Synthesis of Lactose-Containing Glycopolymer-Grafted Silica Gel Particles

Tian-Ying Guo, Ping Liu, Yong-Qing Xia, Mou-Dao Song

Key Laboratory of Functional Polymer Materials, Ministry of Education, Institute of Polymer Chemistry, College of Chemistry, Nankai University, Tianjin 300071, China

Received 20 November 2008; accepted 21 September 2009
DOI 10.1002/app.31525
Published online 5 January 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The surface free-radical graft polymerization of 2-*O*-meth-acryloyloxyethoxyl-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside onto silica gel particles has been carried out with azobis (isobutyronitrile) as initiator. The grafting reaction conditions and the glycopolymer-grafted silica gel particles have been investigated in detail. Chromatographic experiments have been attempted on glycopolymer-modified silica gel particles as a stationary phase under normal phase conditions, and it was found that a certain separation effect of the quercetin and its derivant isorhamnetin was achieved. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 1611–1616, 2010

Key words: glycopolymer; graft polymerization; chromatographic properties

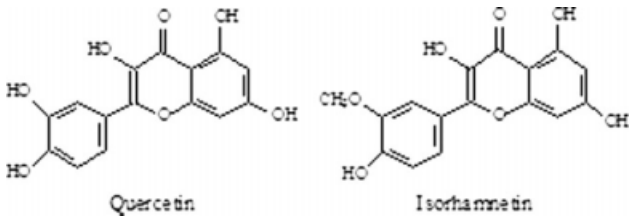
INTRODUCTION

The surface modification of inorganic substrate by polymer represents a new class of polymeric materials, which combine the properties of the inorganic particles (in terms of mechanical strength, modulus, and thermal stability) with the compatible and the chemical activity of the organic polymer matrix. The main practical applications of such systems include the specific stationary phases for gas or liquid chromatography,[1,2] the supported catalysis,[3,4] the colloid stabilization,[5,6] the adhesion of biocompatible materials,[7] the reinforcement of elastomers,[8] and so forth. Usually, covalent bonding of the polymeric phase onto silica substrates has been used to resist swelling effects and operate at high temperatures. Among various methods for covalent bonding of polymer chains onto inorganic substrate, free-radical surface graft polymerization is a simple and efficient method of obtaining a dense grafted polymer layer. Free-radical graft polymerization typically involves the formation of both free polymer chains in the solution and grafted polymer chains on the substrate. To decrease the free polymer chains, it usually requires surface activation by the introduction of the surface active sites, for example, vinyl groups, epoxy groups,[9–11] or by the direct bonding initiator molecules.[12–14]

Although the sugar portions of the glycoconjugates have been found to play essential roles as recognition sites between cells or as factors controlling the generation of biological functions,[15] many studies are aiming at developing functional polymers based on the synthetic polymers containing sugar moieties, herein referred to as "glycopolymers." Glycopolymers are considered as high-value polymeric materials, because of their solubility in water, strong hydrogen bonding ability, and high hydrophilicity. These kinds of polymers have potential applications in a variety of functional materials, for example, surfactants, hydrogels, drug-delivery systems, cell-specific culture substrata, and chromatographic supports.[16–22]

Flavonoid compounds, which contain about several tens of different compounds, such as quercetin and isorhamnetin, are one of the largest groups of naturally occurring phenols commonly present in medicinal plants. It has been shown that quercetin, as a typical member of the large family of flavonoids, has multiple biological and pharmacological activities, including antiviral,[23] anti-inflammatory,[24–26] antiallergy, and anticancer properties.[27–29] Usually, extraction or separation of active components from herb is tedious and inefficient because of poor affinity and selectivity of conventional separation materials (i.e., silica gel,[30] modified silica gel,[31] or polyamides[32]). For example, quercetin and isorhamnetin are all the main components of flavonoid compounds and very similar in structure (see Scheme 1),

Correspondence to: T.-Y. Guo (tyguo@nankai.edu.cn).
Contract grant sponsor: National Natural Science Foundation of China; contract grant number: 20874052.



