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CATIONS IN HUMAN HEALTH AND TREATMENT OF DISEASE

(57) Abstract: Provided herein are polymer materials that find use in, for example, delivery of short-chain fatty acids. In particular, polymers are provided that form stable nanoscale structures and release their payload, for example, by cleavage of a covalent bond (e.g., via hydrolysis or enzymatic cleavage). The polymers are useful, for example, for delivery of payloads (e.g., SCFAs) to the intestine for applications in health and treatment of disease, and have broad applicability in diseases linked to changes in the human microbiota including inflammatory, autoimmune, allergic, metabolic, and central nervous system diseases, among others.



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**POLYMER MATERIALS FOR DELIVERY OF SHORT-CHAIN FATTY ACIDS TO THE INTESTINE FOR APPLICATIONS IN HUMAN HEALTH AND TREATMENT OF DISEASE**

**5 FIELD**

Provided herein are polymer materials that find use in, for example, delivery of short-chain fatty acids. In particular, polymers are provided that form stable nanoscale structures and release their payload, for example, by cleavage of a covalent bond (e.g., via hydrolysis or enzymatic cleavage). The polymers are useful, for example, for delivery of payloads (e.g., short-chain fatty acids (SCFAs)) to the intestine for applications in health and treatment of disease, and have broad applicability in diseases linked to changes in the human microbiota including inflammatory, autoimmune, allergic, metabolic, and central nervous system diseases, among others.

**15 BACKGROUND OF THE INVENTION**

Host-microbiota interactions are essential for establishing appropriate immune homeostasis, and perturbations of naturally-selected bacterial populations, a condition called dysbiosis, have been linked to many different pathologies. Dysbiosis can lead to reduced concentrations of bacterial metabolites crucial for maintaining gut homeostasis, in particular short-chain fatty acids that are produced by bacterial fermentation of carbohydrates in the gut. Shortages of these acids have been correlated to a variety of conditions, including inflammatory, autoimmune, and allergic disease (Sandin et al., 2009, Nylund et al., 2015, Berni-Canani et al., 2016, incorporated by reference in their entirety), metabolic disease (Meijer et al., 2010, incorporated by reference in its entirety), and central nervous system disorders (Haghikia et al., 2015, MacFabe, 2015, incorporated by reference in their entirety), among others. Short-chain fatty acids derived from gut bacteria fermentation regulate both the proportions and functional capabilities of intestinal Treg cells (Arpaia et al., 2013; Furusawa et al., 2013; Smith et al., 2013, incorporated by reference in their entirety). As the primary fuel source of intestinal epithelial cells, they also promote barrier function and reduce permeability by increasing proliferation and mucus production (Berni-Canani et al. 2015, incorporated by reference in its entirety). Supplementing diets with short-chain fatty acids has demonstrated therapeutic effects in animal models of allergy, obesity, and others.

Translating short-chain fatty acid treatments to clinical use has been challenging because degradation occurs rapidly during transit through the gut and the molecules

themselves, both as free base and acid salts, are unpalatable, malodorous, and upsetting to the stomach. Enterically-coated short-chain fatty acid products are commercially available but not widely used, partly due to the aforementioned drawbacks, as well as their inability to densely pack the pharmaceutically-active short-chain fatty acid in sufficient quantities to demonstrate therapeutic effects. None of the products have been specifically evaluated for the treatment or prevention of any specific disease.

Delivery systems that overcome the above limitations would be useful for all the known clinical applications of short-chain fatty acids enumerated above.

## 10 SUMMARY OF THE INVENTION

Provided herein are copolymers (e.g., random or block) that are delivery vehicles for short- to medium-chain hydrophobic or amphiphilic carboxylic acids (e.g., 3-12 carbon atoms in the chain, collectively referred to herein are short-chain fatty acids [“SCFA”s]) and functionalized derivatives of those acids. The polymers provide delivery of the SCFAs to the gut, including the mucosal lining of the small and large intestine, and in particular embodiments, the ileum. The SCFAs and/or their derivatives are attached to the copolymer backbone with a covalent bond, which is cleavable by hydrolysis or enzyme, thereby releasing the SCFA to have a desired therapeutic effect on human disease. The therapeutic effect is targeted at the barrier function of the intestine and the mucus layer of the gut and all diseases in which mucus layer thickness or barrier function are implicated may be treated. Exemplary human diseases that are treatable with the polymers described herein include, but are not limited to, autoimmune diseases (e.g. rheumatoid arthritis, celiac disease), allergic and atopic diseases (e.g., food allergies of all types, eosinophilic esophagitis, allergic rhinitis, allergic asthma, pet allergies, drug allergies), inflammatory conditions (e.g. inflammatory bowel disease, ulcerative colitis, Crohn’s disease), infectious diseases, metabolic disorders, diseases of the central nervous system (e.g. multiple sclerosis, Alzheimer’s disease, Parkinson’s disease), blood disorders (e.g. beta-thalassemia) colorectal cancer, diseases effecting gut motility (e.g. diarrhea), Type I diabetes, and autism spectrum disorders, among others. The copolymers are administered by any suitable route of administration (e.g., orally, rectally, etc.), and overcome the known limitations associated with the administration of short-chain fatty acids on their own.

Embodiments herein relate to copolymers (e.g., random or block) of (i) a polymer of hydrophilic monomers and (ii) a monomer that displays an SCFA moiety and is attached to the copolymer by a methacrylate or methacrylamide group, supramolecular assemblies

thereof, nanoparticles comprising such copolymers, and methods of use thereof. In some embodiments, the copolymers comprise a random, or pseudo-random distribution of the two types of monomers. In other embodiments, the copolymer is a block copolymer comprising a hydrophilic block and a block comprising monomers that display an SCFA moiety and are  
5 attached to the copolymer by a methacrylate or methacrylamide group (e.g., SFCA-displaying poly(N-oxyethyl methacrylate) block, SFCA-displaying poly(N-oxyethyl methacrylamide) block, SFCA-displaying poly(N-(4-hydroxybenzoyloxy)alkyl methacrylamide) block, SFCA-displaying poly(N-(4-hydroxybenzoyloxy)alkyl methacrylate) block, etc.).

Advantages of the polymer drug delivery systems described herein, in which the  
10 pharmaceutically-active SCFAs are covalently attached to the polymer chain, include: masking odor of SCFAs, enhancing palatability of SCFAs, and increasing the bioavailability of SCFAs, especially in the distal gut, which are otherwise ill-suited for therapeutic use. In some embodiments, polymer nanoparticles carrying SCFAs can further pack more densely, delivering therapeutically relevant doses of the bioactive molecule. In some embodiments,  
15 the delivery systems described herein can survive stomach transit and deliver a therapeutic payload of SCFAs targeted at the intestinal barrier upon hydrolysis, triggered by pH change, or by enzymatic cleavage, e.g. by bacterial or host esterases, and therefore represent attractive options for short-chain fatty acid delivery.

In some embodiments, provided herein are copolymers (e.g., block or random) of: (i)  
20 a polymer of hydrophilic monomers (or a poly-hydrophilic block) and (ii) a N-oxyalkyl methacrylamide monomer (or poly(N-oxyalkyl methacrylamide) block) with a SCFA moiety or other pharmaceutically-relevant small molecule attached to this block via a covalent bond.

In some embodiments, the N-oxyalkyl methacrylamide monomer (or poly(N-oxyalkyl methacrylamide) block) comprises monomers selected from the group consisting of  
25 oxymethyl methacrylamide, 2-oxyethyl methacrylamide, 3-oxypropyl methacrylamide, N-oxyisopropyl methacrylamide, 4-oxybutyl methacrylamide, N-oxyisobutyl methacrylamide, or N-oxyalkyl methacrylamide with longer or otherwise branched or substituted alkyl chains. In some embodiments, the N-oxyalkyl methacrylamide (or poly(N-oxyalkyl methacrylamide) block) comprises a linear alkyl chain of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12,  
30 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)). In some embodiments, the N-oxyalkyl methacrylamide (or poly(N-oxyalkyl methacrylamide) block) comprises a branched alkyl group of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)), such as 2-methylpentyl, 3-ethylpentyl, 3,3-dimethylhexyl, 2,3-dimethylhexyl, 4-ethyl-2-methylhexyl, or any other suitable branched

alkyl groups. In some embodiments, the N-oxyalkyl methacrylamide (or poly(N-oxyalkyl methacrylamide) block) comprises one or more double or triple carbon-carbon bonds (e.g., alkenyl or alkynyl instead of alkanyl). In some embodiments, the N-oxyalkyl methacrylamide (or poly(N-oxyalkyl methacrylamide) block) comprises a hetero alkyl group  
5 comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more heteroatoms (e.g., O, S, NH, etc.) substituted for one of the carbons in the alkyl group (e.g.,  $(\text{CH}_2)_n\text{X}(\text{CH}_2)_m$ , wherein m and n are independently 1-10 and X is O, S, or NH). In some embodiments, the N-oxyalkyl methacrylamide (or poly(N-oxyalkyl methacrylamide) block) comprises a substituted alkyl group comprising one of the aforementioned alkyl  
10 groups (e.g., linear or branched) with one or more pendant substituent groups (e.g., OH, NH<sub>2</sub>, =O, halogen, (e.g., Cl, F, Br, I), CN, CF<sub>3</sub>, etc.). In some embodiments, the poly(N-oxyalkyl methacrylamide) comprises a linear or branched alkyl group comprising any suitable combination of heteroatoms, pendant substituents, double bonds, etc. In particular  
15 is 2-oxyalkyl methacrylamide (or poly(2-oxyalkyl methacrylamide) block).

