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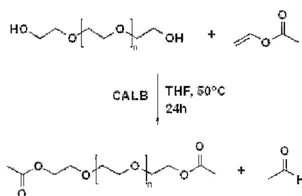

FIG. 1

(57) Abstract: Abstract The invention relates to functionalized, telechelic polymers synthesized by enzymatic catalysis and methods, and the functionalization of polymers via Michael addition with a lipase catalyst, and the crosslinking of mono- or difunctional (telechelic) polymers made by enzymatic catalysis, such as by using multifunctional coupling agents and enzyme catalysts. Quantitative transesterification of vinyl methacrylate with poly(ethylene glycol), poly(isobutylene) and poly(dimethylsiloxane) was achieved using Candida antarctica lipase B. In addition, methacrylate-functionalized poly(ethylene glycol) monomethyl ether has been successfully coupled to aminoethoxy poly(ethylene glycol) monomethyl ether via Michael addition using Candida antarctica lipase B. Amine-functionalized poly(ethylene glycol)s have also been used for the preparation of poly(ethylene glycol)-based dendrimers and gels through Michael addition of the polymer onto triacryloyl hexahydro-triazine using the same enzyme. 1H and 13C NMR spectroscopy verified the structure of the functionalized polymers.

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PROCESS OF PREPARING FUNCTIONALIZED POLYMERS VIA ENZYMATIC CATALYSIS

Cross Reference

[0001] This application claims priority to U.S. Provisional Application Serial No. 60/975,625 filed on September 27, 2007 and U.S. Provisional Application Serial No. 61/040,395 filed on March 28, 2008, which are incorporated herein by reference in its entirety.

Technical Field

[0002] The invention relates to methods of functionalizing polymers via enzymatic catalysis, and the polymer materials produced thereby. More particularly, the invention relates to methods of preparing functionalized polymers via a transesterification process with a catalyst. In examples, the invention relates to a method of preparing functionalized glycol ethers, functionalized polyisobutylenes and functionalized polysiloxanes via a transesterification process with a lipase catalyst. In other examples, the invention relates to functionalization of polymers via Michael addition with a lipase catalyst, and the crosslinking of mono- or difunctional (telechelic) polymers made by enzymatic catalysis as outlined in this invention, using multifunctional coupling agents and enzyme catalysts.

Background of the Invention

[0003] The modification of natural or synthetic polymers with enzymes is an environmentally friendly alternative to classical chemical modification reactions which generally require harsh reaction conditions. Despite the advantage of using enzymes in functionalization reactions, i.e. milder reaction conditions and highly specific transformations, only a few examples have been reported in the literature involving either natural or synthetic polymers.

[0004] Mushroom tyrosinase has been used to introduce phenolic functionalities into chitosan. The synthetic pathway involved enzyme-catalyzed oxidation of phenol to an *o*-quinone which dissociated from the enzyme and freely diffused to the nucleophilic amine site of the chitosan polymer. In addition to the incorporation of quinone into the chitosan, subsequent polymerization of quinone into oligomeric phenols was also observed as determined by UV-Vis

spectroscopy. The effectiveness of the reactions was not discussed. Polysaccharides have been functionalized with fatty acids by transesterification using ion-paired subtilisin Carlsberg protease in organic solvents. It has been observed that the enzyme selectively acylated the primary hydroxyl groups on polysaccharides and that the degrees of substitution per glucose moiety in amylose and β-cyclodextrin were 0.185 and 0.250, respectively. Lactose was attached to the hydroxyl groups of hydroxyethylcellulose (HEC) by transglycosylation in sodium acetate buffer using *Aspergillus oryzae*. Although the number of sites available in each HEC unit is 3, the maximum degree of substitution obtained by this method was only 0.033. The same natural polymer, HEC, has recently been modified by enzymatic transesterification with both vinyl propionate and vinyl acrylate in the presence of subtilisin Carlsberg in anhydrous pyridine. These biotransformations of cellulose were promising; however, low conversions limited their viability. It has been reported that the phosphorylation of cotton cellulose using bakers' yeast hexokinase as the enzyme and ATP as the phosphoryl donor. The phosphorylation of 0.03 % of the glycopyranose units in the cellulose resulted in improved dyeability and flame resistance. All of the examples listed here were hindered by low conversion.

[0005] Even fewer examples have been reported for synthetic polymer functionalization using enzymatic catalysis, which are also characterized by low conversion. For instance, 16% of the pendant nitrile groups of polyacrylonitrile fibers were converted to the corresponding amides by a nitrile hydratase enzyme, resulting in a significant increase in the hydrophilicity of the fiber surface. Mushroom tyrosinase has been utilized to graft poly(4-hydroxystyrene) (PHS) onto chitosan. In this approach, first 1-2 % of the phenolic moieties of PHS were enzymatically oxidized and then the resulting polymer was reacted with the amine groups of chitosan. Lipase-catalyzed acylation of poly[N-(2-hydroxypropyl)-11-methacryloylaminoundecanamide-*co*-styrene] and the corresponding monomer with vinyl acetate, phenyl acetate, 4-fluorophenyl acetate and phenyl stearate has been reported. ^{1}H NMR results revealed that the reactivity of the monomer was higher than that of the copolymer and that the copolymer could be acylated with up to 40% conversion. In addition, it was found that the reactivity of copolymer was dependent on copolymer composition which indicated the effect of steric hindrance and hydrophobicity on reaction kinetics. The synthesis of organosilicon carbohydrate macromers by *Candida antarctica* lipase B catalyzed esterification has been reported. Diacid-endblocked siloxanes were reacted with α,β-ethyl glucoside under vacuum in bulk. Esterification occurred with high

regioselectivity (>98%) at the primary hydroxyl (C6) of the glucoside, but electrospray ionization mass spectrometry (ESI MS) showed the presence of a mixture of mono- and diesters.

[0006] The preparation of polyolefin-based telechelic polymers, such as methacrylate-terminated polyisobutylenes (PIB-MAs), has been shown in the prior art by a variety of strategies. In one example, PIB-Cl^{t} prepared by the *inifer* technique was first converted to the corresponding *exo*-olefin by dehydrochlorination with *t*-BuOK, followed by hydroboration/oxidation to yield PIB-OH which was subsequently converted to PIB-MA by acylation with methacryloyl chloride. In another process, PIB-OH was synthesized by hydroboration/oxidation of allyl-terminated PIB, prepared by end-quenching of living PIB^{+} with allyltrimethylsilane, and the latter was converted to PIB-MA with methacryloyl chloride. A further process describes how PIB-MA was synthesized by nucleophilic substitution of PIB-CH_2-CH_2-CH_2-Br (prepared by *anti-Markovnikov* hydrobromination of allyl-terminated PIB) with sodium methacrylate. This same method was also applied to Glissopal®2300, a commercially available PIB with olefinic end groups. Quantitative syntheses of *primary* hydroxyl terminated PIB and Glissopal-OH has also been described.

[0007] Notwithstanding the state of the art as described herein, there is a need for further improvements in the preparation of functionalized polymers (monofunctional or difunctional (telechelic) polymers) via enzymatic catalysis which is an environmentally friendly method when compared with toxic acylating agents such as methacryloyl chloride, and catalysts; and is useful in biomaterials prepared from these functionalized polymers.

Summary of the Invention

[0008] The invention provides methods and functionalized polymers that are prepared through enzymatic catalysis, and the polymer materials produced thereby. In one embodiment of the invention, the functionalized polymer is prepared by reacting a polymer and at least one acyl donor in the presence of an effective amount of an enzymatic catalyst in a transesterification reaction. Other functionalized polymers may be prepared from other chemical reactions, such as epoxidation, Michael addition, hydrolysis, or other techniques, through enzymatic catalysis.

[0009] In another embodiment of the invention, poly(ethylene glycol) is transesterified with at least one ester in the presence of an effective amount of a lipase to form a functionalized, telechelic polymer.

[0010] Furthermore, the invention provides methods of preparing a telechelic polymer through enzymatic catalysis. The method includes the steps of reacting a glycol ether, such as poly(ethylene) glycol, with at least one ester in the presence of an effective amount of a lipase in a transesterification reaction.

[0011] The invention also provides methods and functionalized polyolefin-based polymers that are prepared through enzymatic catalysis, and the polymer materials produced thereby. In one embodiment of the invention, the functionalized polymer is prepared by reacting a polyolefin-based polymer and at least one acyl donor in the presence of an effective amount of an enzymatic catalyst in a transesterification reaction. Other functionalized polymers may be prepared from other chemical reactions, such as epoxidation, Michael addition, hydrolysis, or other techniques, through enzymatic catalysis.

[0012] In another embodiment of the invention, hydroxyl-functionalized polyisobutylene is transesterified with at least one ester in the presence of an effective amount of a lipase to form mono- or difunctionalized (telechelic) polymer.

[0013] In yet another embodiment of the invention, a block copolymer is prepared. The method of preparing the block copolymer through enzymatic catalysis includes the steps of reacting a methacrylate functionalized polymer prepared by enzymatic transesterification with another polymer bearing amine functionality in the presence of an effective amount of an enzyme catalyst via Michael addition reaction.

[0014] In still yet another embodiment of the invention, a dendrimer is prepared. The method of preparing the dendrimer includes the steps of reacting a polyether diamine with a functionalized nitrogen heterocycle in the presence of an effective amount of the enzyme catalyst. Depending on the stoichiometry of the reaction, a polymer gel will be formed.

[0015] In another embodiment of the invention, another dendrimer is prepared. The method of preparing the dendrimer includes the steps of reacting an aminoethoxy glycol ether

with a functionalized nitrogen heterocycle in the presence of an effective amount of the enzyme catalyst.

Brief Description of the Drawings

[0016] FIG. 1 illustrates the scheme of the transesterification of vinyl acetate with poly(ethylene glycol);

[0017] FIG. 2 is the ^{1}H NMR spectra of PEG (M_n=1000 g/mol, M_w/M_n =1.06) before (A) and after (B) acylation;

[0018] FIG. 3 is the ^{13}C NMR spectra of PEG (M_n=1000 g/mol, M_w/M_n =1.06) before (A) and after (B) acylation; and

[0019] FIG. 4 is the ^{1}H NMR spectrum of methacrylated-PEG (M_n=2000 g/mol, broad MWD);

[0020] FIG. 5 is the ^{13}C NMR spectrum of methacrylated-PEG (M_n=2000 g/mol, broad MWD);

[0021] FIG. 6 illustrates the scheme of the transesterification of vinyl methacrylate with polyisobutylene;

[0022] FIGS. 7a and 7b are the ^{1}H NMR spectra of polyisobutylene before and after methacrylation;

[0023] FIGS. 8a and 8b are the ^{13}C NMR spectra of polyisobutylene before and after methacrylation;

[0024] FIG. 9 is a size exclusion chromatography trace of a polyisobutylene-methacrylate polymer according to one embodiment of the invention;

[0025] FIGS. 10a and 10b are the ^{1}H NMR spectra of Glissopal-OH before and after methacrylation;

[0026] FIGS. 11a and 11b are the ^{13}C NMR spectra of Glissopal-OH before and after methacrylation;

[0027] FIG. 12a is the ^{1}H NMR spectrum of Glissopal-OH;

[0028] FIG. 12b is the ^{1}H NMR spectrum of the reaction product of Glissopal-OH with vinylmethacrylate in bulk;

[0029] FIG. 13 illustrates the scheme of the transesterification of vinyl methacrylate with poly(dimethylsiloxane)s;

[0030] FIG. 14a is the ^{1}H NMR of poly(dimethylsiloxane) diol;

[0031] FIG. 14b is the ^{1}H NMR of the reaction product of poly(dimethylsiloxane) diol with vinylmethacrylate;

[0032] FIG. 15a is the ^{1}H NMR spectrum of poly(dimethylsiloxane) monocarbinol;

[0033] FIG. 15b is the ^{1}H NMR spectrum of the reaction product of poly(dimethylsiloxane) monocarbinol with vinylmethacrylate;

[0034] FIG. 16a is the ^{1}H NMR of poly(dimethylsiloxane) diol;

[0035] FIG. 16b is the ^{1}H NMR of the reaction product of poly(dimethylsiloxane) diol with vinylmethacrylate in bulk;

[0036] FIG. 17a is the ^{1}H NMR spectrum of poly(dimethylsiloxane) monocarbinol;

[0037] FIG. 17b is the ^{1}H NMR spectrum of the reaction product of poly(dimethylsiloxane) monocarbinol with vinylmethacrylate in bulk;

[0038] FIG. 18 illustrates the scheme of the vinyl methacrylate linking reaction between two poly(ethylene glycol)s;

[0039] FIG. 19 is the ^{1}H NMR spectrum of the product of the first step: the reaction between poly(ethylene glycol) monomethyl ether and vinyl methacrylate, according to one embodiment of the invention;

[0040] FIG. 20 is the ^{1}H NMR spectrum of the product of the reaction depicted in FIG. 18.