Scheme 1 Chemical structures of quercetin and isorhamnetin.

but they are different in physiological functions. It is very difficult to separate them effectively. Thus, much research has been directed toward the variation of the sorption materials to achieve the selective interaction with the target molecule.[33]

In this article, our aim is to synthesize the surface glycopolymer-functionalized silica gel particles. Herein, we describe the preparation of *O*-acetyl-lactose-containing glycomonomer and report for the free-radical graft polymerization of glycomonomer onto γ-methacryloxypropyl-trimethoxy silane (MPTMS)-modified silica particles. The glycopolymer, because of their solubility in water, strong hydrogen bonding ability, high hydrophilicity, and grafted silica particles, should present a particular interesting model system as chromatographic support for the isolation of the substance with biological activity or hydrophilicity, such as separation of natural products or separation and assay of biomacromolecules.[16–22] Thus, in this work, we have tried to use the glycopolymer-grafted silica gel particles as HPLC supports to separate the structure, which is closely similar to flavonoid compounds of quercetin and isorhamnetin.

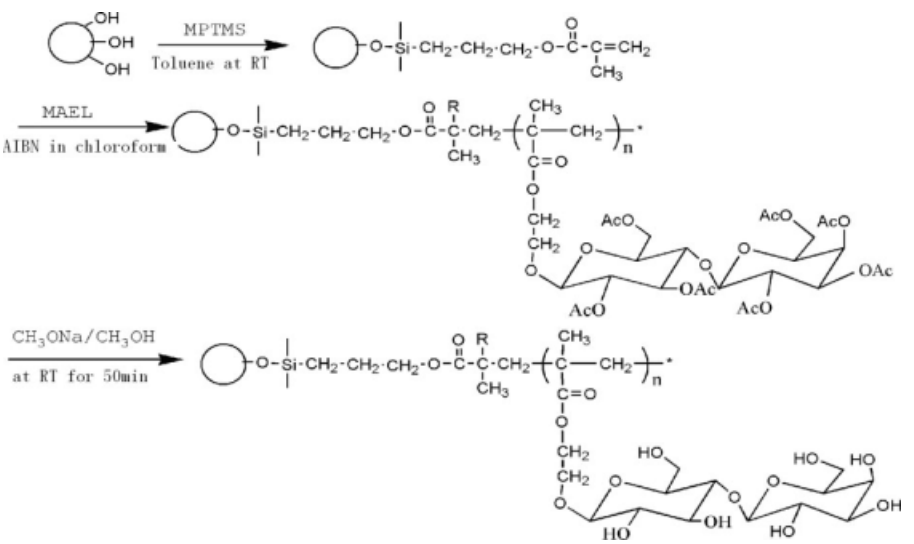
EXPERIMENTAL

Materials and reagents

Silica particles (Meigao Chemical, Qingdao, China) with an average diameter of 10 μm and a specific surface area of 129 m^2/g (BET method) were boiled in 20% HCl for 4 h and washed extensively with distilled water and dried at 120°C for 24 h. Hydroxyethyl methacrylate (Tianjin Chemreagent Institute, Tianjin, China) was distilled under reduced pressure before use. Lactose octaacetate 2-*O*-meth-acryloyloxyethoxyl-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1-4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (MAEL) was synthesized by the reaction of lactose and acetic anhydride in our lab.[34] γ-Methacryloxypropyltrimethoxy silane (MPTMS) was purchased from Nanjing Shuguang Reagent Factory (Nanjing, China). Azobis (isobutyronitrile) (AIBN) was purchased from Tianjin Fuchen Chemical Reagent Factory (Tianjin, China) and purified by recrystallization in ethanol before use. Quercetin and isorhamnetin were purchased from Sigma. Other chemicals used as received were of analytical grade.

Graft polymerization and deprotection reaction

Introduction of double bonds onto the surfaces of silica particles was achieved by the reaction of MPTMS with the hydroxyl groups of silica gel particles. One gram of silica gel particles was suspended in 15 mL of dry toluene. Then, 1 g of MPTMS and a trace amount of triethylamine were added. This mixture was refluxed for 10 h under nitrogen atmosphere. The silica gel was isolated by



Scheme 2 Schematic illustration of glycomonomer grafted onto silica gel particles.