In some embodiments, provided herein are copolymers (e.g., block or random) of: (i) a polymer of hydrophilic monomers (or a poly-hydrophilic block) and (ii) a N-oxyalkyl phenol ester methacrylamide (or poly(N-oxyalkyl phenol ester methacrylamide) block) with a SCFA moiety or other pharmaceutically-relevant small molecule attached to this block via a  
20 covalent bond.

In some embodiments, the N-oxyalkyl 4-phenol ester methacrylamide monomer (or poly(N-oxyalkyl 4-phenol ester methacrylamide) block) comprises monomers selected from the group consisting of oxymethyl 4-phenol methacrylamide, 2-oxyethyl 4-phenol methacrylamide, 3-oxypropyl 4-phenol methacrylamide, 4-oxybutyl 4-phenol  
25 methacrylamide, or N-oxyalkyl 4-phenol methacrylamide with longer or otherwise branched or substituted alkyl chains. In some embodiments, the N-oxyalkyl 4-phenol ester methacrylamide monomer (or poly(N-oxyalkyl 4-phenol ester methacrylamide) block) comprises a linear alkyl chain of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)). In some embodiments, the N-  
30 oxyalkyl 4-phenol ester methacrylamide monomer (or poly(N-oxyalkyl 4-phenol ester methacrylamide) block) comprises a branched alkyl group of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)), such as 2-methylpentyl, 3-ethylpentyl, 3,3-dimethylhexyl, 2,3-dimethylhexyl, 4-ethyl-2-methylhexyl, or any other suitable branched alkyl groups. In some embodiments, the N-

oxyalkyl 4-phenol ester methacrylamide monomer (or poly(N-oxyalkyl 4-phenol ester methacrylamide) block) comprises one or more double or triple carbon-carbon bonds (e.g., alkenyl or alkynyl instead of alkanyl). In some embodiments, the N-oxyalkyl 4-phenol ester methacrylamide monomer (or poly(N-oxyalkyl 4-phenol ester methacrylamide) block) comprises a hetero alkyl group comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more heteroatoms (e.g., O, S, NH, etc.) substituted for one of the carbons in the alkyl group (e.g.,  $(\text{CH}_2)_n\text{X}(\text{CH}_2)_m$ , wherein m and n are independently 1-10 and X is O, S, or NH). In some embodiments, the N-oxyalkyl 4-phenol ester methacrylamide monomer (or poly(N-oxyalkyl 4-phenol ester methacrylamide) block) comprises a substituted alkyl group comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more pendant substituent groups (e.g., OH, NH<sub>2</sub>, =O, halogen, (e.g., Cl, F, Br, I), CN, CF<sub>3</sub>, etc.). In some embodiments, the poly(N-oxyalkyl methacrylamide) comprises a linear or branched alkyl group comprising any suitable combination of heteroatoms, pendant substituents, double bonds, etc. In particular embodiments, the N-oxyalkyl 4-phenol methacrylamide is poly(2-oxyethyl 4-phenol methacrylamide).

In some embodiments, poly(N-oxyalkyl 4-phenol methacrylamide), with or without any alkyl modifications described above, is substituted at any position on the phenol ring with moieties selected from the groups including, but not limited to, alkyl, hydroxyl, alkoxy, amine, N-alkyl amine, carboxyl, halogen, nitro, and derivatives thereof.

In some embodiments, provided herein are copolymers (e.g., block or random) of: (i) a polymer of hydrophilic monomers (or a poly-hydrophilic block) and (ii) a N-oxyalkyl methacrylate monomer (or poly(N-oxyalkyl methacrylate) block) with a SCFA moiety or other pharmaceutically-relevant small molecule attached to this block via a covalent bond.

In some embodiments, the N-oxyalkyl methacrylate monomer (or poly(N-oxyalkyl methacrylate) block) comprises monomers selected from the group consisting of oxymethyl methacrylate, 2-oxyethyl methacrylate, 3-oxypropyl methacrylate, N-oxyisopropyl methacrylate, 4-oxybutyl methacrylate, N-oxyisobutyl methacrylate, or N-oxyalkyl methacrylate with longer or otherwise branched or substituted alkyl chains. In some embodiments, the N-oxyalkyl methacrylate (or poly(N-oxyalkyl methacrylate) block) comprises a linear alkyl chain of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)). In some embodiments, the N-oxyalkyl methacrylate (or poly(N-oxyalkyl methacrylate) block) comprises a branched alkyl group of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)), such as 2-methylpentyl, 3-ethylpentyl, 3,3-dimethylhexyl,

2,3-dimethylhexyl, 4-ethyl-2-methylhexyl, or any other suitable branched alkyl groups. In some embodiments, the N-oxyalkyl methacrylate (or poly(N-oxyalkyl methacrylate) block) comprises one or more double or triple carbon-carbon bonds (e.g., alkenyl or alkynyl instead of alkanyl). In some embodiments, the N-oxyalkyl methacrylate (or poly(N-oxyalkyl  
5 methacrylate) block) comprises a hetero alkyl group comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more heteroatoms (e.g., O, S, NH, etc.) substituted for one of the carbons in the alkyl group (e.g.,  $(\text{CH}_2)_n\text{X}(\text{CH}_2)_m$ , wherein m and n are independently 1-10 and X is O, S, or NH). In some embodiments, the N-oxyalkyl methacrylate (or poly(N-oxyalkyl methacrylate) block) comprises a substituted alkyl group  
10 comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more pendant substituent groups (e.g., OH, NH<sub>2</sub>, =O, halogen, (e.g., Cl, F, Br, I), CN, CF<sub>3</sub>, etc.). In some embodiments, the poly(N-oxyalkyl methacrylate) comprises a linear or branched alkyl group comprising any suitable combination of heteroatoms, pendant substituents, double bonds, etc. In particular embodiments, the N-oxyalkyl methacrylate (or  
15 poly(N-oxyalkyl methacrylate) block) is 2-oxyalkyl methacrylate (or poly(2-oxyalkyl methacrylate) block).

In some embodiments, provided herein are copolymers (e.g., block or random) of: (i) a polymer of hydrophilic monomers (or a poly-hydrophilic block) and (ii) a N-oxyalkyl phenol ester methacrylate (or poly(N-oxyalkyl phenol ester methacrylate) block) with a  
20 SCFA moiety or other pharmaceutically-relevant small molecule attached to this block via a covalent bond.

In some embodiments, the N-oxyalkyl 4-phenol ester methacrylate monomer (or poly(N-oxyalkyl 4-phenol ester methacrylate) block) comprises monomers selected from the group consisting of oxymethyl 4-phenol methacrylate, 2-oxyethyl 4-phenol methacrylate, 3-  
25 oxypropyl 4-phenol methacrylate, 4-oxybutyl 4-phenol methacrylate, or N-oxyalkyl 4-phenol methacrylate with longer or otherwise branched or substituted alkyl chains. In some embodiments, the N-oxyalkyl 4-phenol ester methacrylate monomer (or poly(N-oxyalkyl 4-phenol ester methacrylate) block) comprises a linear alkyl chain of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)).  
30 In some embodiments, the N-oxyalkyl 4-phenol ester methacrylate monomer (or poly(N-oxyalkyl 4-phenol ester methacrylate) block) comprises a branched alkyl group of 1-20 carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or ranges therebetween (e.g., 2-8)), such as 2-methylpentyl, 3-ethylpentyl, 3,3-dimethylhexyl, 2,3-dimethylhexyl, 4-ethyl-2-methylhexyl, or any other suitable branched alkyl groups. In some

embodiments, the N-oxyalkyl 4-phenol ester methacrylate monomer (or poly(N-oxyalkyl 4-phenol ester methacrylate) block) comprises one or more double or triple carbon-carbon bonds (e.g., alkenyl or alkynyl instead of alkanyl). In some embodiments, the N-oxyalkyl 4-phenol ester methacrylate monomer (or poly(N-oxyalkyl 4-phenol ester methacrylate) block) comprises a hetero alkyl group comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more heteroatoms (e.g., O, S, NH, etc.) substituted for one of the carbons in the alkyl group (e.g.,  $(\text{CH}_2)_n\text{X}(\text{CH}_2)_m$ , wherein m and n are independently 1-10 and X is O, S, or NH). In some embodiments, the N-oxyalkyl 4-phenol ester methacrylate monomer (or poly(N-oxyalkyl 4-phenol ester methacrylate) block) comprises a substituted alkyl group comprising one of the aforementioned alkyl groups (e.g., linear or branched) with one or more pendant substituent groups (e.g., OH, NH<sub>2</sub>, =O, halogen, (e.g., Cl, F, Br, I), CN, CF<sub>3</sub>, etc.). In some embodiments, the poly(N-oxyalkyl methacrylate) comprises a linear or branched alkyl group comprising any suitable combination of heteroatoms, pendant substituents, double bonds, etc. In particular embodiments, the N-oxyalkyl 4-phenol methacrylate is poly(2-oxyethyl 4-phenol methacrylate).