[0041] FIG. 21 illustrates a scheme of a reaction for forming the core of a dendrimer according to one embodiment of the invention; transesterification with HO-PEG-OH will yield the first generation.

[0042] FIG. 22 illustrates a scheme of a reaction for a selective reaction according to another embodiment of the invention;

[0043] FIG. 23 is the ^{1}H NMR spectrum of a monosubstituted compound prepared according to the reaction of FIG. 22;

[0044] FIG. 24 illustrates the synthesis of a dendrimer using Michael addition;

[0100] FIG. 25 illustrates a scheme of a reaction for conjugation of folic acid onto poly(ethylene glycol) monomethyl ether.

Detailed Description of the Invention

[0045] An embodiment of the invention relates to methods preparing functionalized polymers through enzymatic catalysis. In an example of the invention, the functionalized polymer is prepared by reacting a polyol with an ester and an effective amount of an enzymatic catalyst in a transesterification reaction. Other chemical reactions, such as epoxidation, Michael addition, hydrolysis, or other techniques, may also be used to prepare functionalized polymers through enzymatic catalysis.

[0046] In an embodiment of the invention the polyol includes, but is not limited to, unsaturated diols such as polybutadiene diol or saturated diols such as ethylene glycol, diethylene glycol, polyethylene glycol, aminoethoxy glycol ether, such as aminoethoxy polyethylene glycol monomethyl ether, polyethylene glycol monomethyl ether, propylene glycol, dipropylene glycol, polypropylene glycol, 2-methyl-1,3-propanediol, 1,2-, 1,3-, 1,4-, or 2,3-butanediols, 2-methyl-1,4-butanediol, 2,3-dimethyl-2,3-butanediol, 1,5-pentanediol, 1,6-hexanediol; unsaturated triols such as castor oil (i.e., triricinoleoyl glycerol); saturated triols such as 1,2,4-butanetriol, 1,2,6-hexanetriol, trimethylolethane (i.e., 1,1,1-tri(hydroxymethyl)ethane),

trimethylolpropane (i.e., 2,2-di(hydroxymethyl)-1-butanol), triethanolamine, triisopropanolamine; unsaturated tetraols such as 2,4,6-tris(N-methyl-N-hydroxymethyl-aminomethyl)phenyl; saturated tetraols such as pentaerythritol (i.e., tetramethylolmethane), tetrahydroxypropylene ethylenediamine (i.e., N,N,N',N'-tetrakis(2-hydroxypropyl)-ethylenediamine); and other polyols such as mannitol (i.e., 1,2,3,4,5,6-hexanehexyl) and sorbitol.

[0047] Another embodiment of the invention relates to methods preparing functionalized polymers through enzymatic catalysis. In an example of the invention, the functionalized polymer is prepared by reacting a polyolefin or a polyolefin-derived material with an ester and an effective amount of an enzymatic catalyst in a transesterification reaction. Other chemical reactions, such as epoxidation, hydrolysis, or other techniques, may also be used to prepare functionalized polymers through enzymatic catalysis.

[0048] In another embodiment of the invention the polyolefin or polyolefin-derived material includes, but is not limited to, polychloroprene, polybutadiene, polyisoprene, polyisobutylene, polysiloxanes, such as polydimethylsiloxane, nitrile-butadiene rubber, styrene-butadiene rubber, chlorinated polyethylene, chlorosulfonated polyethylene, epichlorohydrin rubber, butyl rubber, or halobutyl rubber. The above-mentioned materials may be hydroxyl functionalized for use in preparing the functionalized polymer.

[0049] In yet a further embodiment of the invention, the acyl donor refers to a compound that is capable of leading to the formation of an ester in the presence of an enzymatic catalyst and a substrate. Examples of acyl donors include butyl acetate, ethyl phenyl acetate, ethyl acetate, ethyl trichloroacetate, ethyl trifluoroacetate, isopropenyl acetate, vinyl acetate, ethyl methoxy acetate, 2,2,2-trifluoroethyl butyrate, diketene, vinyl propionate and vinyl methacrylate.

[0050] In further embodiment of the invention, the enzymatic catalyst is capable of catalyzing a transesterification reaction. In particular the enzymatic catalyst is a lipase or an esterase. Examples of such lipases and esterases are Candida cylindracea, Candida lipolytica, Candida rugosa, Candida antarctica, Candida utilis, Chromobacterium viscosum, Geotrichum viscosum, Geotrichum candidum, Mucor javanicus, Mucor mihei, pig pancreas, Pseudomonas spp., Pseudomonas fluorescens, Pseudomonas cepacia, Rhizopus arrhizus, Rhizopus delemar, Rhizopus niveus, Rhizopus oryzae, Aspergillus niger, Penicillium roquefortii, Penicillium

camembertii or esterases of Bacillus spp. and Bacillus thermoglucosidasius. Amanolipase was also found to work in catalyzing Michael addition. The enzymes mentioned are commercially available, for example from Novozymes Biotech Inc., Denmark.

Materials, Methods and Characterization

Materials

[0051] *Candida antarctica* lipase B (CALB) immobilized on macroporous polyacrylic resin beads (Novozyme 435; Aldrich), Amano lipase M from *Mucor javanicus* (Aldrich), poly(ethylene glycol) (M_n=2000, 4600, 10,000 g/mol, broad molecular weight distribution MWD, Aldrich; and M_n=1000, 10,100 g/mol, $M_w/M_n < 1.1$, American Polymer Standards Corp.), α-ω-diamino terminated poly(ethylene glycol) (M_n=2,000 g/mol, $M_w/M_n < 1.1$, Polymer Source Inc.), ω-amino terminated poly(ethylene glycol) monomethyl ether (M_n=2,000 g/mol, $M_w/M_n <$ 1.1, Polymer Source Inc.), polyethylene glycol monomethyl ether (M_n=2,000 g/mol, broad MWD, Aldrich) silanol-terminated poly(dimethyl siloxane) (M_n = 3,300 g/mol, Aldrich), monocarbinol-terminated poly(dimethyl siloxane) (M_n = 5,000 g/mol, Gelest), vinyl methacrylate (98%, Alfa Aesar), triethylamine (≥99.5 %, Aldrich) and folic acid (98 %, Aldrich) were used as received. Tetrahydrofuran (Fischer Scientific) and hexane (Fisher Scientific) were distilled over sodium benzophenone (Aldrich) and vinyl acetate (≥99 %, Aldrich) was distilled from calcium chloride (Aldrich) prior to use.

[0052] Glissopal®2300 (nominal M_n = 2,145 g/mol, M_w/M_n = 1.98) was obtained courtesy of BASF. Primary hydroxyl-functionalized PIBs (PIB-OH and Glissopal-OH) were prepared as described in S. Ummadisetty, J. P. Kennedy *J. Polym. Sci. Part A Polym. Chem.* **2008,** *46,* 4236.

Characterization

[0053] ^1H and ^{13}C NMR spectra were recorded on a Varian Mercury-300 NMR spectrometer at 300 and 75 MHz respectively. Deuterated chloroform (Chemical Isotope Laboratories, 99.8% $CDCl_3$) and dimethyl sulfoxide-d_6 [Chemical Isotope Laboratories, (D, 99.9%)] were used as the solvents. The resonances of non-deuterated chloroform at δ7.27 ppm and δ77.23 ppm were internal references for the ^1H and ^{13}C NMR spectra, respectively; and the

resonances of non-deuterated dimethyl sulfoxide peaks at δ2.5 ppm and δ39.51 ppm were used as internal reference for ^{1}H and ^{13}C NMR spectra respectively. The molecular weight (MW) and molecular weight distribution (MWD) of the PEGs and PIBs before and after acylation or methacrylation were determined by size exclusion chromatography (SEC) using two different SEC set-up. The first system consisted of a Waters 515 HPLC pump, a Waters 2487 Dual Absorbance UV Detector, a Wyatt OPTILAB DSP Interferometric Refractometer, a Wyatt DAWN EOS multi-angle light scattering detector, a Wyatt ViscoStar viscometer, a Wyatt QELS quasi-elastic light scattering instrument, a Waters 717plus autosampler and 6 Styragel® columns (HR0.5, HR1, HR3, HR4, HR5 and H6). The RI detector and the columns were thermostated at 35°C and THF freshly distilled from CaH_2 was used as the mobile phase at a flow rate of 1 mL/min. The second set-up was a Waters 150C-Plus system equipped with a Waters 410 differential refractometer, a Wyatt DAWN EOS light scattering detector and 3 Styragel® columns (HR1, HR4E and HR5E) thermostated at 35°C, with THF as the mobile phase at a flow rate of 1 mL/min. The results were analyzed using the ASTRA software (Wyatt Technology) with *dn/dc* (specific refractive index increment)= 0.068 and 0.108 for PEG and PIB respectively, and assuming 100% mass recovery.

Transesterification method

[0054] In the transesterification method, an ester was reacted with the polyol in the presence of an effective amount of the enzyme catalyst. In one embodiment of the invention, the ester is vinylacetate, vinyl methacrylate or combination thereof. In another embodiment of the invention, the polyol is poly(ethylene glycol). In yet a further embodiment of the invention, the enzyme catalyst is a lipase, wherein the lipase is CALB. In particular, the transesterification method proceeded when about 3 equivalents of an ester, vinylacetate (about 0.11 mL, 1.2 mmol) or vinyl methacrylate (about 0.14 mL, 1.2 mmol), was reacted with poly(ethylene glycol) (about 0.2 g, 0.2 mmol) in THF (about 4.5 mL) in the presence of CALB (about 10 mg/mL), and the reaction mixture was stirred at about 300 rpm for 24 hours at about 50 °C. After the reaction, the mixture was filtered to remove the enzyme. THF, unreacted vinyl acetate and the by-product acetaldehyde were removed by a rotary evaporator and the polymer was dried in a vacuum oven at room temperature for about 24 hours. The methacrylate-functionalized polymer was

precipitated into hexane after filtering off the enzyme, and dried in a vacuum oven at room temperature for about 24 hours.

[0055] For the transesterification reactions, vinyl acetate (VA) and vinyl methacrylate (VMA) were chosen as suitable acyl donors since they rendered the transesterification reaction substantially irreversible by forming an unstable enol, i.e. vinyl alcohol, which rapidly tautomerized to acetaldehyde. Among several lipases used in transesterification reactions CALB was found to be one of the most active and stable lipases. THF was used as the solvent due to its relatively low polarity, ability to maintain the catalytically active conformation of the enzyme, as well as being a good solvent for the polymer. Anhydrous conditions were employed to prevent possible hydrolysis; and the reaction was run at 50 °C because the optimum temperature range of enzyme stability is 40-60 °C.

[0056] It was determined that under the experimental conditions that were developed (FIG. 1) both narrow and broad MWD PEGs reacted quantitatively with vinyl acetate. The ^{1}H NMR spectra of the PEG with M_n = 1,000 g/mol, M_w/M_n = 1.06) before and after acylation are shown in FIG. 2. The peak at δ4.6 ppm (a) attributed to the protons of the terminal hydroxyl groups of the polymer disappeared and new peaks corresponding to the methyl protons of the acyl end group and the methylene protons next to the ester bond at δ2.0 (e) and 4.1 ppm (b), respectively, were observed. From the integration ratio of the three methyl protons of the acyl end group (e) to two methylene protons adjacent to the acyl end group (b) the conversion was readily calculated as 100%. The reactions proceeded smoothly with all PEG samples, yielding α-ω telechelic PEG-acetates.

[0057] The ^{13}C NMR spectrum corresponding to FIG. 2 is shown in FIG. 3. The carbons connected to the hydroxyl-end groups observed at δ60 ppm in the ^{13}C NMR spectrum of the starting material disappeared after the reaction and the new resonances observed at δ20.8 and δ170.7 ppm were attributed to the methyl carbon and the carbonyl carbon of the acyl end group, respectively.

[0058] Following the same strategy, poly(ethylene glycol) dimethacrylate was prepared using vinyl methacrylate as the ester. The ^{1}H and ^{13}C NMR spectra of a methacrylated poly(ethylene glycol) (M_n = 2,000 g/mol, broad MWD) are shown in FIGS. 4 and 5,

respectively. The integration ratios of the two vinylidene (δ5.7 (a) and δ6.0 (b)) and the three methyl δ1.9 (c) protons in the methacrylate groups, and the two methylene protons adjacent to the methacrylate end group (d) were 1:1:3:2 which indicated quantitative functionalization.

[0059] In the ^{13}C NMR spectrum, similarly to the acyl-functionalized PEG, no peaks at δ60 ppm attributed to carbons adjacent to hydroxyl-end groups in the starting material were observed and the new resonances appeared at the expected positions confirming the structure of the product. All PEG samples reacted quantitatively, yielding α-ω telechelic PEG-methacrylates.