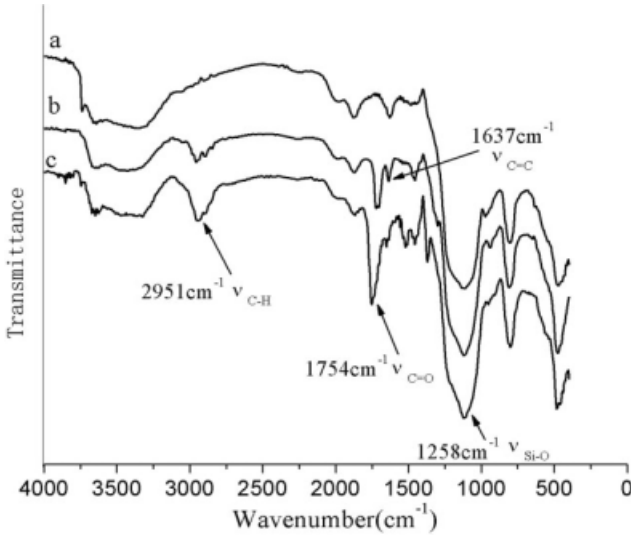


Figure 1 FTIR spectra of (a) pristine silica gel, (b) MPTMS-modified silica gel, and (c) glycopolymer-modified silica gel.

centrifugation and extracted with acetone for 24 h to remove the excessively absorbed MPTMS.

The graft density of the MPTMS on the silica surface was determined by thermogravimetric analysis (TGA) using eq. 1:

$$\text{Graft density}(\text{umol}/\text{m}^2) = \frac{\left(\frac{W_{\text{silane}}}{100-W_{\text{silane}}}\right) \times 100 - W_{\text{silica}}}{M \times 100} \times 10^3, \quad (1)$$

where W_{silane} is the weight loss between room temperature and 800°C corresponding to the decomposition of the MPTMS, and M is the molecular weight of the grafted silane. W_{silica} is the weight loss of silica determined before grafting.

Free-radical graft polymerization of MAEL glycomonomer onto MPTMS-modified silica particles, initiated by AIBN, was carried out in a three-necked flask in which the reaction temperature was controlled at 65°C. Then, the reaction was conducted under a nitrogen blanket to exclude oxygen. To remove ungrafted but physically adsorbed polymers, the grafted substrate was extracted with acetone for at least 24 h by a Soxhlet apparatus.

The grafted silica particles were suspended in dry methanol in a glass flask. After adding some amount of sodium methoxide, the flask was stirred for 50 min at room temperature. The suspension was isolated by centrifugation and purified with distilled water by a Soxhlet apparatus for 2 days to obtain lactose-carrying polymer-modified silica gel particles.

Preparation of the HPLC columns

Three grams of pristine silica gel and the MAEL-grafted silica particles were sonicated in methanol and were packed into stainless steel HPLC columns (250 mm × 4.6 mm, I.D.), respectively, with methanol at 41 MPa using an air-driven fluid pump (CGY-100).

Characterization

IR spectra were recorded with a Bio-Rad FTS 135 Fourier-transform infrared (FTIR) spectrometer in the range of 3500–500 cm^{-1} using KBr pellets.

TGA was carried out with NETZSCH TG 209 (Germany) at a heating rate of 10°C/min in N_2 atmosphere and temperature range from 0 to 800°C. The experimental baseline of the instrument was calibrated using an empty crucible, and the temperature was calibrated using indium and zinc as standard samples.

Field emission scanning electron microscope (SEM) images were recorded on a LEO-1530 VP microscope (German).

HPLC analysis was carried out on a SSI HPLC (Scientific System) system equipped with a UV detector (Lab Alliance) and the detection was carried out at 256 nm. The evaluations of the columns were performed at room temperature. The mobile phase was acetonitrile–methanol (85 : 15, v/v). The flow rate was 1.0 mL/min. The sample volume and concentration injected were 20 μL and 100 μg mL, respectively. Toluene was used as a void marker.