In some embodiments, poly(N-oxyalkyl 4-phenol methacrylate), with or without any alkyl modifications described above, is substituted at any position on the phenol ring with moieties selected from the groups including, but not limited to, alkyl, hydroxyl, alkoxy, amine, N-alkyl amine, carboxyl, halogen, nitro, and derivatives thereof.

In some embodiments, the polymer of hydrophilic monomers is selected from the group consisting of poly(ethylene oxide) ("PEG"), poly(ethylene oxide)-co-poly(propylene oxide) random, di- or multiblock copolymers, poly(vinyl alcohol), poly(ethylene-co-vinyl alcohol), poly(N-vinyl pyrrolidone), poly(acrylic acid), poly(methyloxazoline) ("PMOXA"), poly(ethyloxazoline), poly(alkylacrylates), poly(acrylamide), poly(N-alkylacrylamides), hydrophilic polypeptide, polysaccharide, poly(N,N-dialkylacrylamides), hyaluronic acid, alginate, cyclodextrin, or poly(N-acryloylmorpholine). In some embodiments, polymer of hydrophilic monomers (whether in a block or random copolymer) are attached to the copolymer by a methacrylate or methacrylamide group. In particular embodiments, the hydrophilic block is poly(N-(2-hydroxypropyl)methacrylamide) ("PHPMA" or "polyHPMA"). In certain embodiments, the molecular weight of the polyHPMA block is 7000-15,000 Da (e.g., 7000 Da, 8000 Da, 9000 Da, 10000 Da, 11000 Da, 12000 Da, 13000 Da, 14000 Da, 15000 Da, or ranges therebetween (e.g., 9000-14000 Da)).

In particular embodiments, the copolymer comprises a covalently-attached SCFA moiety or other pharmaceutically-relevant small molecule. In some embodiments, the SCFA



moiety is selected from the group consisting of acetic acid, propionic acid, isopropionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, caprylic acid, capric acid, lauric acid, and derivatives thereof. In some embodiments, any fatty acids with an aliphatic tail of 12 or fewer carbons (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or any ranges  
5 therein (e.g., 3-10) may find use in embodiments herein. In certain embodiments, the SCFA moiety is butyrate (butyric acid) or iso-butyrate (iso-butyric acid).

In some embodiments, the ratio of the hydrophilic block to the SCFA-displaying (or other pharmaceutically-relevant-small-molecule-displaying) block is between 0.25 and 3.5 (e.g., 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8,  
10 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, or ranges therebetween (e.g., 0.7-1.8)). In some embodiments, the ratio of the hydrophilic monomer to the SCFA-displaying (or other pharmaceutically-relevant-small-molecule-displaying) monomer is between 0.5 and 2.0 (e.g., 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, or ranges therebetween (e.g., 0.7-1.8)).

In some embodiments, a polymer comprises hydrophilic monomer to hydrophobic monomer incorporation ratio of 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20 (or any ranges therebetween).

In some embodiments, a polymer comprises 20-80 percent by weight (e.g., 20 wt%,  
20 25 wt%, 30 wt%, 35 wt%, 40 wt%, 45 wt%, 50 wt%, 55 wt%, 60 wt%, 65 wt%, 70 wt%, 75 wt%, 80 wt%, or ranges therebetween) hydrophilic monomer. In some embodiments, a polymer comprises 20-80 percent by weight (e.g., 20 wt%, 25 wt%, 30 wt%, 35 wt%, 40 wt%, 45 wt%, 50 wt%, 55 wt%, 60 wt%, 65 wt%, 70 wt%, 75 wt%, 80 wt%, or ranges therebetween) hydrophobic monomer.

In some embodiments, provided herein are copolymers comprising HPMA monomers (or a polyHPMA block), and SCFA moieties (e.g., butyrate or iso-butyrate) or other pharmaceutically-relevant small molecule covalently attached, via a linker group, to the copolymer by a methacrylate or methacrylamide group.

In some embodiments, provided herein are supramolecular assemblies comprising a  
30 plurality of the copolymers (e.g., comprising SCFAs or other small molecular cargo) described herein (e.g. dispersed in a liquid). In some embodiments, an assembly is a nanoparticle between 10-1000 nm in diameter (e.g., 10, 20, 50, 100, 200, 500, 1000nm, or ranges therebetween (e.g., 50-500nm)). In certain embodiments, the plurality of block copolymers comprises linear and branched copolymers self-assembled or covalently linked to

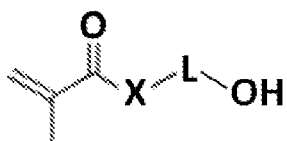
form the nanoparticle. In other embodiments, the assembly is a micelle. In yet other embodiments, the supramolecular assemblies are isolated (e.g. as a powder) and redispersed (e.g. in a liquid).

In some embodiments, provided herein are methods of delivering a target molecule (e.g., SCFA) to a subject (e.g., a human subject, a male subject, a female subject, etc.), the method comprising providing a supramolecular assembly of the copolymers described herein, wherein the supramolecular assembly comprises the target molecule (e.g., SCFA); and contacting the subject with the supramolecular assembly, thereby delivering the target molecule to the subject. In some embodiments, a composition (e.g., pharmaceutical composition) comprising the block copolymers described herein and/or supramolecular assemblies thereof are administered to a subject by any suitable route of administration. In some embodiments, the target molecule (e.g., SCFA) is covalently attached to the supramolecular assembly. In particular embodiments, the supramolecular assembly is contacted (e.g., administered) orally when given to the subject. In some embodiments, the supramolecular assembly is dispersed in a liquid carrier when contacted with the subject. In other embodiments, the supramolecular assembly is a solid when contacted with the subject. In some embodiments the supramolecular assembly is for use as a medicament.

In some embodiments, provided herein is the use of a supramolecular assembly of the copolymers described herein in the manufacture of a medicament.

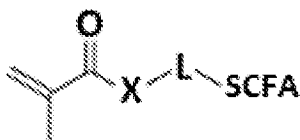
In some embodiments, provided herein are pharmaceutical compositions comprising the supramolecular assemblies described herein. In particular embodiments, a supramolecular assembly is combined with a pharmaceutically acceptable carrier (e.g., considered to be safe and effective) and is administered to a subject (e.g., without causing undesirable biological side effects or unwanted interactions).

In some embodiments, provided herein are compositions comprising a copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (I):



, wherein X is O, NH, or S; wherein L is a linker selected from an alkyl chain, a heteroalkyl chain, a substituted alkyl chain, or a substituted heteroalkyl chain; wherein the copolymer displays one or more short-chain fatty acid (SCFA) moieties.

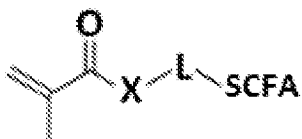
In some embodiments, provided herein are compositions comprising a copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (II):



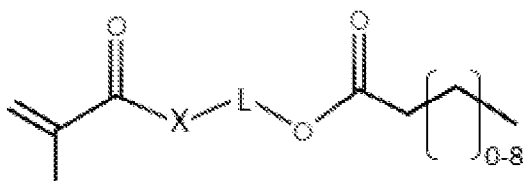
, wherein X is O, NH, or S; wherein L is a linker selected from an alkyl chain, a heteroalkyl chain, a substituted alkyl chain, or a substituted heteroalkyl chain; and wherein SCFA is a short-chain fatty acid.

