) protected amino acid is used to determine the loading capacities of Merrifield resin-based supports [19]. In this case, a more reactive dye, *p*-nitrophenyl chloroformate, is employed, and its UV absorption at 435 nm is used to determine the total concentration of the functional groups attached to the resin. The resin-containing hydroxyl groups help to swell in DMF and react with a 10-fold excess *p*-nitrophenyl chloroformate in the presence of TEA (Scheme 2). Then, the nitrophenol was cleaved from resin under basic conditions to determine its loading capacities from the UV calibration curve. The loading capacity of the copolymerized beads can be as high as 1.8 mmol g^{-1} (Table 1). These results demonstrate that the copolymerized resins contain several folds of more reactive hydroxyl functionalities than the commercially available TentaGel resins.

4. Conclusions

In summary, grafting hydrophilic monomers by controlled/living ATRP from polystyrene-based resins can yield a novel structure designed polymer supports with high polar solvent swellability and high hydroxyl functionality. Copper(II) salt can be effectively removed from the resin by washing the resin with an organic soluble EDTA salt to yield white resins. The favorable mechanical characteristics of DMA-containing resins were obtained after the ATRP method was introduced. The resin's swelling behavior in hydrophilic solvents is similar



Scheme 2. Determination of loading capacities by reacting the beads with 4-nitrophenyl chloroformate.

to that of TentaGel resins while the copolymerized **DP3** sample has the advantages of ease of preparation and a significantly higher loading capacity ($\sim$1.8 mmol g^{-1}). These types of resin have potential application in peptide synthesis; this work is currently under investigation in the authors' laboratory.

Acknowledgments

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References

[1] Merrifield RB. J Am Chem Soc 1966;85:2149.
[2] Sherrington DC. J Polym Sci Part A Polym Chem 2001;39:2364.
[3] Gauthier MA, Luo J, Calvet D, Ni C, Zhu XX, Garon M, et al. Polymer 2004;45:8201.
[4] Kita R, Svec F, Frechet JMJ. J Comb Chem 2001;3:564.
[5] Ayres N, Haddleton DM, Shooter AJ, Pears DA. Macromolecules 2002;35:3849.
[6] Bayer E, Rapp W. Chem Pept Proteins 1986;3:3.
[7] Wang JS, Matyjaszewski K. J Am Chem Soc 1995;117:5614.
[8] Miller PJ, Matyjaszewski K. Macromolecules 1999;32:8760.
[9] Kamigaito M, Ando T, Sawamoto M. Chem Rev 2001;101:3689.
[10] Angot S, Ayres N, Bon SAF, Haddleton DM. Macromolecules 2001; 34:768.
[11] Zheng G, Stover HDH. Macromolecules 2002;35:7612.
[12] Zheng G, Stover HDH. Macromolecules 2003;36:1808.
[13] Meldal M. Tetrahedron Lett 1992;33:3077.
[14] Auzanneau FI, Meldel M, Bock K. J Peptide Sci 1995;1:31.
[15] Cavalli G, Shooter AG, Pears DA, Steinke JHG. J Comb Chem 2003;5:637.
[16] Huang X, Wirth MJ. Macromolecules 1999;32:1694.
[17] Santini R, Griffith MC, Qi M. Tetrahedron Lett 1998;39:8951.
[18] Akelah A, Heffernan JG, Kingston SB, Sherrington DC. J Appl Polym Sci 1983;28:3137.
[19] Fruchtel JS, Jung G. Angew Chem Int Ed Engl 1996;35:17.