TABLE I
TGA Data of MPTMS-Modified Silica Gel Particles

Samples	Amount of silica gel (g)	MPTMS (g)	Triethylamine	Weight loss (wt %)	Graft density[a] (μmol m^2)
A1	0.5	0	–	3.03	0
A2	0.5	0.5	–	7.40	1.55
A3	0.5	1	–	9.99	2.52
A4	0.5	0.5	3–4 Drops	9.61	2.38
A5	0.5	1	3–4 Drops	10.08	2.56

[a] Determined using eq. 1

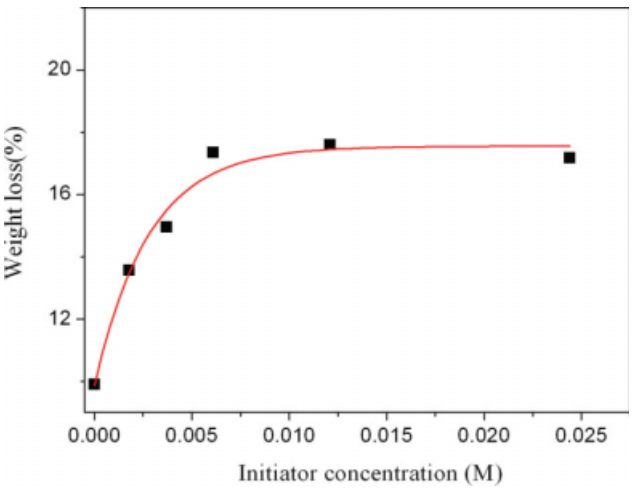


Figure 2 Effect of the initiator concentration on weight loss of glycopolymer-grafted silica gel particles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

RESULTS AND DISCUSSION

The synthesis of glycopolymer-modified silica particles

The synthesis procedure for glycomonomer grafted on the silica gel particles is shown in Scheme 2. In the first step, we chose a convenient and effective method to synthesize glycomonomer. The lactose octaacetate was used as a glycosylation donor, which reacted with hydroxyethyl methacrylate to give corresponding space-armed lactose.

The modification of the MPTMS and grafting of glycomonomer onto silica particles were qualitatively confirmed by FTIR spectroscopy. The FTIR spectra of pristine, MPTMS-modified, and glycopolymer-modified silica gel particles are reported in Figure 1. The spectrum of Figure 1(b) shows characteristic vibrations of the carbonyl ($\nu_{C=O}$ 1721 cm^{-1}), the double bond ($\nu_{C=C}$ 1637 cm^{-1}), and the aliphatic groups (ν_{CH} 2846, 2895, and 2955 cm^{-1}) of the MPTMS molecule. Compared to MPTMS-modified silica particles, the spectrum of Figure 1(c) gives clear evidence of glycomonomer grafting with the absorption at the aliphatic groups (ν_{CH} 2894–2951 cm^{-1}, δ_{CH} 1370 cm^{-1}, 1454 cm^{-1}) and the strong absorption bond at the carbonyl ($\nu_{C=O}$ 1754 cm^{-1}) of the glycopolymer.

To find optimum grafting conditions, increasing amount of MPTMS or adding a trace of triethylamine was introduced in the silica suspension. The graft density was calculated using TGA data. Table I shows the evolution of the graft density as MPTMS concentration or adding triethylamine. The results indicate that the graft density increases with increasing the silane content and reaches a plateau at high concentrations, which is coincident with Guyot et al.'s reports.[35] In our experiment, we added a trace amount of triethylamine during the grafting reaction at a lower MPTMS concentration, and then the graft density had increased much. This indicates that the methoxyl groups of MPTMS are easy to remove in the basic surrounding, which are facile to graft onto silica gel. But adding a trace of triethylamine at high MPTMS concentration, the surface concentration of bonded organosilanes had not obviously increased.

In the following steps, a series of experiments with varying glycomonomer-to-initiator molar ratios were carried out to obtain further insight into the graft polymerization process. Figure 2 shows the dependence of polymer graft density on the initiator concentration. From Figure 2, the polymer graft density increases sharply at rather low concentrations and then remains unchanged when the concentration is greater than 0.012*M*. Drastic increase is understandable, because a higher initiator concentration would lead to a higher initiator rate and so more double bonds on the surface can be initiated. The unchanged trend at a higher initiator rate implies that the initiator is redundant, which is facile to the termination of the initiator. Therefore, a suitable initiator concentration of 0.012*M* was chosen for the polymer grafting modification of silica particles.