In some embodiments, the hydrophilic monomer forms a polymer selected from the group consisting of poly(ethylene oxide) (“PEG”), poly(ethylene oxide)-co-poly(propylene oxide) random, di- or multiblock copolymers, poly(vinyl alcohol), poly ethylene-co-vinyl alcohol), poly(N-vinyl pyrrolidone), poly(acrylic acid), poly(methyloxazoline) (“PMOXA”), poly(ethyloxazoline), poly(alkylacrylates), poly(acrylamide), poly(N-alkylacrylamides), hydrophilic polypeptide, polysaccharide, poly(N,N-dialkylacrylamides), hyaluronic acid, alginate, cyclodextrin, poly(N-acryloylmorpholine), and poly(N-(2-hydroxypropyl)methacrylamide) (“polyHPMA”). In some embodiments, the hydrophilic monomer comprises a methacrylamide or methacrylate terminus for polymerization. In some embodiments, the hydrophilic monomer comprises HPMA.

In some embodiments, L of formula (I) or formula (II) is  $(\text{CH}_2)_n$ , wherein n is 1-16 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or ranges therebetween). In some embodiments, L is  $(\text{CH}_2)_n\text{O}(\text{CO})$ -benzene. In some embodiments, the SCFA is covalently attached to the monomer of formula (I). In some embodiments, the SCFA attached to the monomer of formula (I) comprises formula (II):



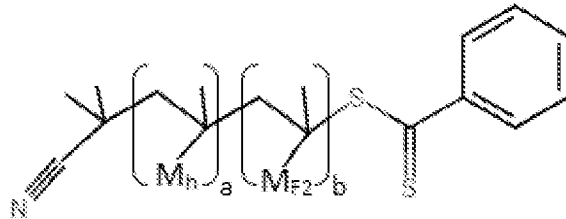
. In some embodiments, the SCFA attached to the monomer of formula (I) or formula (II) comprises formula (III):



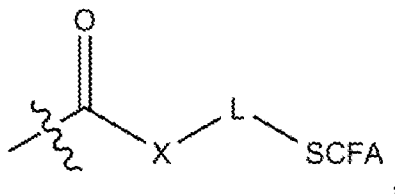
. In some embodiments, the SCFA is selected from the group consisting of acetic acid, propionic acid, isopropionic acid, butyric

acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, caprylic acid, capric acid, lauric acid, branched versions thereof, and derivatives thereof. In some embodiments, the SCFA is butyric acid.

In some embodiments, the copolymer is a block copolymer comprising a hydrophilic  
 5 block and a block of formula (I) or formula (II). In some embodiments, the block copolymer

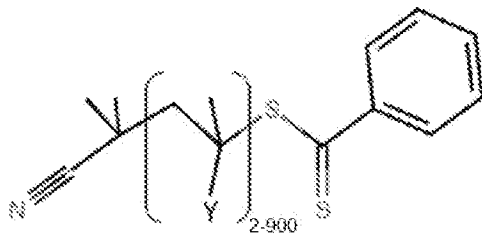


comprises the formula (IV) ;  
 wherein  $M_h$  is the side chain of the hydrophilic monomer,  $M_{F2}$  is the side chain of the monomer of Formula (II):

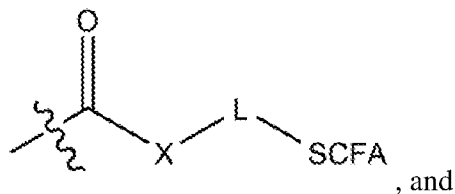


10 wherein a is 1-1000 (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 133, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, or ranges therebetween) and b is 1-1000 (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 133, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, or ranges therebetween).

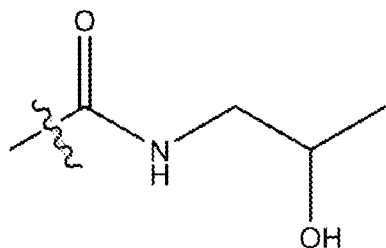
In some embodiments, the copolymer is a random copolymer. In some embodiments,  
 15 the random copolymer comprises formula (V):



; wherein each Y is independently selected from the side chain of a polymer formed from formula (II):

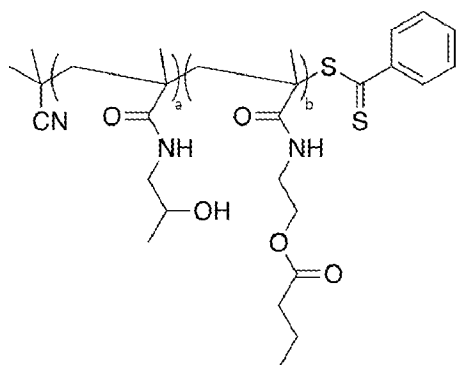


the side chain of polyHPMA:

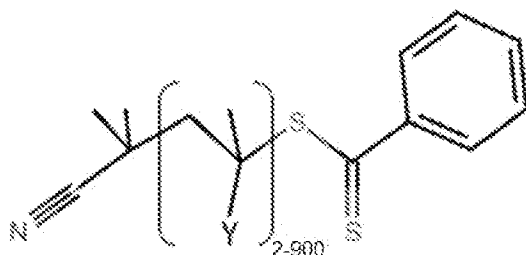


. In some embodiments, there are 2, 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or ranges therebetween of the repeated Y-displaying groups.

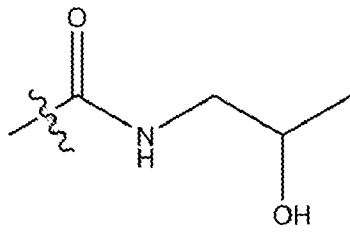
5 In some embodiments, the monomer of formula (II) comprises N-butanoyloxyalkyl methacrylamide. In some embodiments, the N-butanoyloxyalkyl methacrylamide monomer is 2-butanoyloxyethyl methacrylamide. In some embodiments, the copolymer is a block copolymer and comprises formula (VI):



10 ; wherein a and b are independently 1-1000 (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 133, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, or ranges therebetween). In some embodiments, the copolymer is a random copolymer and comprises formula (V):

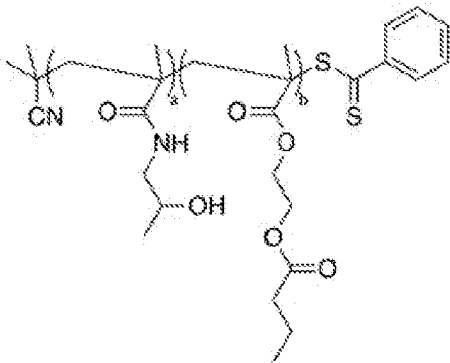


; wherein each Y is independently selected from (i) the side chain of polyHPMA:

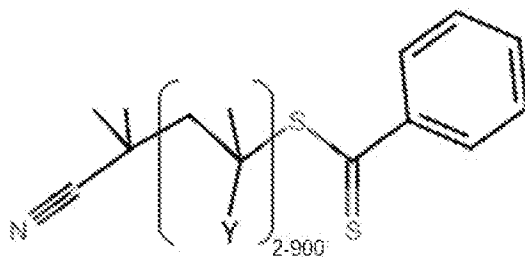


, and (ii) the side chain of poly(2-butanoyloxyethyl methacrylate). In some embodiments, there are 2, 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or ranges therebetween of the repeated Y-displaying groups.

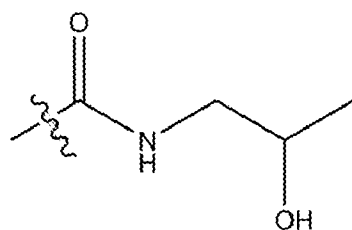
5 In some embodiments, the monomer of formula (II) comprises an N-butanoyloxyalkyl methacrylate. In some embodiments, the N-butanoyloxyalkyl methacrylate monomer is an 2-butanoyloxyethyl methacrylate. In some embodiments, the copolymer is a block copolymer and comprises formula (VII):



10 ; wherein a and b are independently 1-1000 (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 133, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, or ranges therebetween). In some embodiments, the copolymer is a random copolymer and comprises formula (V):

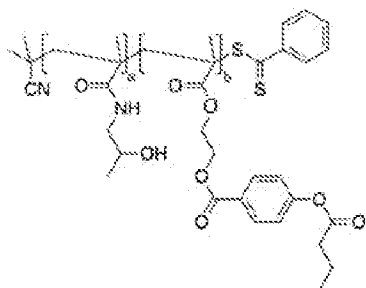


; wherein each Y is independently selected from (i) the side chain of polyHPMA:

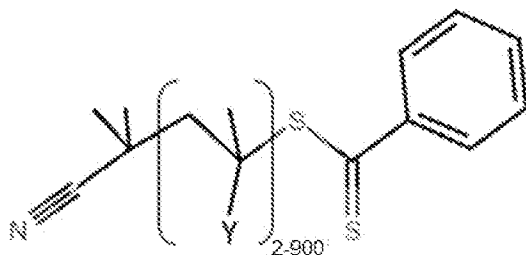


, and (ii) the side chain of poly(2-butanoyloxyethyl methacrylate). In some embodiments, there are 2, 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or ranges therebetween of the repeated Y-displaying groups.