[0060] From the data presented herein, α-ω telechelic PEG-acetates and PEG-methacrylates were successfully synthesized and characterized. The synthetic strategy involved enzyme-catalyzed transesterification using vinyl esters which rendered the reactions irreversible.

[0061] In another embodiment of the invention, the transesterification method includes reacting an ester with a polyolefin in the presence of an effective amount of the enzyme catalyst. In one embodiment of the invention, the ester may be selected from vinylacetate, vinyl methacrylate and combinations thereof. In another embodiment of the invention, the polyolefin is polyisobutylene. In yet a further embodiment of the invention, the enzyme catalyst is a lipase, wherein the lipase is CALB.

[0062] In particular, the primary hydroxyl terminated PIB (PIB-OH) (M_n=5,240 g/mol and M_n/M_w=1.09) was enzymatically methacrylated according to the scheme as shown in FIG. 6. Hexane solvent was used since the catalytically active conformation of the enzyme is best maintained in low polarity solvents. The transesterification method proceeded with PIB-OH (about 0.4 g, 0.08 mmol) being placed in a 10 mL round bottom flask containing hexane (about 5 mL), CALB (about 10mg/mL) and vinyl methacrylate (about 0.1 mL, 0.8 mmol). The flask was sealed with a septum and purged with nitrogen. The resulting solution was stirred at 300 rpm for 24 hours at 50 °C. After filtering off the enzyme, the polymer was precipitated into methanol and dried in vacuum for 24 hours at room temperature (yield: about 0.25 g, conversion: ~100%).

[0063] The ^1H NMR spectrum of the PIB before, as seen in FIG. 7a, and after, as seen in FIG. 7b, methacrylation are provided. The resonance at δ3.6 ppm, corresponding to the $-CH_2-$

protons next to the hydroxyl group, as seen in FIG. 7a, disappeared and new resonances attributed to the vinylidene [δ5.6 (c), δ6.1 (d)], methyl [δ2.0 (b)] and methylene protons adjacent to the methacrylate end group [δ4.2 (a)] appeared at the expected positions. The integration ratios of (c):(d):(a):(b) were 1:1:2:3 which demonstrated quantitative functionalization. The use of THF solvent gave incomplete conversion after 24 hours, although PEG-MA was obtained in quantitative yield under similar conditions. At this point we do not understand the reason for this discrepancy. We hope to get a better understanding of the mechanism of enzymatic polymerizations, which we are actively investigating.

[0064] The ^{13}C NMR spectrum of PIB before, as seen in FIG. 8a, and after, as seen in FIG. 8b, methacrylation are provided. The carbon connected to the $-CH_2-$ protons next to the hydroxyl group at δ62.0 in the starting material shifted downfield to δ66.0 upon methacrylation, and new resonances appeared at the expected positions, confirming the structure of the product.

[0065] The SEC trace of PIB-MA with M_n=5,260 g/mol and M_n/M_w=1.11 is shown in FIG. 9. Since the dn/dc of PIB-OH and PIB-MA are not known the M_n values may not be accurate; however, the discrepancy should be minor and the measured values agree with the expected values within experimental error.

[0066] In another embodiment of the invention, Glissopal-OH made from Glissopal®2300, a commercially available PIB produced by BASF with ~82% exo- and ~18% endo-terminal unsaturated portions, was also methacrylated enzymatically as seen according to the scheme as shown in FIG. 6. Measurements revealed that the Glissopal®2300 had M_n = 3,520 g/mol and M_n/M_w=1.37, and the resulting Glissopal-OH had M_n=3,570 g/mol, M_n/M_w=1.34.

[0067] The ^{1}H NMR spectrum of Glissopal-OH before, as seen in FIG. 10a, and after, as seen in FIG. 10b, methacrylation are provided. As seen in FIG. 10b, the $-CH_2-$ proton resonances shifted downfield from δ3.3-3.6 to δ3.8-4.0 ppm, and the new peaks corresponding to the vinylidene [δ5.6 (e) and 6.2 ppm(d)] and methyl protons [δ1.9 ppm (c)] of the methacrylate end group appeared at expected positions, with the integral values confirming quantitative functionalization. The Glissopal-MA had M_n=3,720 g/mol and M_n/M_w=1.45. ^{13}C NMR spectra of Glissopal-OH and Glissopal-MA also confirmed the structure of the product as seen in FIGS. 11a and 11b.

[0068] In another embodiment of the invention, Glissopal-OH made from Glissopal®2300 was enzymatically methacrylated in bulk. Glissopal-OH (about 0.39 g, 0.11 mmol) was placed in a 5 mL round bottom flask containing vinyl methacrylate (about 0.14 mL, 1.11 mmol) and CALB (about 10% by weight w.r.t. total weight of reactants). The flask was sealed with a septum and purged with nitrogen. The resulting mixture was stirred at about 300 rpm for about 24 hours at 50°C. After the reaction, the polymer was dried under reduced pressure for 3 hours at about 50°C. The ^{1}H NMR spectra of Glissopal-OH and that of the reaction product of Glissopal-OH with vinylmethacrylate in bulk are shown in FIGs. 12a and 12b respectively. Similarly to the reaction in THF (FIGs. 10a and 10b), the conversion was quantitative. The resonances at δ3.3-3.5 ppm corresponding to the $-CH_2-$ protons next to the hydroxyl group in Glissopal-OH shifted downfield to δ3.8-4.0 ppm upon methacrylation; and the new peaks corresponding to the vinylidene [δ5.6 (e) and 6.1 ppm(d)] and methyl protons [δ 2.0 ppm (c)] of the methacrylate end group appeared at expected positions.

[0069] In yet another embodiment of the invention, the transesterification method includes reacting an ester with a polysiloxane in the presence of an effective amount of the enzyme catalyst. In one embodiment of the invention, the ester may be selected from vinylacetate, vinyl methacrylate and combinations thereof. In another embodiment of the invention, the polysiloxane is polydimethylsiloxane. In yet a further embodiment of the invention, the enzyme catalyst is a lipase, wherein the lipase is CALB.

[0070] Methacrylate-functionalized polydimethylsiloxanes may be used as macromonomers in the production of hydrogels including soft contact lens applications. They can either be prepared by reacting an amount of a polydimethylsiloxane diol with a predetermined amount of methacryloyl chloride or a diisocyanate followed by reaction with hydroxyethylmethacrylate (HEMA). In the former case the use of methacryloyl chloride is disadvantageous due to its high toxicity. In the latter case, even when an appropriate stoichiometry is used, chain extension is unavoidable. Therefore, an enzymatic transesterification strategy may be an alternative for the production of this macromonomer.

[0071] In one embodiment of the invention, a methacrylate-functionalized poly(dimethylsiloxane) was prepared by introducing polydimethylsiloxane diol (about 0.75 g,

about 0.23 mmol) in a 25 mL round bottom flask containing CALB (about 10 mg/mL), vinyl methacrylate (about 0.17 mL, about 1.4 mmol) and about 10 mL of THF distilled from sodium benzophenone. The flask was sealed with a septum and purged with nitrogen. The resulting solution was stirred at about 300 rpm for about 24 hours at 50°C. After the reaction, the enzyme was filtered, the polymer was dried under reduced pressure overnight at about 50°C (yield: about 0.66 g). In another embodiment of the invention, and following the same procedure, polydimethylsiloxane monocarbinol (about 0.98 g, about 0.2 mmol) was methacrylated and the yield was about 0.85 g. Both of these reactions are shown schematically in FIG. 13.

[0072] As seen in FIGS. 14a and 14b, the ^{1}H NMR of PDMS diol and the reaction product of PDMS diol with VMA are shown. The vinylidene protons at δ 5.65 and 6.20 ppm (a and b), and the methyl protons of the methacrylate group at δ 1.95 ppm (c) proved that the PDMS-diol does react with the VMA. The peaks at δ 1.5 and 2.25 ppm in the starting material NMR spectrum could not be assigned to anything. However, both peaks disappeared upon methacrylation. In order to see if these peaks were coming from hydroxyl protons, deuterium oxide was added to the NMR solution, but no change in the spectrum was observed.

[0073] The ^{1}H NMR of PDMS monocarbinol and the reaction product of PDMS monocarbinol with VMA are shown in FIGS. 15a and 15b. The NMR of the product showed that the desired methacrylated-PDMS was obtained as seen from the vinylidene protons δ 5.60 and 6.20 ppm (l and m), and methyl protons of the methacrylate group (k) at δ 1.95 ppm. The ratio between vinylidene protons (l) and (m) and (b') was 1:1:2 which was in accordance with the expected structure, though in 1H NMR of silicons, the integral ratios may not be good indications of relative amounts of products.

[0074] In yet another embodiment of the invention, the methacrylate-functionalized poly(dimethylsiloxane)s were prepared in bulk by introducing polydimethylsiloxane diol (about 1.04 g, about 0.32 mmol) in a 5 mL round bottom flask containing CALB (about 10% by weight w.r.t. total weight of reactants) and vinyl methacrylate (about 0.23 mL, about 1.9 mmol). The flask was sealed with a septum and purged with nitrogen. The resulting mixture was stirred at about 300 rpm for about 24 hours at 50°C. After the reaction, the enzyme was filtered; the polymer was dried under reduced pressure overnight at about 50°C. In still yet another

embodiment of the invention, polydimethylsiloxane monocarbinol (about 1.12 g, about 0.22 mmol) was methacrylated by following the same procedure. The ^{1}H NMR spectra of silanol-terminated poly(dimethylsiloxane) before and after methacrylation are shown in FIGs. 16a and 16b respectively; and that of monocarbinol-terminated poly(dimethylsiloxane) before and after methacrylation are given in Figs. 17a and 17b respectively.

[0075] It was observed by ^{1}H NMR spectroscopy that the monocarbinol-terminated poly(dimethylsiloxane) reacted completely with vinyl methacrylate resulting in pure methacrylated polymer as indicated in FIG. 17b. Moreover, there was no chain extension in the resulting polymer as the integral value of the peak in the range δ –0.05-0.25 ppm corresponding to the backbone methyl protons stayed approximately the same upon methacrylation.

[0076] In yet another embodiment of the invention, vinyl methacrylate was used as a linker by reacting the transesterification reaction product, prepared by reacting a glycol ether and vinyl methacrylate in the presence of an effective amount of an enzyme catalyst, with an aminoethoxy glycol ether in the presence of an effective amount of an enzyme catalyst via Michael addition. In one embodiment of the invention, the glycol ether may be polyethylene glycol monomethyl ether and the aminoethoxy glycol ether is aminoethoxy polyethylene glycol monomethyl ether. In yet another embodiment of the invention, the enzyme catalyst is a lipase, wherein the lipase is CALB or Amano Lipase M (ALM). The reaction is shown schematically in FIG. 18.

[0077] The transesterification reaction product of a glycol ether and vinyl methacrylate in the presence of an effective amount of an enzyme catalyst was prepared by dissolving about 1.0 g of polyethylene glycol monomethyl ether (about 0.5 mmol) and about 0.168 g of vinyl methacrylate (about 1.5 mmol) in about 22.5 mL of distilled THF. About 225 mg of CALB (about 10 mg/mL) was then added into the solution. The suspension was stirred for about 24 hours at about 50 °C. The CALB was filtered out using a sintered funnel with a vacuum pump. The THF and unreacted VMA were removed by evaporating under the reduced pressure (about 50 mbar) at about 40 °C. The white product was dissolved in distilled THF and precipitated in about 150 mL of hexane. The precipitate was dried in vacuum and the ^{1}H NMR of the product is shown in FIG. 19.

[0078] The polymer coupling reaction was carried out by dissolving about 0.1 g of aminoethoxy polyethylene glycol monomethyl ether (about 50 μmol) and 0.1575 g of methacrylate-functionalized poly(ethylene glycol) monomethyl ether (about 1.5 eq.) in about 5 mL of anhydrous DMSO. About 200 mg of CALB was added into the solution and stirred for about 72 hours. The product was centrifuged and the upper solution was collected into volumetric flask. The solvent was removed under vacuum at about 70 °C. The precipitate was dried in vacuum and the ^1H NMR of the product is shown in FIG. 20. Using the same method, block copolymers such as PEG-PIB, PEG-PDMS and their variations (diblocks, triblocks or multiblocks) can be prepared.

[0079] In still yet another embodiment of the invention, the transesterification method includes preparing a dendrimer by reacting a polyether diamine or an aminoethoxy glycol ether with a functionalized nitrogen heterocycle in the presence of an effective amount of the enzyme catalyst. In one embodiment of the invention, the polyether diamine is a polyethylene glycol diamine and the aminoethoxy glycol ether is aminoethoxy polyethylene glycol monomethyl ether. In another embodiment of the invention, the functionalized nitrogen heterocycle includes functionalized pyridines, functionalized diazines, functionalized triazines and functionalized tetrazines. An example of a functionalized triazine is triacryloyl hexahydro-triazine. In yet a further embodiment of the invention, the enzyme catalyst is a lipase, wherein the lipase is CALB.