Sample A4 (see Table I) was selected as MPTMS modified silica gel to continue the nest modification. For a similar system like above, the effects of the monomer concentration on the grafting

TABLE II
Experimental Data for Glycopolymer-Modified Silica Particles with Different Amount of Glycomonomer

Samples	Amount of silica (g)	Initiator AIBN (g)	Glycomonomer MAEL (g)	Weight loss (wt %)
B1	0.2	0.02	0	9.61
B2	0.2	0.02	0.3	20.13
B3	0.2	0.02	0.6	22.46
B4	0.2	0.02	1.0	17.59
B5	0.2	0.02	1.5	19.88
B6	0.2	0.02	2.0	22.14

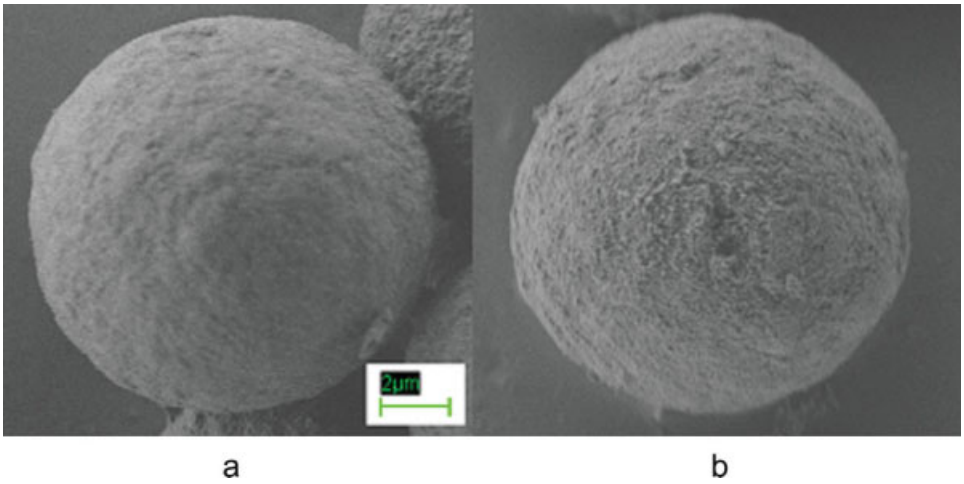


Figure 3 Scanning electron microphotographs of pristine (a) and glycopolymer-modified silica particles (b). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

polymerization reaction are shown in Table II. The data showed to obtain better grafted number of glycopolymer on inorganic substrates, although there are not clearly pertinence between the monomer concentration and the weight loss of glycopolymer-modified silica particles. However, grafting reactions are very sensitive to experimental conditions (moisture content, silane chemical composition and