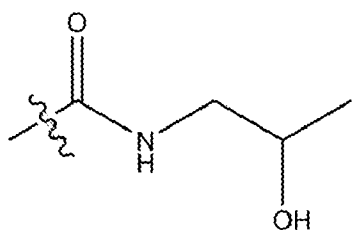
5 In some embodiments, the monomer of formula (II) comprises an N-(4-butanoyloxybenzoyloxy)alkyl methacrylate. In some embodiments, the N-(4-butanoyloxybenzoyloxy)alkyl methacrylate monomer is 2-(4-butanoyloxybenzoyloxy)ethyl methacrylate. In some embodiments, the copolymer is a block copolymer and comprises formula (VIII):



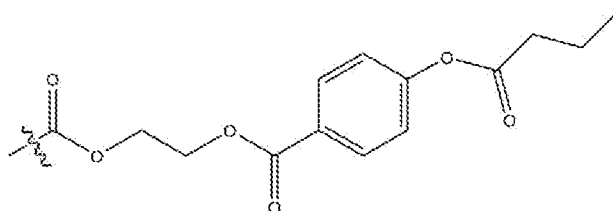
10 ; wherein a and b are independently 1-1000 (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 133, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, or ranges therebetween). In some embodiments, the copolymer is a random copolymer and comprises formula (V):



15 ; wherein each Y is independently selected from (i) the side chain of polyHPMA:

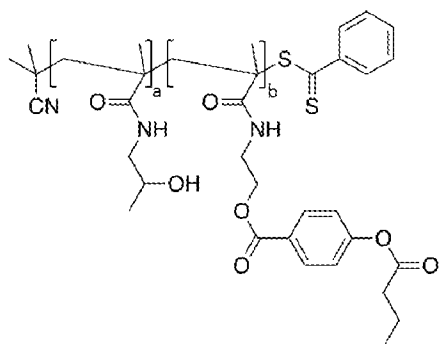


and (ii) the side chain of poly(2-(4-butanoyloxybenzoyloxy)ethyl methacrylate):



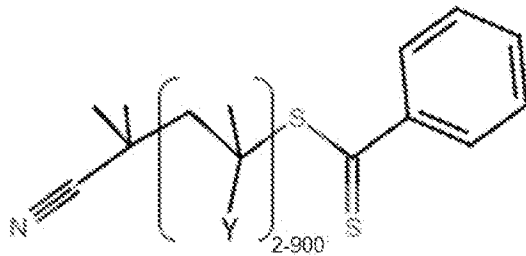
. In some embodiments, there are 2, 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or ranges therebetween of the repeated Y-displaying groups.

In some embodiments, the monomer of formula (II) comprises an N-(4-butanoyloxybenzoyloxy)alkyl methacrylamide. In some embodiments, the N-(4-butanoyloxybenzoyloxy)alkyl methacrylamide monomer is 2-(4-butanoyloxybenzoyloxy)ethyl methacrylamide. In some embodiments, the copolymer is a block copolymer and comprises formula (IX):



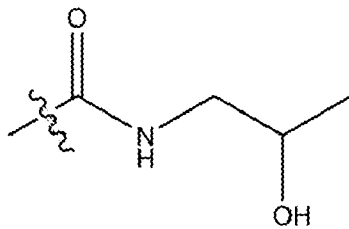
; wherein a and b are independently 1-1000 (e.g., 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 133, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1000, or ranges therebetween). In some embodiments, the copolymer is a random copolymer and comprises formula (V):





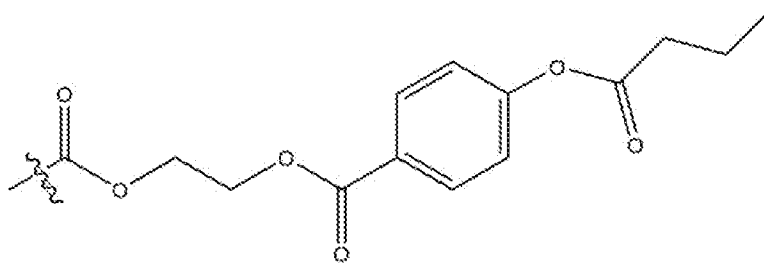
; wherein each Y is independently

selected from (i) the side chain of polyHPMA:



, and (ii) the side chain of poly(2-(4-

butanoyloxybenzoyloxy)ethyl methacrylamide):



5

. In some

embodiments, there are 2, 5, 10, 20, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or ranges therebetween of the repeated Y-displaying groups.

In some embodiments, provided herein are supramolecular assemblies of the copolymers described herein. In some embodiments, the supramolecular assembly is a micelle or nanoparticle.

In some embodiments, provided herein are pharmaceutical compositions comprising the supramolecular assemblies or copolymers described herein and a pharmaceutically-acceptable carrier.

In some embodiments, provided herein are foods or nutraceutical compositions comprising the supramolecular assemblies or copolymers described herein.

In some embodiments, provided herein are methods comprising administering to a subject a pharmaceutical composition, food, or nutraceutical composition described herein.

In some embodiments, the method is performed to treat or prevent a disease or condition. In some embodiments, the disease or condition is selected from the group consisting of autoimmune diseases, allergies, inflammatory conditions, infections, metabolic disorders, diseases of the central nervous system, colon cancer, diabetes, autism spectrum disorders.

5 In some embodiments, provided herein are methods of synthesizing or manufacturing a copolymer, supramolecular assembly, pharmaceutical composition, food, and/or nutraceutical composition described herein.

In some embodiments, provided herein is the use of a copolymer, supramolecular assembly, pharmaceutical composition, food, and/or nutraceutical composition described  
10 herein for the treatment or prevention of a disease or condition.

### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Synthesis of N-oxyalkyl methacrylamide monomer with pharmaceutically-active small molecule

15 Figure 2A-B: Synthesis of (A) 2-(4-hydroxybenzoyloxy)alkyl methacrylamide) and (B) 2-(4-hydroxybenzoyloxy)alkyl methacrylate monomers with pharmaceutically-active small molecule.

Figure 3: Synthesis of the monomer for the control vehicle.

Figure 4: Synthesis of the block copolymer pHPMA-b-pBMA.

20 Figure 5: Synthesis of the block copolymer pHPMA-b-pBBOMA.

Figure 6:  $^1\text{H-NMR}$  of N-(2-hydroxyethyl) methacrylamide (**2**) (500 MHz,  $\text{CDCl}_3$ ).

Figure 7:  $^1\text{H-NMR}$  of N-butanoyloxyethyl methacrylamide (**3**) (500 MHz,  $\text{CDCl}_3$ ).

Figure 8:  $^1\text{H-NMR}$  of pHPMA (**5**) (500 MHz,  $\text{DMSO-d}_6$ ).

Figure 9:  $^1\text{H-NMR}$  of pHPMA-b-pBMA (**6**) (500 MHz,  $\text{DMSO-d}_6$ ).

25 Figure 10: Dynamic light scattering data of copolymer pHPMA-b-pBMA (HPMA/BMA = 0.71) solutions at different concentrations. At 80 mg/mL, the solution showed a sharp peak with the diameter of 64.6 nm.

Figure 11: Concentration of butyrate in feces for one day after oral administration of block copolymer at time 0.

30 Figure 12: Concentration of butyrate in feces after daily oral administration of block copolymer for five days, followed by block copolymer withdrawal for two days.

Figure 13: Core body temperature over time after challenge with peanut protein with and without polymer treatment. "Allergic positive control" or "allergic" means an allergy to peanuts was induced in mice by sensitization via intragastric gavage with 5 mg peanut protein

+ 10 µg cholera toxin as adjuvant. “Non-allergic negative control” means 10 µg cholera toxin adjuvant only was used and did not induce an allergy.

Figure 14: Concentration of peanut-specific IgE in serum 24 hours after challenge with peanut protein with and without polymer treatment. “Allergic positive control” or  
5 “allergic” means an allergy to peanuts was induced in mice by sensitization via intragastric gavage with 5 mg peanut protein + 10 µg cholera toxin as adjuvant. “Non-allergic negative control” means 10 µg cholera toxin adjuvant only was used and did not induce an allergy.

Figure 15: Concentration of peanut-specific IgG1 in serum 24 hours after challenge with peanut protein with and without polymer treatment. “Allergic positive control” or  
10 “allergic” means an allergy to peanuts was induced in mice by sensitization via intragastric gavage with 5 mg peanut protein + 10 µg cholera toxin as adjuvant. “Non-allergic negative control” means 10 µg cholera toxin adjuvant only was used and did not induce an allergy.