[0080] In one embodiment of the invention, a dendrimer may be prepared via a Michael addition reaction, as seen in FIG. 21. However, based on the stoichiometry, a polymer gel formed when about 0.2 g (about 0.1 mmol) of polyethylene glycol diamine and about 0.0977 g (about 0.4 mmol) of triacryloyl hexahydro-triazine was reacted in about 5 mL of anhydrous DMSO. About 50 mg of CALB (about 10 mg/mL) was added into the solution. The suspension was stirred for about 24 hours at about 50 °C. The product was a gelled, crosslinked polyethylene glycol.

[0081] In another embodiment of the invention, the dendrimer may be prepared via a Michael addition reaction, as seen in FIG. 22, by dissolving about 0.1 g (about 50 μmol) aminoethoxy polyethylene glycol monomethyl ether and about 0.0125 g (about 50 μmol) of triacryloyl hexahydro-triazine in about 5 mL of anhydrous DMSO. About 50 mg (about 10

mg/mL) of CALB was added into the solution. The suspension stirred for about 24 hours at about 50 °C. ^{1}H NMR analysis of the product is seen in FIG. 23.

[0082] In another embodiment of the invention, it is envisioned that functionalized polymers prepared via a transesterification process with an enzymatic catalyst as described above, may be further modified for the purposes of drug delivery. In one example, it is envisioned that a methacrylate-functionalized glycol ether, such as a methacrylate-functionalized poly(ethylene glycol) monomethyl ether, may be reacted with a vitamin, such as folic acid, a mineral and combinations thereof, in the presence of an enzymatic catalyst to form a drug that may be administered to a patient. In one aspect of the invention, the drug is prepared by reacting a methacrylate–functionalized poly(ethylene glycol) monomethyl ether with folic acid via a Michael addition reaction in the presence of Amano Lipase M to form a folic acid modified methacrylate–functionalized poly(ethylene glycol) monomethyl ether compound (FIG 24).

[0083] Fig 25. illustrates a scheme of a reaction for conjugation of folic acid onto poly(ethylene glycol) monomethyl ether. In this example, folic acid (0.33 g, 0.75 mmol) was dissolved in DMSO (10 mL) at room temperature and then methacrylate–functionalized poly(ethylene glycol) monomethyl ether (0.5 g, 0.25 mmol) dissolved in 2.5 mL of THF was added dropwise to the folic acid solution. To neutralize the reaction medium, 0.3 mL of triethylamine was added and then CALB (125 mg) was added. The reaction flask was sealed with a septum, purged with nitrogen and the mixture was stirred at 300 rpm for 4 days at 50°C. The enzyme was removed by centrifuging the solution. The solvent was removed under reduced pressure and THF was added to the crude product to dissolve the polymer. The polymer was precipitated into hexane.

[0084] Based upon the foregoing disclosure, it should now be apparent that the method of preparing functionalized polymers through enzymatic catalysis as described herein will carry out the objects set forth hereinabove. It is, therefore, to be understood that any variations evident fall within the scope of the claimed invention and thus, the selection of specific component elements can be determined without departing from the spirit of the invention herein disclosed and described.

Claims

What is claimed is:

1. A method of preparing a functionalized polymer through enzymatic catalysis, the method comprising the steps of:

reacting a polymer with at least one acyl donor in the presence of an enzymatic catalyst in a transesterification reaction

2. The method of claim 1, wherein the polymer is a glycol ether.

3. The method of claim 2, wherein the glycol ether is poly(ethylene glycol).

4. The method of claim 2, wherein the glycol ether is aminoethoxy polyethylene glycol monomethyl ether.

5. The method of claim 1, wherein the polymer is a polysiloxane.

6. The method of claim 5, wherein the polysiloxane is polydimethylsiloxane.

7. The method of claim 1, wherein the polymer is a hydroxy-functionalized polyisobutylene.

8. The method of claim 1, wherein the at least one acyl donor is an ester.

9. The method of claim 8, wherein the ester is vinyl acetate, vinyl methacrylate and mixtures thereof.

10. The method of claim 1, wherein the enzyme catalyst is *Candida antarctica* lipase B.

11. The method of claim 1, wherein the at least one acyl donor renders the transesterification reaction substantially irreversible.

12. The method of claim 11, wherein the transesterification reaction is rendered substantially irreversible through formation of an unstable enol which tautomerizes to an acetaldehyde.

13. A method of preparing a telechelic polymer through enzymatic catalysis, the method comprising the steps of:

reacting a glycol ether with at least one ester in the presence of an effective amount of a lipase in a transesterification reaction in a transesterification reaction.

14. The method of claim 13, wherein the glycol ether is poly(ethylene) glycol.

15. The method of claim 14, wherein the glycol ether is aminoethoxy polyethylene glycol monomethyl ether.

16. The method of claim 13, wherein the at least one ester is vinyl acetate, vinyl methacrylate and mixtures thereof.

17. The method of claim 13, wherein the lipase is *Candida antarctica* lipase B.

18. The method of claim 13, wherein the transesterification reaction is irreversible.

19. A method of preparing a functionalized polymer through enzymatic catalysis, the method comprising the steps of:

reacting a polyolefin-based material with at least one acyl donor in the presence of an enzymatic catalyst in a transesterification reaction.

20. The method of claim 19, wherein the polyolefin-based material is hydroxy-functionalized polyisobutylene.

21. The method of claim 19, wherein the at least one acyl donor is an ester.

22. The method of claim 21, wherein the ester is selected from the group consisting of vinyl acetate, vinyl methacrylate and mixtures thereof.

23. The method of claim 19, wherein the enzyme catalyst is *Candida antarctica* lipase B.

24. The method of claim 19, wherein the at least one acyl donor renders the transesterification reaction substantially irreversible.

25. The method of claim 24, wherein the transesterification reaction is rendered substantially irreversible through formation of an unstable enol which tautomerizes to an acetaldehyde.

26. A method of preparing a block copolymer through enzymatic catalysis, the method comprising the steps of:

reacting a functionalized polymer with an aminoethoxy glycol ether in the presence of an effective amount of an enzyme catalyst via Michael addition reaction.

27. The method of claim 26, wherein the functionalized polymer is prepared by reacting a glycol ether with at least one ester in the presence of an effective amount of a lipase in a transesterification reaction.

28. The method of claim 27, wherein the at least one ester is selected from the group consisting of vinyl acetate, vinyl methacrylate and mixtures thereof.

29. The method of claim 26, wherein the lipase is *Candida antarctica* lipase B.

30. The method of claim 27, wherein the lipase is *Candida antarctica* lipase B.

31. A method of preparing a dendrimer through enzymatic catalysis, the method comprising the steps of:

reacting a polyether diamine with a functionalized nitrogen heterocycle in the presence of an effective amount of the enzyme catalyst.

32. The method of claim 31, wherein the polyether diamine is a polyethylene glycol diamine.

33. The method of claim 31, wherein the functionalized nitrogen heterocycle includes functionalized pyridines, functionalized diazines, functionalized triazines and functionalized tetrazines.

34. The method of claim 33, wherein the functionalized nitrogen heterocycle is triacryloyl hexahydro-triazine.

35. The method of claim 31, wherein the enzyme catalyst is a lipase.

36. The method of claim 35, wherein the lipase is *Candida antarctica* lipase B.

37. A method of preparing a dendrimer through enzymatic catalysis, the method comprising the steps of:

reacting an aminoethoxy glycol ether with a functionalized nitrogen heterocycle in the presence of an effective amount of the enzyme catalyst.

38. The method of claim 37, wherein the aminoethoxy glycol ether is aminoethoxy polyethylene glycol monomethyl ether.

39. The method of claim 37, wherein the functionalized nitrogen heterocycle includes functionalized pyridines, functionalized diazines, functionalized triazines and functionalized tetrazines.