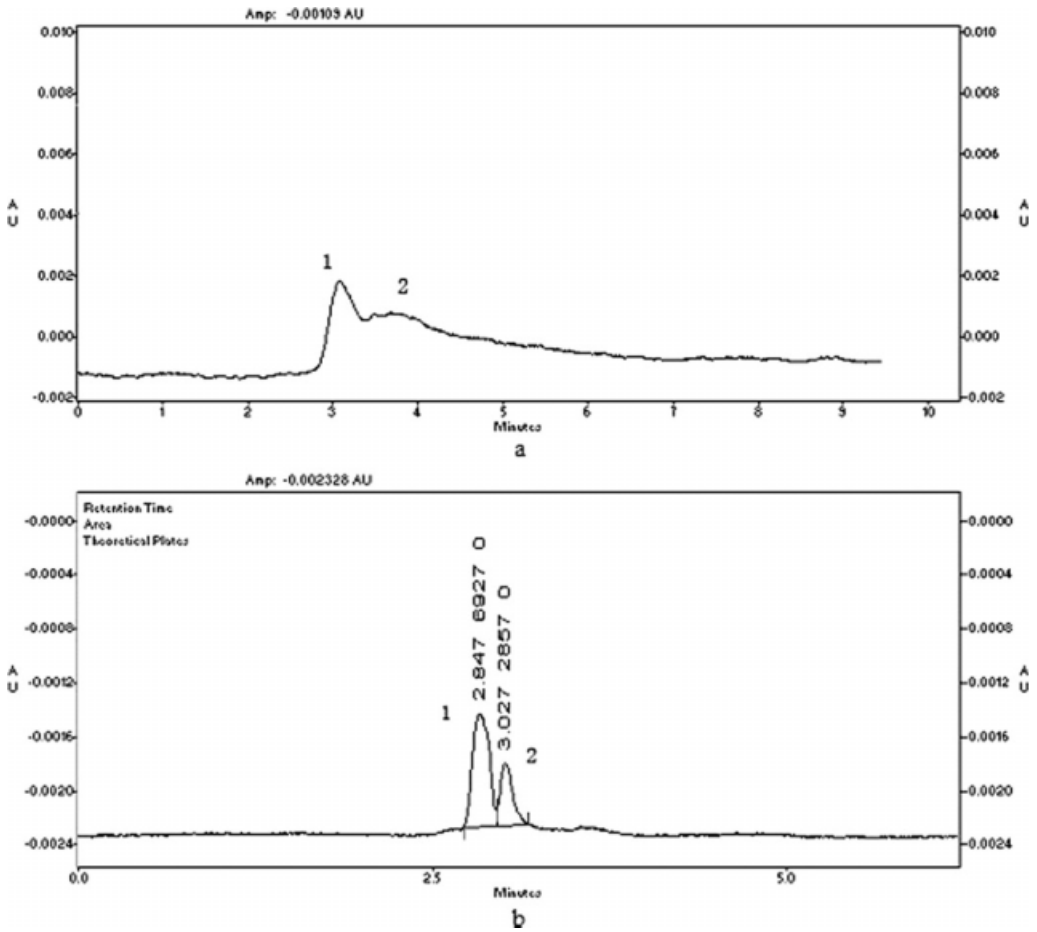


Figure 4 Chromatograms for (a) unmodified silica gel and (b) glycopolymer-modified silica gel with acetonitrile–methanol (85/15, v/v) as mobile phase; flow rate was 1 mL/min; the wavelength was set at 256 nm; the column temperature was ambient; isorhamnetin (1) and quercetin (2).

functionality, washing procedures and curing treatments, etc.), such sensitivity makes it difficult to ensure reproducible surface coverage.[14]

To the better observation of the surface pattern of silica particles, we used the SEM. Figure 3 shows the scanning electron microphotograph of pristine [Fig. 3(a)] and glycopolymer-modified silica particles [Fig. 3(b)]. It can be observed that the surface morphology of the modified silica particle is somewhat rougher than the pristine silica particles, which overlay of the glycopolymer on the silica particles after grafting polymerization. But the profile of glycopolymer-grafted silica gel is steel uniform and clear.

HPLC measurements

The separating effects to flavonoid compounds of quercetin and isorhamnetin for the glycopolymer-grafted silica gel (sample B3) were examined in the HPLC system. Figure 4 shows the chromatographic profiles of pristine and glycopolymer-grafted silica gel particles as supports. It can be seen that quercetin (2) and isorhamnetin (1) can be separated obviously on the glycopolymer-grafted silica gel column as seen in Figure 4(b), when acetonitrile–methanol (85 : 15, v/v) was used as mobile phase. And Figure 4(a) showed that under the same condition, the mixture of isorhamnetin (1) and quercetin (2) could not be separated by the pristine silica gel column. This indicates that the surface property of the silica gel particles was improved, when the lactose-containing glycopolymer was grafted onto the silica gel surface. This result is still a primary investigation at present state. The related work is still continuing in our lab, such as the detailed chromatographic conditions to these separation objects and different types of natural product compounds or biomacromolecules for the applications based on glycopolymer-grafted silica gel.

CONCLUSIONS

The graft polymerization of MAEL glycomonomer on MPTMS-modified silica gel particles was accomplished by the surface-initiated free-radical polymerization. The grafted density can be regulated by adjusting the concentrations of initiator and monomer for the reaction system. The glycopolymer-grafted silica gel, as a HPLC stationary phase under normal phase conditions, was found to have obvious separation effect for quercetin and its derivant isorhamnetin in our experiment. The properties of the inorganic particles, combined with the biological activity or hydrophilicity of glycopolymer, would have many potential applications in materials science.