Figure 16: Concentration of mouse mast cell protease-1 in serum 90 minutes after challenge with peanut protein with and without polymer treatment. “Allergic positive  
15 control” or “allergic” means an allergy to peanuts was induced in mice by sensitization via intragastric gavage with 5 mg peanut protein + 10 µg cholera toxin as adjuvant. “Non-allergic negative control” means 10 µg cholera toxin adjuvant only was used and did not induce an allergy.

Figure 17: Comparison of markers of allergic sensitivity in mice treated with sodium  
20 butyrate or not treated (labeled as “positive control” or “PN + CT” or “CT-only”) after challenge with peanut protein. Left is the change in mouse core body temperature, middle is amount of peanut-specific IgE, and right is amount of peanut-specific IgG1.

Figure 18: DLS of polymer particles with the HPMA to BMA ratio listed in 1X PBS  
at 53.3 mg/mL

25 Figure 19: Core body temperature over time after challenge with peanut protein with and without polymer and sodium butyrate treatment showing no protection with HPMA/BMA = 1.01 (“butyrate polymer”) or sodium butyrate without polymer.

Figure 20: Comparison of pHPMA-b-pBMA formulations as characterized by GPC,  
NMR, and DLS.

30 Figures 21A-C: **A.** GPC trace of pHPMA-b-pBMA Formulation 1; **B.** DLS trace of pHPMA-b-pBMA Formulation 1; **C.** NMR spectrum and integrations used to determine the molecular weight and HPMA:BMA block ratio for Formulation 1.

Figures 22A-C: **A.** GPC trace of pHPMA-*b*-pBMA Formulation 4; **B.** DLS trace of pHPMA-*b*-pBMA Formulation 4; **C.** NMR spectrum and integrations used to determine the molecular weight and HPMA:BMA block ratio for Formulation 4.

Figures 23A-C: **A.** GPC trace of pHPMA-*b*-pBMA Formulation 3; **B.** DLS trace of  
5 pHPMA-*b*-pBMA Formulation 3; **C.** NMR spectrum and integrations used to determine the molecular weight and HPMA:BMA block ratio for Formulation 3.

Figures 24A-C: **A.** Overlaid GPC traces of different batches of polymer pHPMA-*b*-  
pBMA; **B.** Overlaid detail of peak in (A.) at about 16 min corresponding to polymer  
pHPMA-*b*-pBMA demonstrating differences in molecular weight and elution time; **C.**  
10 Overlaid DLS traces of different batches of polymer pHPMA-*b*-pBMA demonstrating that  
different molecular weights lead to different particle sizes.

Figure 25: Photographs of different pHPMA-*b*-pBMA solutions showing differences  
in solution color and opacity depending on the particle size and molecular weight.

Figure 26: Percent of butyrate released from polymer backbone, either Formulation 1  
15 or Formulation 3, after a single dose to mice.

Figures 27A-D: Comparison of (A) core body temperature over time after challenge,  
(B) concentration of peanut-specific IgE in serum 24 hours before challenge, (C)  
concentration of peanut-specific IgG1 in serum 24 hours before challenge, and (D)  
concentration of mouse mast cell protease-1 in serum 90 minutes after challenge with peanut  
20 protein when mice are treated daily with Formulation 3 or sodium butyrate or are untreated.

## DEFINITIONS

Although any methods and materials similar or equivalent to those described herein  
can be used in the practice or testing of embodiments described herein, some preferred  
25 methods, compositions, devices, and materials are described herein. However, before the  
present materials and methods are described, it is to be understood that this invention is not  
limited to the particular molecules, compositions, methodologies or protocols herein  
described, as these may vary in accordance with routine experimentation and optimization. It  
is also to be understood that the terminology used in the description is for the purpose of  
30 describing the particular versions or embodiments only, and is not intended to limit the scope  
of the embodiments described herein.

Unless otherwise defined, all technical and scientific terms used herein have the same  
meaning as commonly understood by one of ordinary skill in the art to which this invention  
belongs. However, in case of conflict, the present specification, including definitions, will

control. Accordingly, in the context of the embodiments described herein, the following definitions apply.

As used herein and in the appended claims, the singular forms “a”, “an” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to “a block copolymer” is a reference to one or more block copolymers and equivalents thereof known to those skilled in the art, and so forth.

As used herein, the term “comprise” and linguistic variations thereof denote the presence of recited feature(s), element(s), method step(s), etc. without the exclusion of the presence of additional feature(s), element(s), method step(s), etc. Conversely, the term “consisting of” and linguistic variations thereof, denotes the presence of recited feature(s), element(s), method step(s), etc. and excludes any unrecited feature(s), element(s), method step(s), etc., except for ordinarily-associated impurities. The phrase “consisting essentially of” denotes the recited feature(s), element(s), method step(s), etc. and any additional feature(s), element(s), method step(s), etc. that do not materially affect the basic nature of the composition, system, or method. Many embodiments herein are described using open “comprising” language. Such embodiments encompass multiple closed “consisting of” and/or “consisting essentially of” embodiments, which may alternatively be claimed or described using such language.

As used herein, the term “short-chain fatty acid” (“SCFA”) refers to a carboxylic acid attached to an aliphatic chain, which is either saturated or unsaturated, the aliphatic chain being 12 carbons or less in length.

As used herein the term “fatty acid derivative” (specifically “SCFA derivative”) refers to a small molecular compounds that are obtained by making simple modifications (e.g., amidation, methylation, halogenation, etc.) to fatty acid molecules (e.g., SCFA molecules). For example, butyramide, D- or L-amino-n-butyric acid, alpha- or beta-amino-n-butyric acid, arginine butyrate, butyrin, phenyl butyrates (e.g., 4-, 3-, 2-), dimethylbutyrate, 4-halobutyrate (e.g., fluoro-, chloro-, bromo-, iodo-), 3-halobutyrate (e.g., fluoro-, chloro-, bromo-, iodo-), 2-halobutyrate (e.g., fluoro-, chloro-, bromo-, iodo-), oxybutyrate, and methylbutyrates are exemplary butyrate derivatives. Other butyrate derivatives and similar derivatives of other SCFAs are within the scope of the SCFA derivatives described herein.

As used herein, the term “copolymer” refers to a polymer formed from two or more different monomer subunits. Exemplary copolymers include alternating copolymers, random copolymers, block copolymers, etc.

As used herein, the term “block copolymer” refers to copolymers wherein the repeating subunits are polymeric blocks, i.e. a polymer of polymers. In a copolymer of blocks A and B, A and B each represent polymeric entities themselves, obtained by the polymerization of monomers. Exemplary configurations of such block copolymers include  
5 branched, star, di-block, tri-block and so on.

As used herein, the term “supramolecular” (e.g., “supramolecular assembly”) refers to the non-covalent interactions between molecules and/or solution (e.g., polymers, macromolecules, etc.) and the multicomponent assemblies, complexes, systems, and/or fibers that form as a result.

10 As used herein, the term “pharmaceutically acceptable carrier” refers to non-toxic solid, semisolid, or liquid filler, diluent, encapsulating material, formulation auxiliary, excipient, or carrier conventional in the art for use with a therapeutic agent for administration to a subject. A pharmaceutically acceptable carrier is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.  
15 The pharmaceutically acceptable carrier is appropriate for the formulation employed. For example, if the therapeutic agent is to be administered orally, the carrier may be a gel capsule. A “pharmaceutical composition” typically comprises at least one active agent (e.g., the copolymers described herein) and a pharmaceutically acceptable carrier.

As used herein, the term “effective amount” refers to the amount of a composition  
20 (e.g., pharmaceutical composition) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

As used herein, the term “administration” refers to the act of giving a drug, prodrug, or other agent, or therapeutic treatment (e.g., pharmaceutical compositions of the present  
25 invention) to a subject or in vivo, in vitro, or ex vivo cells, tissues, and organs. Exemplary routes of administration to the human body can be through the eyes (e.g., intraocularly, intravitreally, periocularly, ophthalmic, etc.), mouth (oral), skin (transdermal), nose (nasal), lungs (inhalant), oral mucosa (buccal), ear, rectal, by injection (e.g., intravenously, subcutaneously, intratumorally, intraperitoneally, etc.) and the like.

30 As used herein, the terms “co-administration” and “co-administer” refer to the administration of at least two agent(s) or therapies to a subject. In some embodiments, the co-administration of two or more agents or therapies is concurrent (e.g., in the same or separate formulations). In other embodiments, a first agent/therapy is administered prior to a second agent/therapy. Those of skill in the art understand that the formulations and/or routes of

administration of the various agents or therapies used may vary. The appropriate dosage for co-administration can be readily determined by one skilled in the art. In some embodiments, when agents or therapies are co-administered, the respective agents or therapies are administered at lower dosages than appropriate for their administration alone. Thus, co-administration is especially desirable in embodiments where the co-administration of the agents or therapies lowers the requisite dosage of a potentially harmful (e.g., toxic) agent(s).