40. The method of claim 39, wherein the functionalized nitrogen heterocycle is triacryloyl hexahydro-triazine.

41. The method of claim 37, wherein the enzyme catalyst is a lipase.

42. The method of claim 41, wherein the lipase is *Candida antarctica* lipase B.

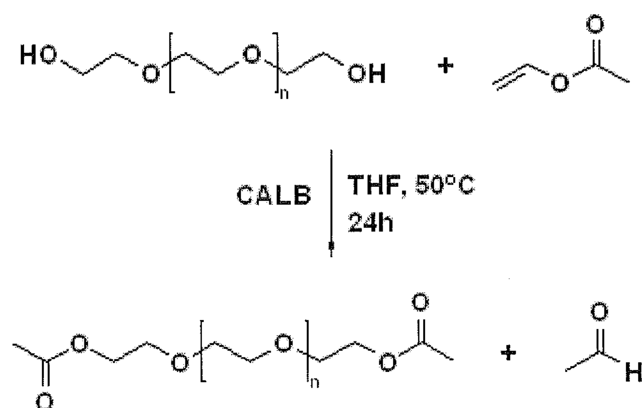


FIG. 1

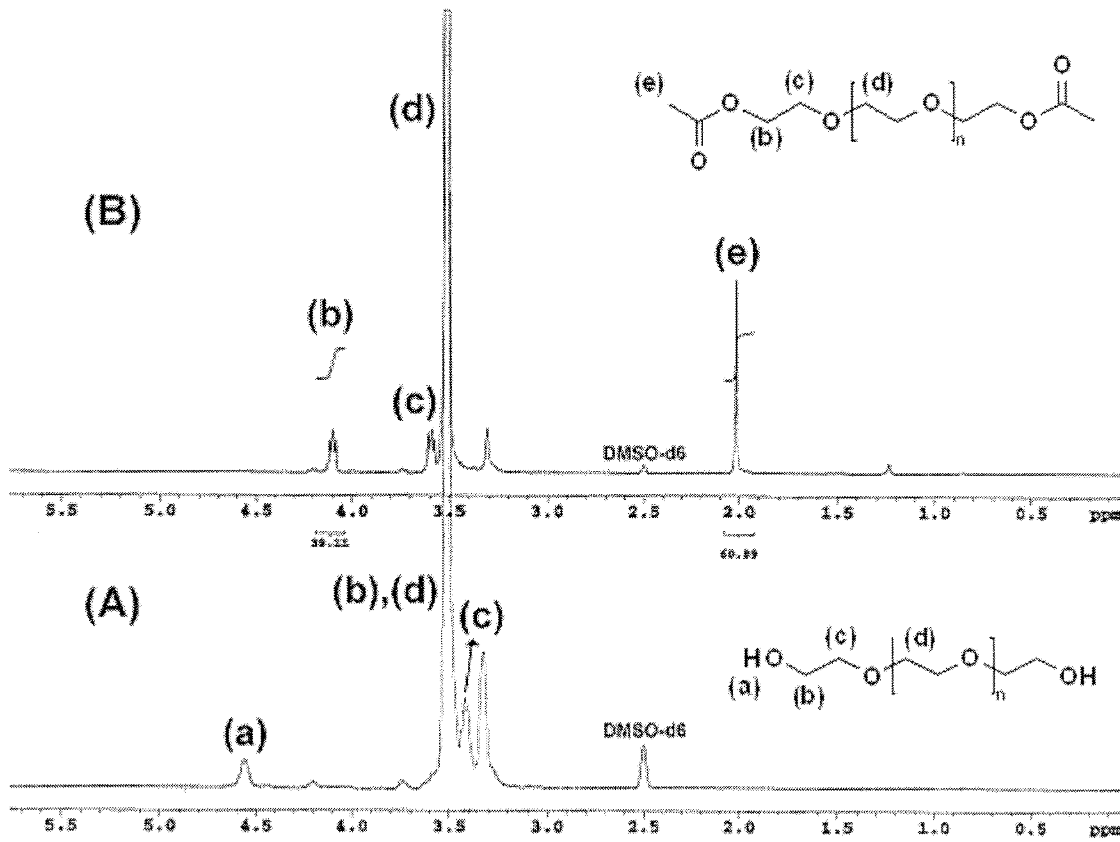


FIG. 2

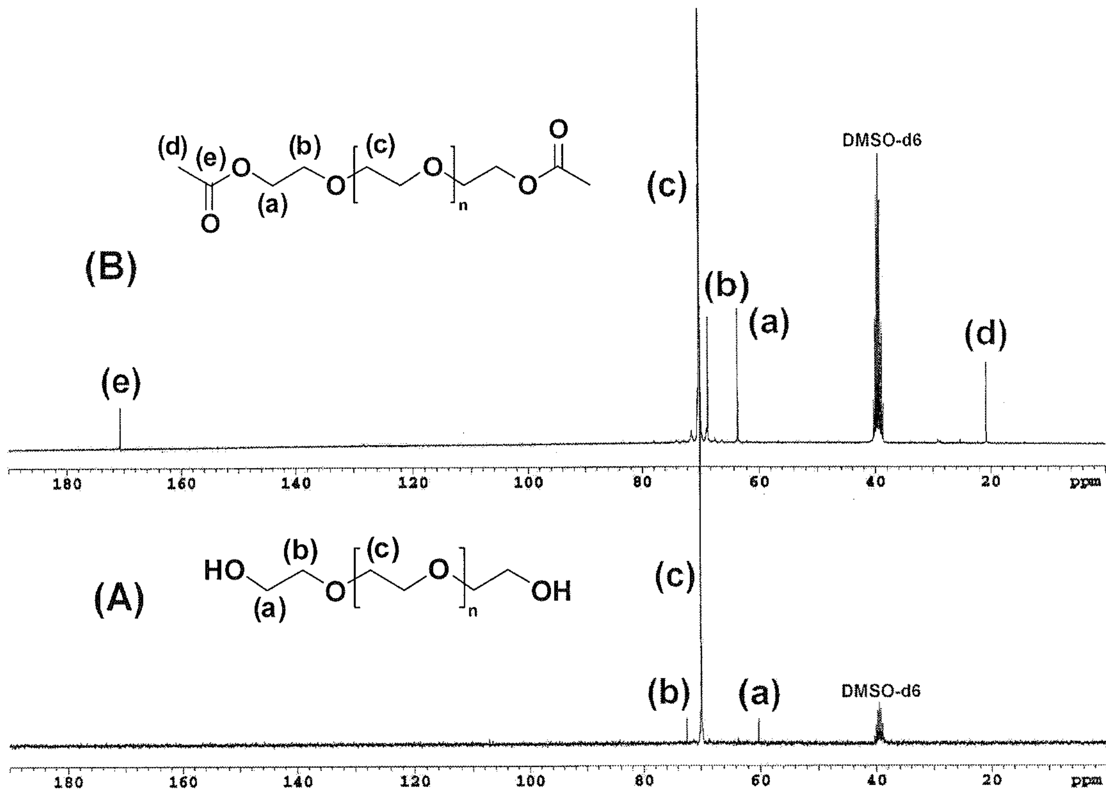


FIG. 3

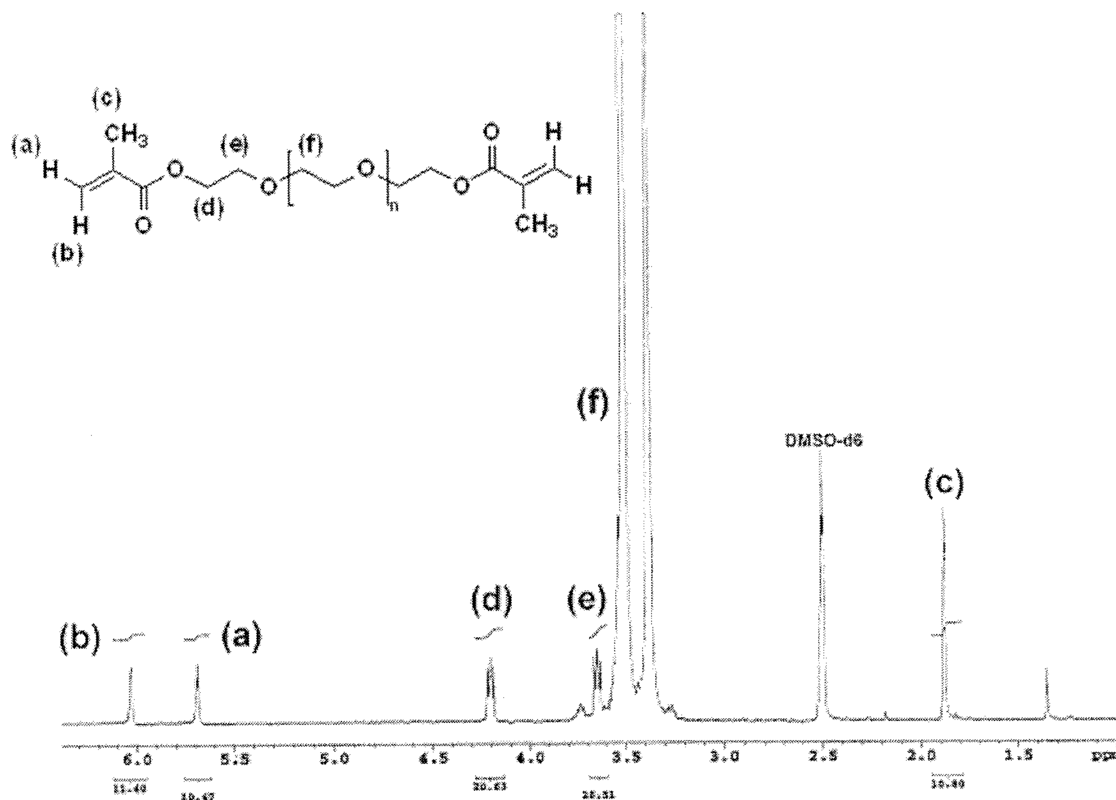


FIG. 4

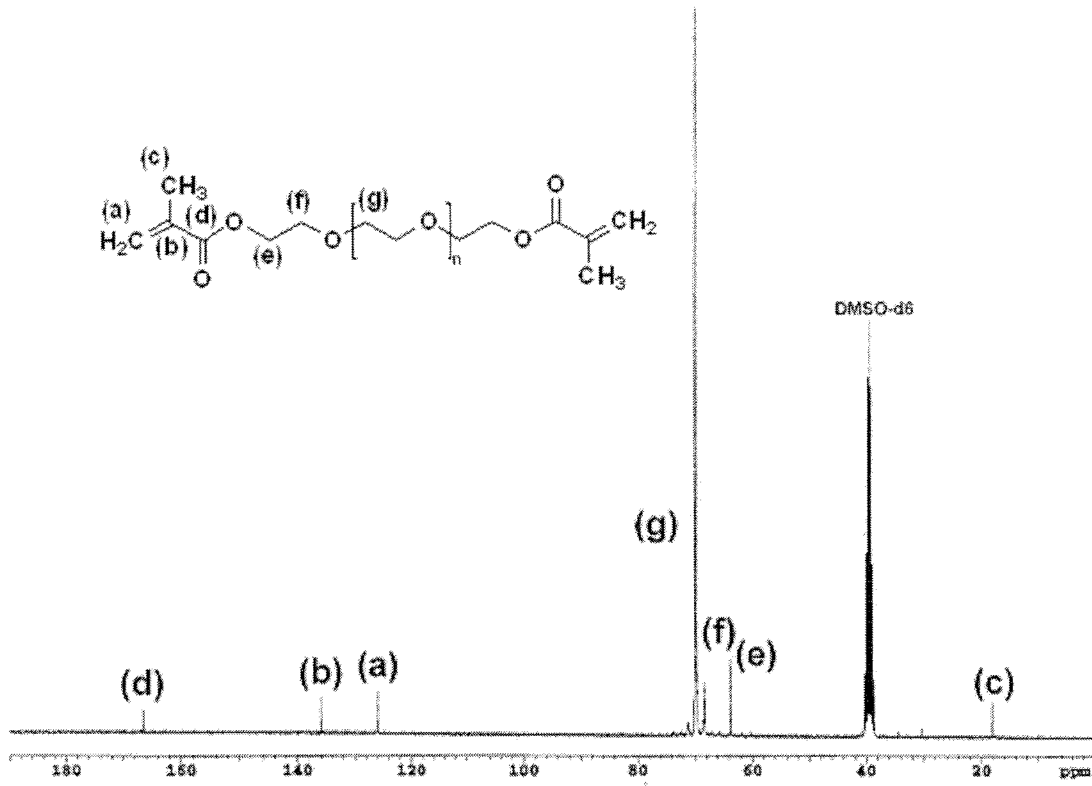


FIG. 5

PIB-OH

Glissopal®2300-OH

(10 eq.)

CALB (10mg/mL)
Hexane
24h, 50⁰C

PIB-MA

Glissopal®2300-MA

FIG. 6

FIG. 7b

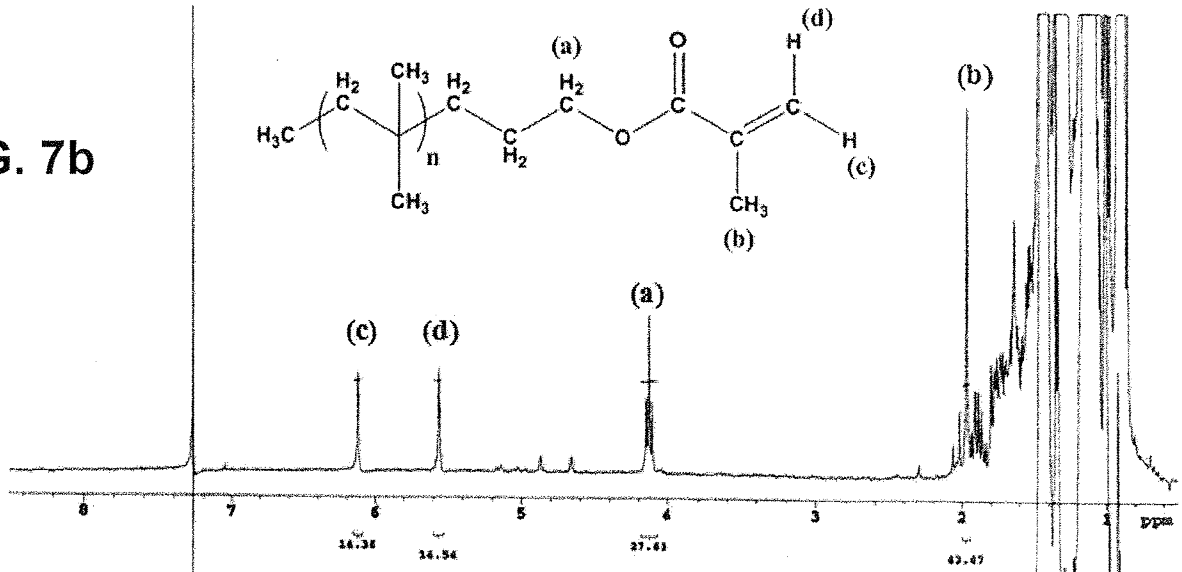
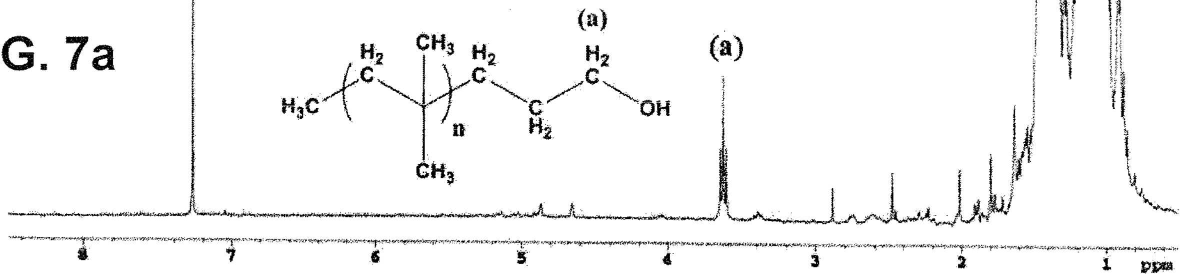