References

1. Kobayashi, J.; Kikuchi, A.; Sakai, K.; Okano, T. Anal Chem 2003, 75, 3244.
2. Sulitzky, C.; Ruckert, B.; Hall, A. J.; Lanza, F.; Unger, K.; Sellergren, B. Macromolecules 2002, 35, 79.
3. Suzuki, K.; Yumura, T.; Mizuguchi, M.; Tanaka, Y.; Chen, C. W.; Akashi, M. J Appl Polym Sci 2000, 77, 2678.
4. Martin, E.; Dubois, Ph.; Jerome, R. Macromolecules 2003, 36, 7094.
5. Poncet-Legrand, C.; Bordes, B.; Lafuma, F. Colloid Polym Sci 2001, 279, 114.
6. Van Ewijk, G. A.; Philipse, A. P. Langmuir 2001, 17, 7204.
7. Haginaka, J.; Sanbe, H. Anal Chem 2000, 72, 5201.
8. Montes, H.; Lequeux, F.; Berriot, J. Macromolecules 2003, 36, 8107.
9. Chaimberg, M.; Parnas, R.; Cohen, Y. J Appl Polym Sci 1989, 37, 2921.
10. Browne, T.; Chaimberg, M.; Cohen, Y. J Appl Polym Sci 1992, 44, 671.
11. Nguyen, V.; Yoshida, W.; Cohen, Y. J Appl Polym Sci 2003, 87, 300.
12. Hussenman, M.; Malmstrom, E. E.; McNamara, M.; Mate, M.; Mecerreyes, D.; Benoit, D. G.; Hedrick, J. L.; Mansky, P.; Huang, E.; Russell, T. P.; Hawker, C. J. Macromolecules 1999, 32, 1424.
13. Prucker, O.; Ruhe, J. Macromolecules 1998, 31, 602.
14. Bartholome, C.; Beyou, E.; Bourgeat-Lami, E.; Chaumont, P.; Zydowicz, N. Macromolecules 2003, 36, 7946.
15. Dwek, R. A. Chem Rev 1996, 96, 683.
16. Albertin, L.; Kohlert, C.; Stenzel, M.; Foster, L. J. R.; Davis, T. P. Biomacromolecules 2004, 5, 255.
17. Narumi, A.; Matsuda, T.; Kaga, H.; Satoh, T.; Kakuchi, T. Polymer 2002, 43, 4835.
18. Grands, D.; Baskaran, S.; Chaikof, E. L. Macromolecules 2001, 34, 1640.
19. Loykulnant, S.; Hirao, A. Macromolecules 2000, 33, 4757.
20. Gotz, H.; Harth, E.; Schiller, S. M.; Frank, C. W.; Knoll. W.; Hawker, C. J. J Polym Sci Part A: Polym Chem 2002, 40, 3379.
21. Kobayashi, A.; Goto, M.; Kobayashi, K.; Akaike, T. J Biomater Sci Polym Ed 1994, 6, 325.
22. Sun, X. L.; Grande, D.; Baskaran, S.; Hanson, S. R.; Chaikof, E. L. Biomacromolecules 2002, 3, 1065.
23. Van Hoof, L.; Van den Berghe, D. A.; Hatfield, G. M.; Vlietinck, A. Planta Med 1984, 50, 513.
24. Brasseur, T. J Pharm Belg 1989, 44, 235.
25. Wei, B. L.; Lu, C. M.; Tsao, L. T.; Wang, J. P.; Lin, C. N. Planta Med 2001, 67, 745.
26. Fischer, M.; Mills, G. D.; Slaga, T. J. Carcinogenesis 1982, 3, 1243.
27. Hertog, M. G. L.; Hollman, P. C. H.; van de Putte, B. J Agric Food Chem 1993, 41, 1242.
28. Birt, D. F.; Hendrich, S.; Wang, W. Pharmacol Ther 2001, 90, 157.
29. Sousa, R. L.; Marletta, M. A. Arch Biochem Biophys 1985, 240, 345.
30. Ali, M.; Qedry, J. S. J Ind Chem Soc 1987, 64, 230.
31. Bhusman, R.; Ali, I. J. Planar Chromatogr 1990, 3, 85.
32. Barcelon, D. J Chromatogr 1982, 238, 175.
33. Sherma, J. Anal Chem 1992, 64, 134.
34. Guo, T. Y.; Liu, P.; Zhu, J. W.; Song, M. D.; Zhang, B. H. Biomacromolecules 2006, 7, 1196.
35. Bourgeat-Lami, E.; Espiard, Ph.; Guyot, A. Polymer 1995, 36, 4385.