As used herein, the term “nanoparticles” refers to particles having mean dimensions (e.g., diameter, width, length, etc.) of less than 1  $\mu\text{m}$  (e.g., <500 nm (“sub-500-nm nanoparticles”), <100 nm (“sub-100-nm nanoparticles”), <50 nm (“sub-50-nm nanoparticles”).

As used herein, the term “biocompatible” refers to materials, compounds, or compositions means that do not cause or elicit significant adverse effects when administered to a subject. Examples of possible adverse effects that limit biocompatibility include, but are not limited to, excessive inflammation, excessive or adverse immune response, and toxicity.

As used herein, the term “biostable” refers to compositions or materials that do not readily break-down or degrade in a physiological or similar aqueous environment. Conversely, the term “biodegradable” refers herein to compositions or materials that readily decompose (e.g., depolymerize, hydrolyze, are enzymatically degraded, disassociate, etc.) in a physiological or other environment.

As used herein, the term “substituted” refers to a group (e.g., alkyl, etc.) that is modified with one or more additional group(s). Non-limiting examples of substituents include, for example: halogen, hydroxy, oxo (=O), thioxo (=S), cyano (-CN), nitro (-NO<sub>2</sub>), imino (=N-H), oximo (=N-OH), hydrazino (=N-NH<sub>2</sub>), NH<sub>2</sub>)-R<sup>b</sup>-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-R<sup>a</sup>, -R<sup>b</sup>-OC(O)-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-C(O)R<sup>a</sup>, -R<sup>b</sup>-C(O)OR<sup>a</sup>, -R<sup>b</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-O-R<sup>c</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2), and -R<sup>b</sup>-S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2); and alkyl, alkenyl, alkynyl, each of which may be optionally substituted by halogen, oxo (=O), thioxo (=S), cyano (-CN), nitro (-NO<sub>2</sub>), imino (=N-H), oximo (=N-OH), hydrazine (=N-NH<sub>2</sub>), -R<sup>b</sup>-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-R<sup>a</sup>, -R<sup>b</sup>-OC(O)-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-C(O)R<sup>a</sup>, -R<sup>b</sup>-C(O)OR<sup>a</sup>, -R<sup>b</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-O-R<sup>c</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2), carbocycle and heterocycle; wherein each R<sup>a</sup> is independently selected from hydrogen, alkyl, alkenyl, alkynyl, carbocycle and heterocycle,

wherein each  $R^a$ , valence permitting, may be optionally substituted with halogen, oxo (=O), thioxo (=S), cyano (-CN), nitro (-NO<sub>2</sub>), imino (=N-H), oximo (=N-OH), hydrazine (=N-NH<sub>2</sub>), -R<sup>b</sup>-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-R<sup>a</sup>, -R<sup>b</sup>-OC(O)-OR<sup>a</sup>, -R<sup>b</sup>-OC(O)-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-C(O)R<sup>a</sup>, -R<sup>b</sup>-C(O)OR<sup>a</sup>, -R<sup>b</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-O-R<sup>c</sup>-C(O)N(R<sup>a</sup>)<sub>2</sub>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)OR<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)C(O)R<sup>a</sup>, -R<sup>b</sup>-N(R<sup>a</sup>)S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>R<sup>a</sup> (where t is 1 or 2), -R<sup>b</sup>-S(O)<sub>t</sub>OR<sup>a</sup> (where t is 1 or 2) and -R<sup>b</sup>-S(O)<sub>t</sub>N(R<sup>a</sup>)<sub>2</sub> (where t is 1 or 2); and wherein each  $R^b$  is independently selected from a direct bond or a straight or branched alkylene, alkenylene, or alkynylene chain, and each  $R^c$  is a straight or branched alkylene, alkenylene or alkynylene chain. Substituent groups may be selected from, but are not limited to: alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocycloalkyl, hydroxyl, alkoxy, mercaptyl, cyano, halo, carbonyl, thiocarbonyl, isocyanato, thiocyanato, isothiocyanato, nitro, perhaloalkyl, perfluoroalkyl, and amino, including mono- and di-substituted amino groups, and the protected derivatives thereof. A “substituted alkyl” encompasses alkynes and alkenes, in addition to alkanes displaying substituent moieties.

As used herein, the term “pseudo-random” refers to sequences or structures generated by processes in which no steps or measures have been taken to control the order of addition of monomers or components.

As used herein, the term “display” refers to the presentation of solvent-exposed functional group by a molecule, monomer, polymer, nanostructure or other chemical entity.

## DETAILED DESCRIPTION OF THE INVENTION

Provided herein are polymer materials that find use in, for example, delivery of short-chain fatty acids. In particular, polymers are provided that form stable nanoscale structures and release their payload, for example, by cleavage of a covalent bond (e.g., via hydrolysis or enzymatic cleavage). The polymers are useful, for example, for delivery of payloads (e.g., SCFAs) to the intestine for applications in health and treatment of disease, and have broad applicability in diseases linked to changes in the human microbiota including inflammatory, autoimmune, allergic, metabolic, and central nervous system diseases, among others.

In some embodiments, provided herein are copolymers (e.g., block or random) of: hydrophilic monomers (or a block thereof) pendant-displaying methacrylamide or methacrylate monomers (or a block thereof). In some embodiments, methods are provided for the assembly of these copolymers into nanoparticles, micelles, or other delivery systems. In some embodiments, methods are provided for the administration of the copolymers, and



delivery systems comprising such copolymers, for the treatment or prevention of various diseases and conditions. In particular, polymers are functionalized to deliver a pharmaceutically-relevant small molecule moiety (e.g., SCFA) relevant for treating human disease with a covalent bond that is broken (e.g., by hydrolysis or enzyme activity). In some 5 embodiments, copolymers are obtained using reversible addition-fragmentation chain-transfer (“RAFT”) polymerization of an appropriate monomer with an initiator.

In some embodiments, polymers are random copolymers comprising N-hydroxypropyl methacrylamide (HPMA) monomers. Other synthetic natural molecules or polymers may be used as hydrophilic monomers.

10 In some embodiments, an HPMA-(N-hydroxyethyl methacrylamide) copolymer is a copolymer (e.g., random copolymer) of HPMA monomers and N-hydroxyethyl methacrylamide monomers. A free HPMA terminus (e.g., not connected to the N-hydroxyethyl acrylamide block) may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer 15 agents, protecting groups, drug, biomolecule, or tissue targeting moiety. Some or all N-hydroxyethyl methacrylamide monomers display a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids (“SCFA”s) and their derivatives containing up to 12 carbon atoms in the chain, for example, between 3 20 and 10 carbon atoms in the chain. The chain may be linear or branched. Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-butyrate, and other SCFAs described herein, as well as derivatives thereof. A free SCFA terminus may be one of a number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

25 In some embodiments, an HPMA-(N-hydroxyethyl methacrylate) copolymer is a copolymer (e.g., random copolymer) of HPMA monomers and N-hydroxyethyl methacrylate monomers. A free HPMA terminus may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or tissue targeting moiety. Some or all 30 N-hydroxyethyl methacrylate monomers display a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids (“SCFA”s) and their derivatives containing, for example, between 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or ranges therebetween) carbon atoms in the chain. The chain may be linear or

branched. Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-butyrate, and other SCFAs described herein, as well as derivatives thereof. A free SCFA terminus may be one of a number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

5           In some embodiments, an HPMA-(N-(4-hydroxybenzoyloxy)alkyl methacrylate) copolymer is a copolymer (e.g., random copolymer) of HPMA monomers and N-(4-hydroxybenzoyloxy)alkyl methacrylate monomers. A free HPMA terminus may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or  
10 tissue targeting moiety. Some or all N-(4-hydroxybenzoyloxy)alkyl methacrylate monomers display a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids (“SCFA”s) and their derivatives containing, for example, between 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or ranges therebetween) carbon atoms in  
15 the chain. The chain may be linear or branched. Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-butyrate, and other SCFAs described herein, as well as derivatives thereof. A free SCFA terminus may be one of a number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

20           In some embodiments, an HPMA-(N-(4-hydroxybenzoyloxy)alkyl methacrylamide) copolymer is copolymer (e.g., random copolymer) of HPMA monomers and N-(4-hydroxybenzoyloxy)alkyl methacrylamide monomers. A free HPMA terminus may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or  
25 tissue targeting moiety. Some or all N-(4-hydroxybenzoyloxy)alkyl methacrylamide monomers display a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids (“SCFA”s) and their derivatives containing, for example, between 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or ranges  
30 therebetween)carbon atoms in the chain. The chain may be linear or branched. Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-butyrate, and other SCFAs described herein, as well as derivatives thereof. A free SCFA terminus may be one of a number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

In some embodiments, polymers are block copolymers comprising poly(N-hydroxypropyl methacrylamide) as a hydrophilic block. Other synthetic natural molecules or polymers may be used as hydrophilic blocks.