FIG. 7a



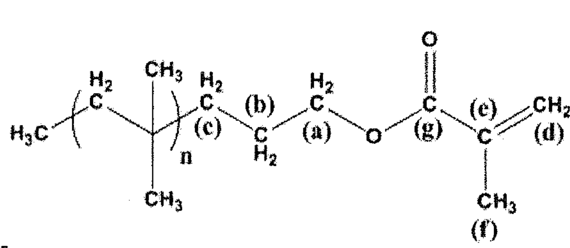


FIG. 8b

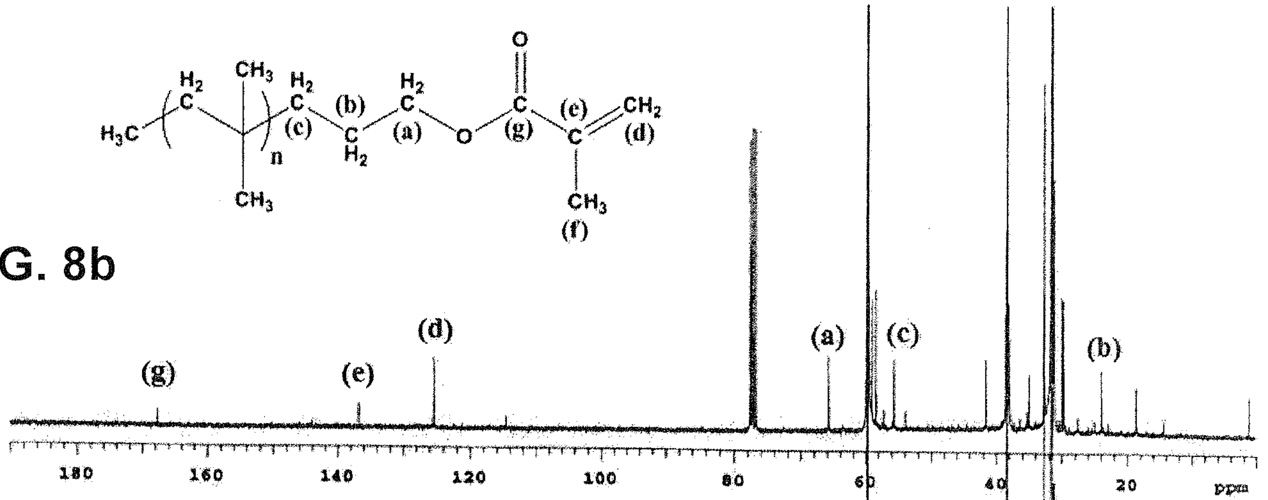
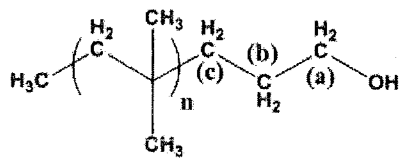
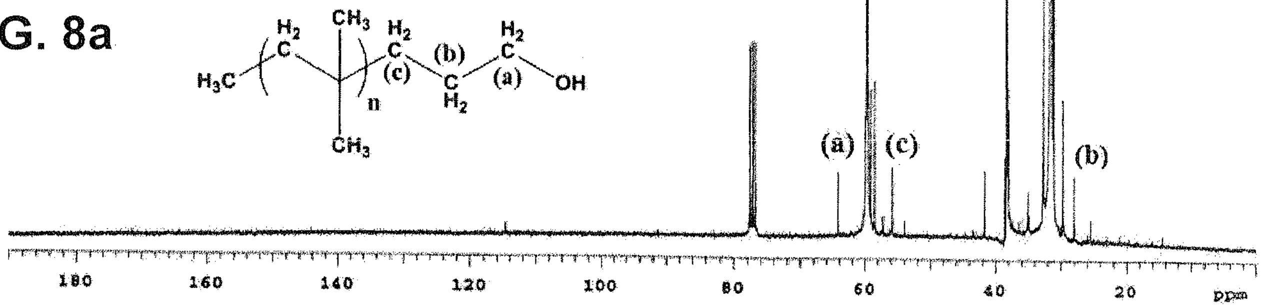


FIG. 8a





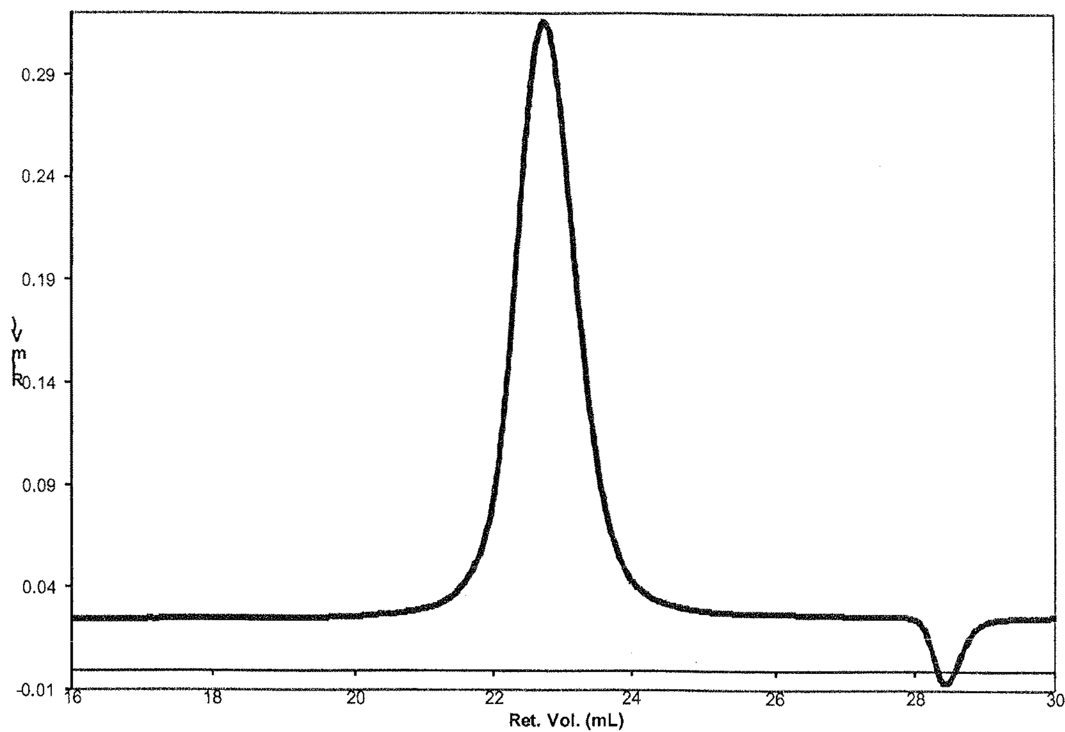


FIG. 9

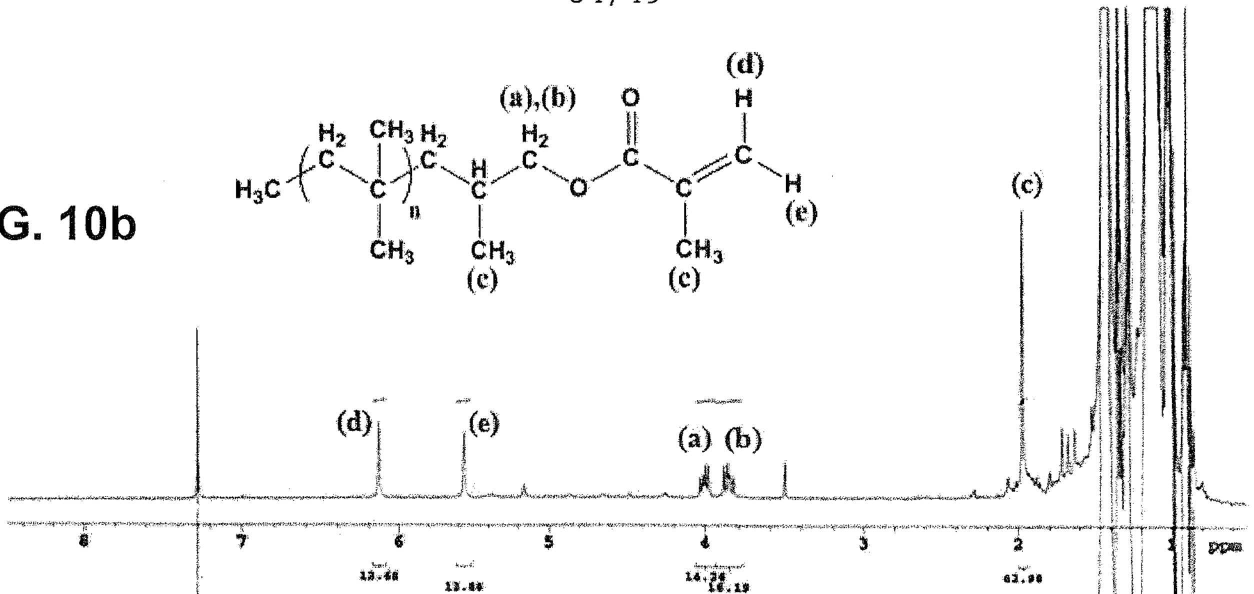


FIG. 10b
(a),(b)
(d)
(c)
(e)
(d)
(e)
(a) (b)
(c)
8
7
6
5
4
3
2
1
ppm

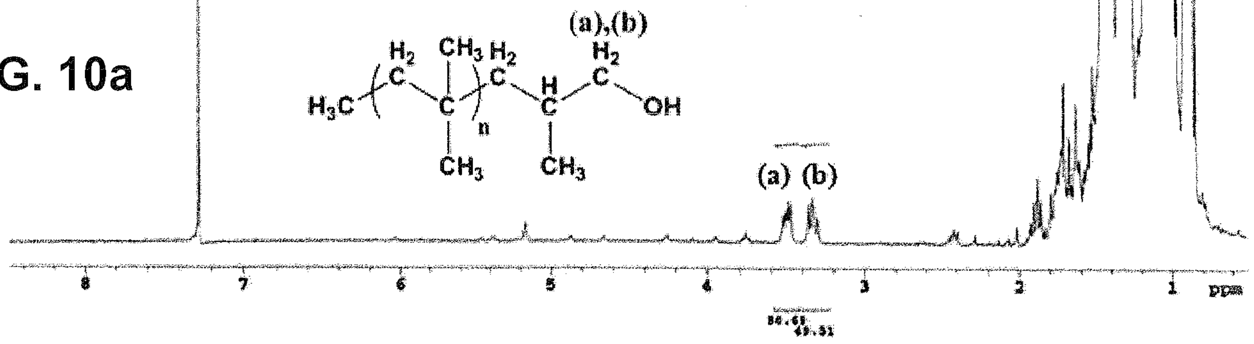


FIG. 10a
(a),(b)
(a) (b)
8
7
6
5
4
3
2
1
ppm

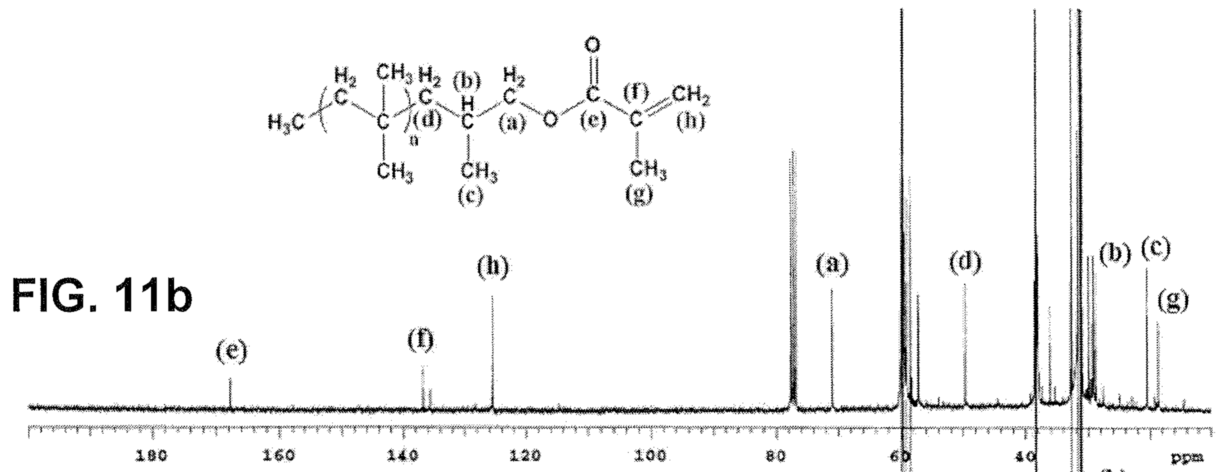


FIG. 11b
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
180
160
140
120
100
80
60
40
ppm

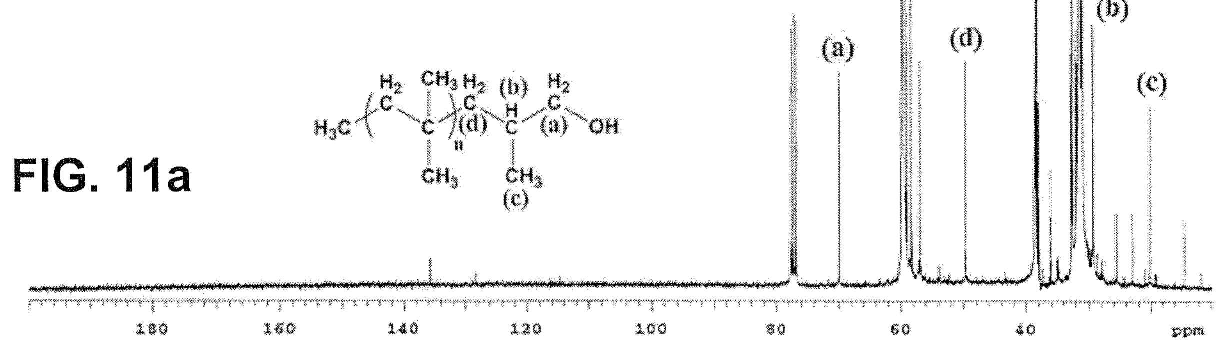


FIG. 11a
(a)
(b)
(c)
(d)
OH
180
160
140
120
100
80
60
40
ppm

FIG. 12b

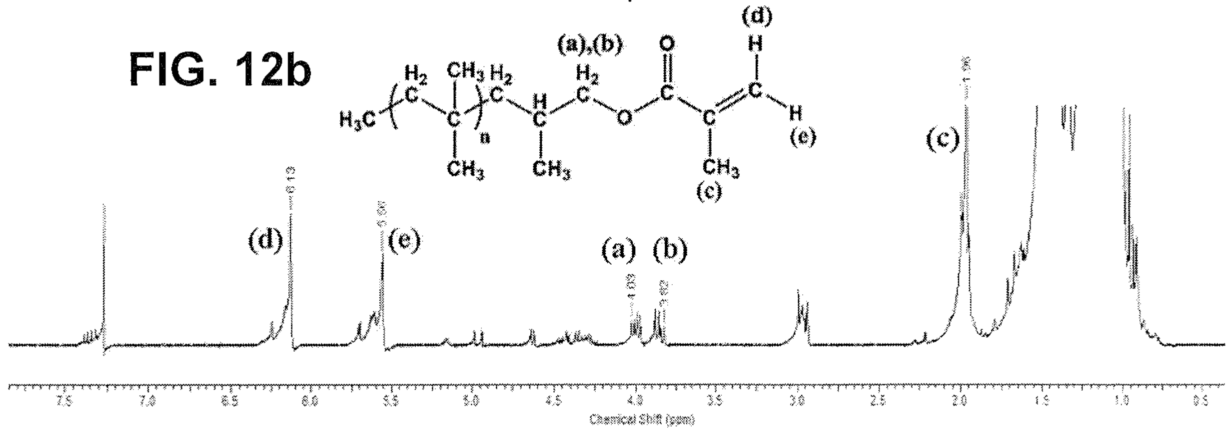
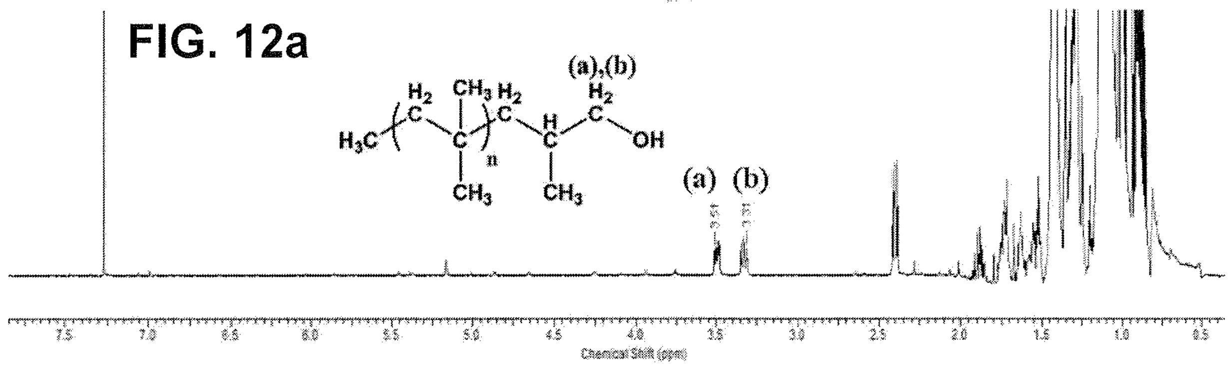


FIG. 12a



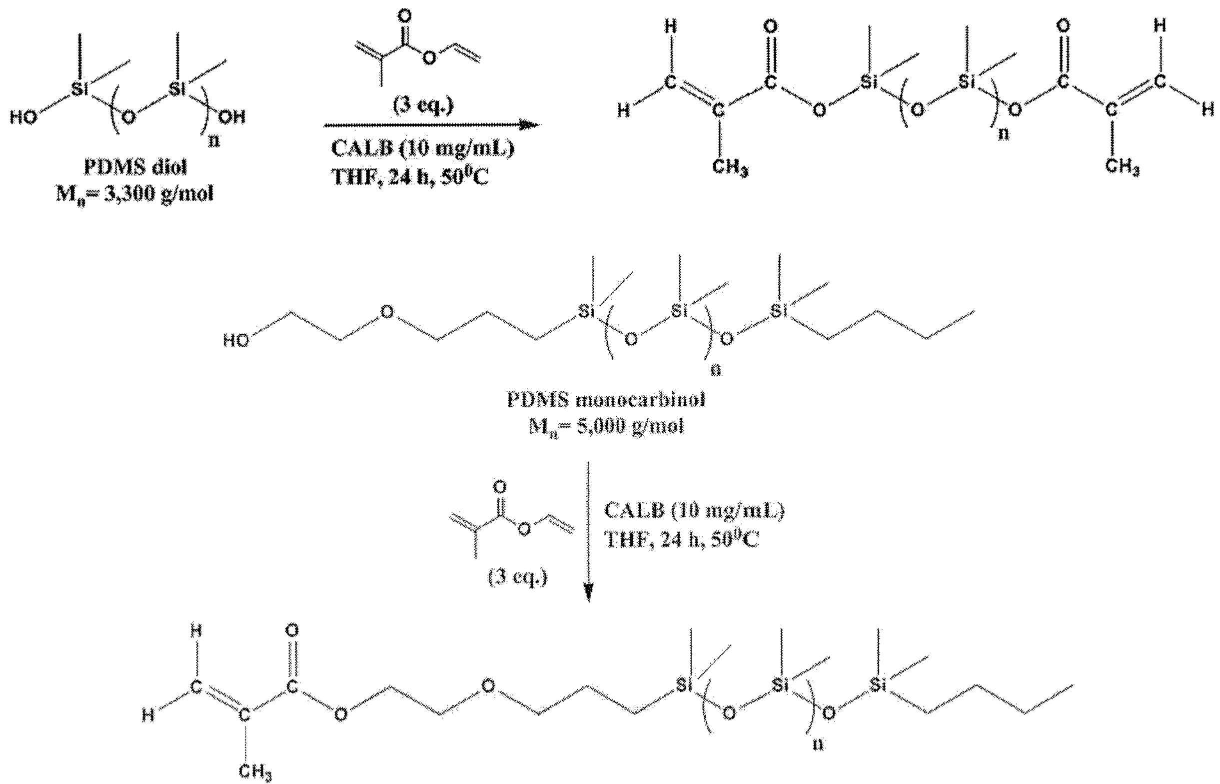


FIG. 13

FIG. 14b

(b) (a) (c) (a) (b) (c)

1.07 1.00 3.07 1023.22

7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0

Chemical Shift (ppm)

FIG. 14a

HO OH n

1.51 1.00 297.10

7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0

Chemical Shift (ppm)

FIG. 15b

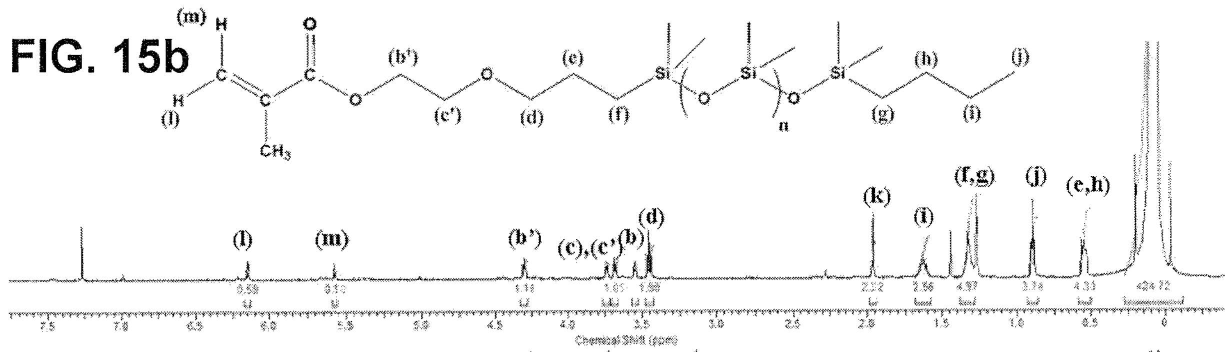


FIG. 15a

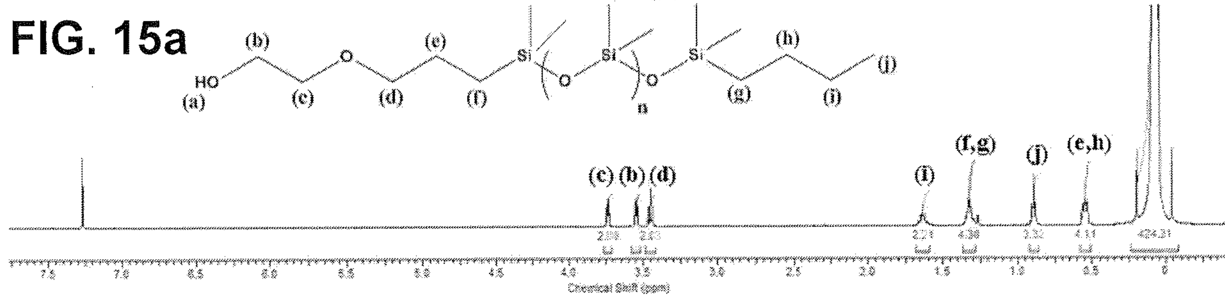


FIG. 16b

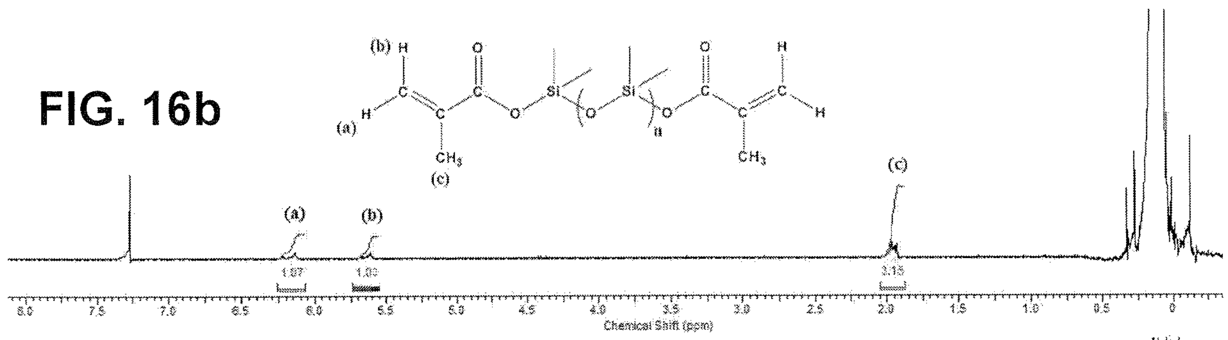


FIG. 16a

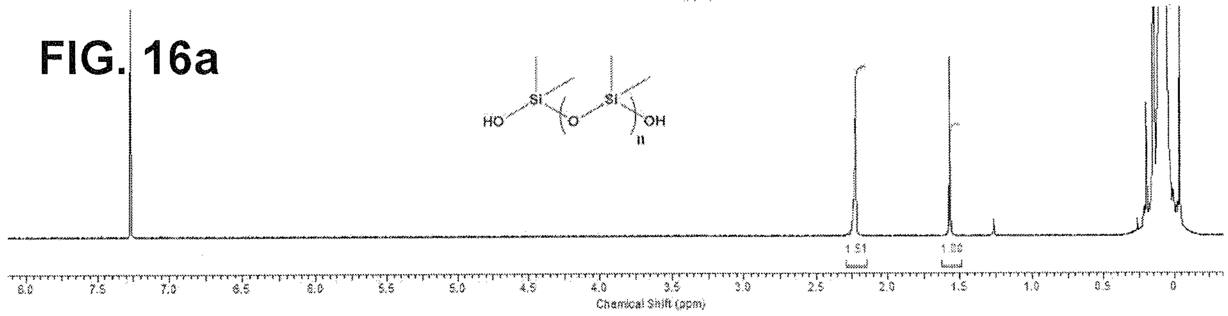


FIG. 17b

(m) H, O, (b'), (e), (h), (j), C, C, O, O, Si, Si, Si, H, (l), (c'), (d), (f), O, O, n, (g), (i), CH_3

(k) (l) (m) (b') (c') (d) (i) (f,g) (j) (e,h)

1.00 1.00 1.84 1.82 1.87 2.98 2.37 4.02 2.35 3.94 380.88

7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0

Chemical Shift (ppm)

FIG. 17a

HO (a) (b) (c) O (d) (e) (f) Si O Si O Si n (g) (h) (i) (j)

(c) (b) (d) (i) (f,g) (j) (e,h)

2.00 2.02 2.33 2.21 4.30 3.32 4.11 424.31

7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0

Chemical Shift (ppm)

H_3C–O(–CH₂CH₂–O)$_n$–CH₂CH₂–O–C(=O)–C(CH₃)=CH₂ + H_2N–CH₂CH₂–(O–CH₂CH₂)$_n$–O–CH_3

CALB

DMSO, 50°C, 72h

H_3C–O(–CH₂CH₂–O)$_n$–CH₂CH₂–O–C(=O)–CH(CH₃)–CH₂–NH–CH₂CH₂–(O–CH₂CH₂)$_n$–O–CH_3

MPEG-VMA-MPEG

FIG. 18

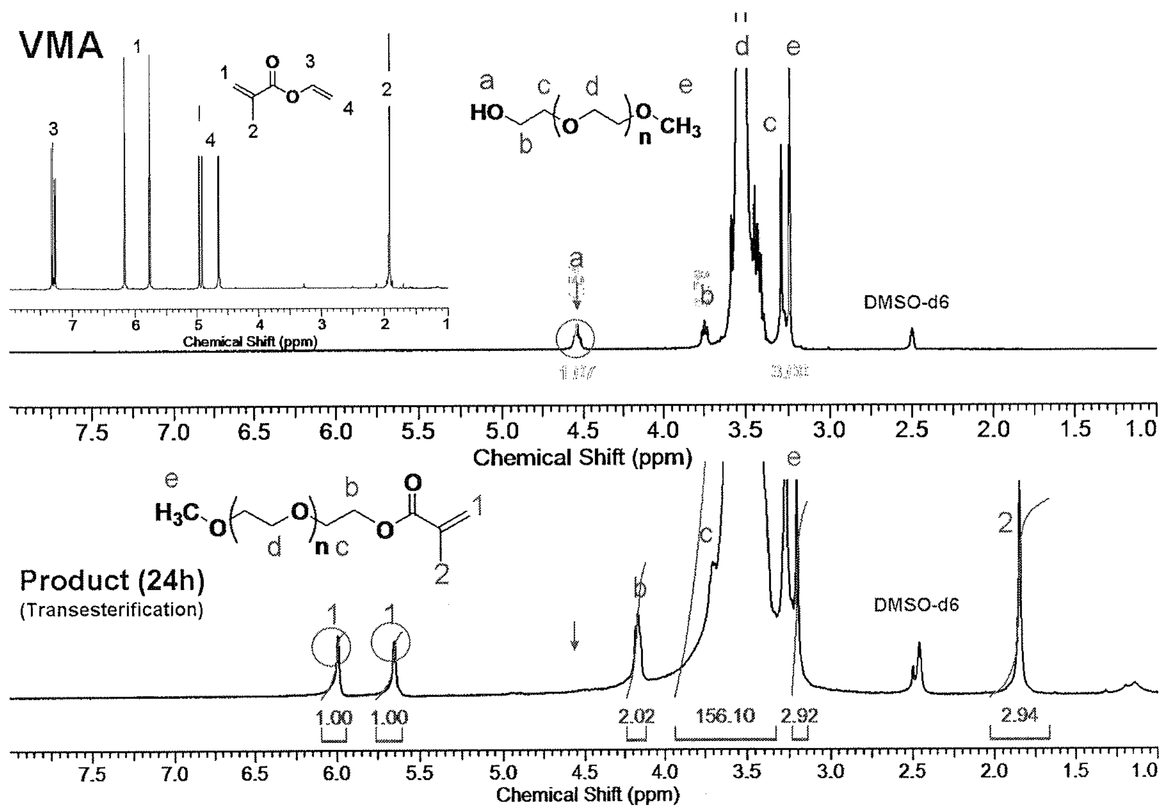


FIG. 19

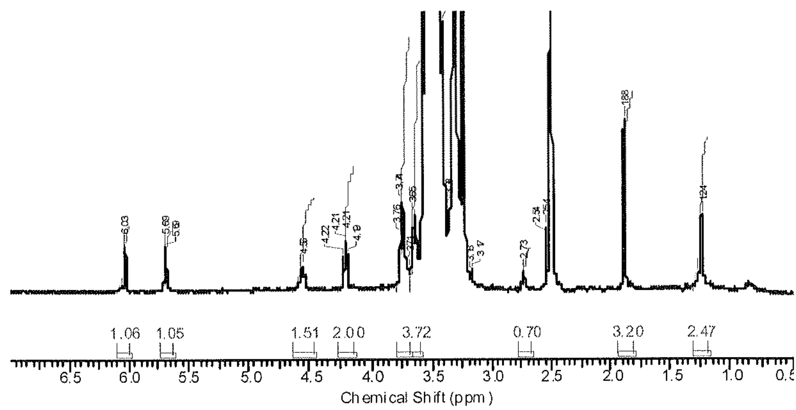


FIG. 20

CALB

DMSO, 24h, 50°C

4 eq.

FIG. 21

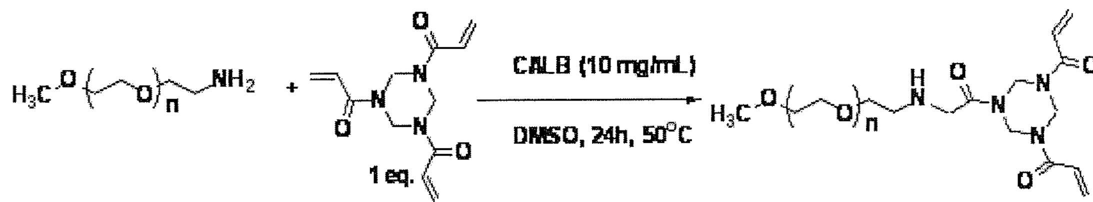


FIG. 22

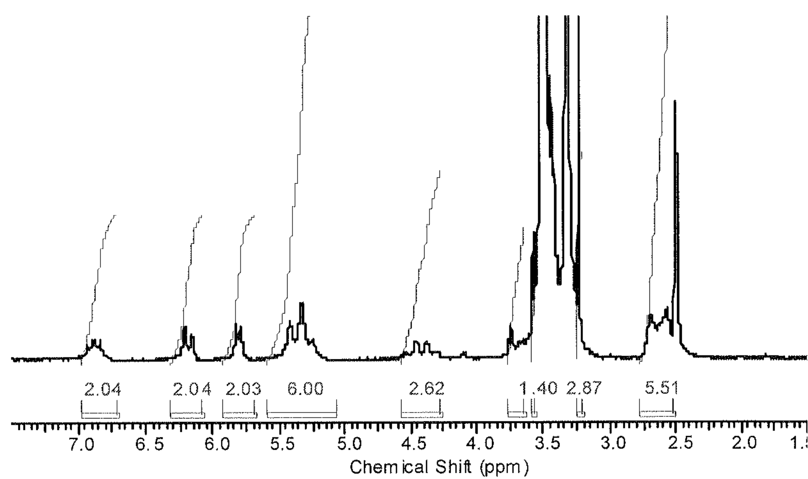


FIG. 23

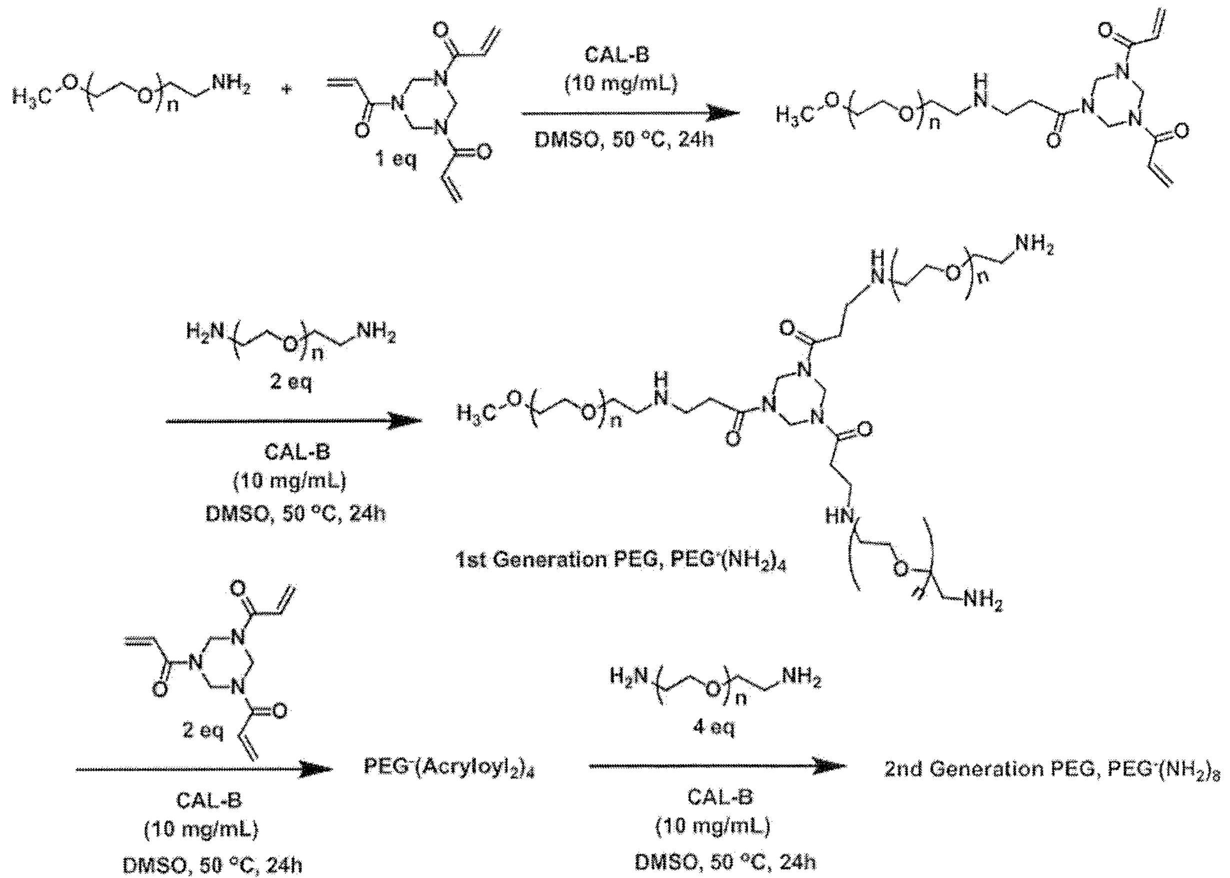


FIG. 24

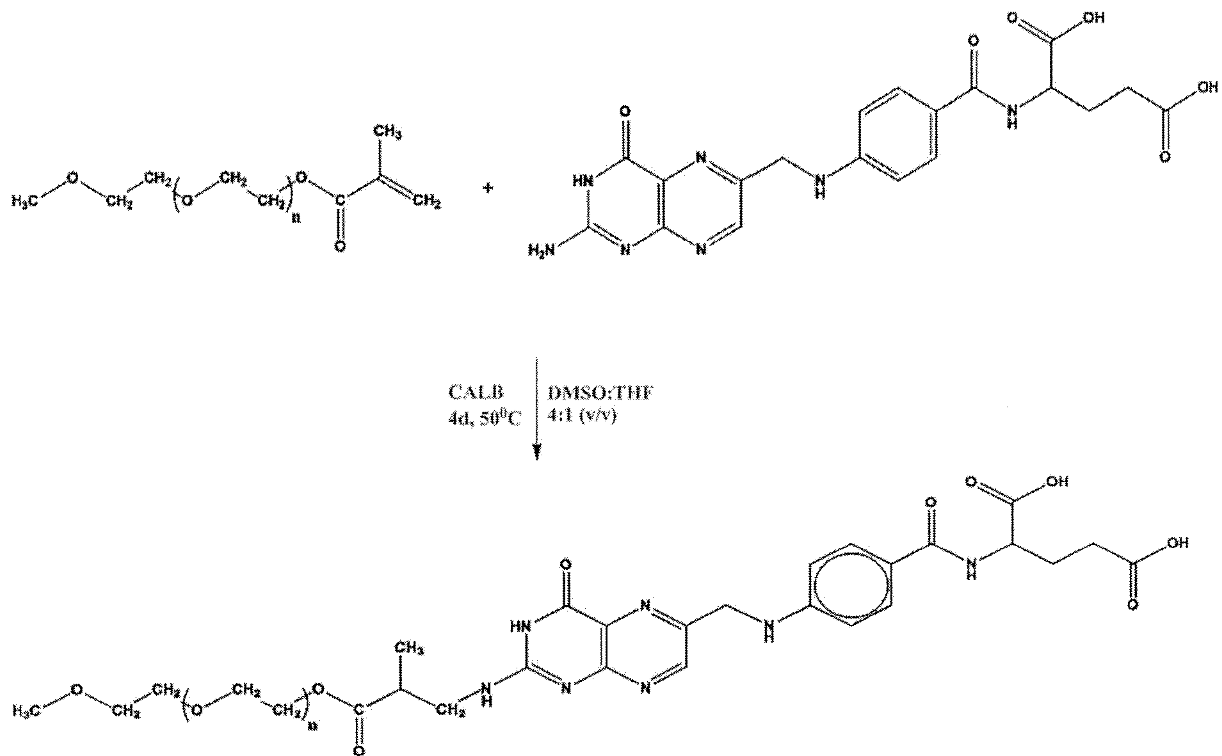


FIG. 25