In some embodiments, an HPMA-(N-hydroxyethyl methacrylamide) copolymer is  
5 composed of the hydrophilic polyHPMA block covalently attached to the N-hydroxyethyl methacrylamide block. The free HPMA terminus not connected to the N-hydroxyethyl methacrylamide block may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or tissue targeting moiety. The N-hydroxyethyl  
10 methacrylamide block displays a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids ("SCFA"s) and their derivatives containing, for example, between 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9 10, 11, 12, or ranges therebetween) carbon atoms in the chain. The chain may be linear or branched.  
15 Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-buturate, and other SCFAs described herein, as well as derivatives thereof. The free SCFA terminus not connect to the N-hydroxyethyl methacrylamide block may be one of a number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

In some embodiments, an HPMA-(N-hydroxyethyl methacrylate) copolymer is  
20 composed of the hydrophilic polyHPMA block covalently attached to the N-hydroxyethyl methacrylate block. The free HPMA terminus not connected to the N-hydroxyethyl methacrylate block may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or tissue targeting moiety. The N-hydroxyethyl  
25 methacrylate block displays a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids ("SCFA"s) and their derivatives containing, for example, between 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9 10, 11, 12, or  
30 ranges therebetween) carbon atoms in the chain. The chain may be linear or branched. Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-buturate, and other SCFAs described herein, as well as derivatives thereof. The free SCFA terminus not connect to the N-hydroxyethyl methacrylate block may be one of a

number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

In some embodiments, an HPMA-(N-(4-hydroxybenzoyloxy)alkyl methacrylate) copolymer is composed of the hydrophilic polyHPMA block covalently attached to the N-(4-  
5 hydroxybenzoyloxy)alkyl methacrylate block. The free HPMA terminus not connected to the N-(4-hydroxybenzoyloxy)alkyl methacrylate block may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or tissue targeting moiety. The N-(4-hydroxybenzoyloxy)alkyl methacrylate block displays a pharmaceutically-  
10 relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids ("SCFA"s) and their derivatives containing, for example, between 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9 10, 11, 12, or ranges therebetween)carbon atoms in the chain. The chain may be linear or branched. Example SCFAs include, but are not limited to, acetate,  
15 propionate, iso-propionate, butyrate, iso-butyrate, and other SCFAs described herein, as well as derivatives thereof. The free SCFA terminus not connect to the N-(4-hydroxybenzoyloxy)alkyl methacrylate block may be one of a number of chemical groups including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

In some embodiments, an HPMA-(N-(4-hydroxybenzoyloxy)alkyl methacrylamide) copolymer is composed of the hydrophilic polyHPMA block covalently attached to the N-(4-  
20 hydroxybenzoyloxy)alkyl methacrylamide block. The free HPMA terminus not connected to the N-(4-hydroxybenzoyloxy)alkyl methacrylamide block may be one of a number of chemical groups, including but not limited to hydroxyl, methoxy, thiol, amine, maleimide, halogen, polymer chain transfer agents, protecting groups, drug, biomolecule, or tissue  
25 targeting moiety. The N-(4-hydroxybenzoyloxy)alkyl methacrylamide block displays a pharmaceutically-relevant small molecule covalently attached to the hydroxyethyl functional group. A preferred embodiment of the pharmaceutically-relevant small molecule is short- and medium-chain fatty acids ("SCFA"s) and their derivatives containing, for example, between  
30 3 and 12 (e.g., 3, 4, 5, 6, 7, 8, 9 10, 11, 12, or ranges therebetween)carbon atoms in the chain. The chain may be linear or branched. Example SCFAs include, but are not limited to, acetate, propionate, iso-propionate, butyrate, iso-butyrate, and other SCFAs described herein, as well as derivatives thereof. The free SCFA terminus not connect to the N-(4-hydroxybenzoyloxy)alkyl methacrylamide block may be one of a number of chemical groups

including but not limited to methyl, hydroxyl, methoxy, thiol, amine, N-alkyl amine, and others.

Blocks may vary in molecular weight and therefore size, the adjustment of which alters the ratio of inert, unfunctionalized, pharmaceutically inactive material and active, functionalized pharmaceutically-active material. Some embodiments are a linear HPMA-(N-oxyethyl methacrylamide) block copolymer whose relative block sizes are between 0.25 and 3.5 (e.g., 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, and ranges therebetween (e.g., 0.7 - 1.8)). Other embodiments are a linear HPMA-(2-(4-hydroxybenzoyloxy)ethyl methacrylate) block copolymer whose relative block sizes are between 0.25 and 3.5 (e.g., 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, and ranges therebetween (e.g., 0.7 - 1.8)). A “relative block size” of 0.25 – 3.5 means that for every 1 mole of HPMA weight, the N-oxyethyl methacrylamide block (or a SCFA functionalized derivative) is 0.25 mole – 3.5 mole). In some embodiments, block copolymers described herein form nanoparticles or micelles of diameter 10-1000 nm (e.g., 10, 20, 50, 100, 200, 500, 1000nm, or ranges therebetween (e.g., 50-500nm) when dispersed (e.g. in a liquid). The nanoparticles or micelles thus formed can then be isolated as a solid (e.g. in a powder, by lyophilization, etc.) with or without stabilizers (e.g. surfactants).

Although specific embodiments herein refer to polyHPMA as the hydrophilic block, other hydrophilic polymers may be used in place of polyHPMA including poly(ethylene oxide)-co-poly(propylene oxide) random, di- or multiblock copolymers, poly(vinyl alcohol), poly ethylene-co-vinyl alcohol), poly(N-vinyl pyrrolidone), poly(acrylic acid), poly(methyloxazoline) (“PMOXA”), poly(ethyloxazoline), poly(alkylacrylates), poly(acrylamide), poly(N-alkylacrylamides), hydrophilic polypeptide, polysaccharide, poly(N,N-dialkylacrylamides), hyaluronic acid, alginate, cyclodextrin, or poly(N-acryloylmorpholine). The hydrophilic block may be present at a molecular weight of between 3000 and 50,000 Da (e.g., 3000, 4000, 5000 Da, 6000 Da, 7000 Da, 8000 Da, 9000 Da, 10000 Da, 11000 Da, 12000 Da, 13000 Da, 14000 Da, 15000 Da, 20000 Da, 25000 Da, 30000 Da, 35000 Da, 40000 Da, 45000 Da, 50000 Da, or ranges therebetween (e.g., 9000-14000 Da, 14000-30000)).

In certain embodiments, the copolymer compositions herein are administered in the form of a pharmaceutical composition, a dietary supplement, or a food or beverage. When the compositions herein are used as a food or beverage, the food or beverage can be, e.g. a health

food, a functional food, a food for a specified health use, a dietary supplement, or a food for patients. The composition may be administered once or more than once. If administered more than once, it can be administered on a regular basis (e.g. two times per day, once a day, once every two days, once a week, once a month, once a year) or on as needed, or irregular basis.

5 The frequency of administration of the composition can be determined empirically by those skilled in the art.

Release of the pharmaceutically-active small molecule (e.g., SCFA) is a necessarily important aspect of the copolymer performance for material processing or downstream biological applications. In some embodiments, the pharmaceutically-active small molecule  
10 may be cleaved from the polymer backbone under suitable biological conditions, including hydrolysis (e.g. at certain pH) and enzyme activity (e.g. an esterase). In this regard, the copolymer may be termed a prodrug. In various embodiments, the pharmaceutical composition includes about 10-80% (e.g., 10%, 11%, 12%, 13%, 14%, 15%, 20%, 25%,  
15 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, or ranges therebetween) of pharmaceutically-active small molecule, e.g. a SCFA or derivative thereof, by weight. Those skilled in the art of clinical pharmacology can readily arrive at dosing amounts using routine experimentation.

Release of the pharmaceutically-active small molecule necessarily has a therapeutic effect recapitulating the therapeutic effects of SCFAs, including targeting the barrier function of the intestine and the mucus layer of the gut and all diseases in which SCFAs have been  
20 implicated to have a therapeutic benefit, including increasing mucus layer thickness or barrier function are implicated may be treated. In some embodiments, the human diseases that are treatable include, but are not limited to, rheumatoid arthritis, celiac disease and other autoimmune diseases, food allergies of all types, eosinophilic esophagitis, allergic rhinitis, allergic asthma, pet allergies, drug allergies, and other allergic and atopic diseases,  
25 inflammatory bowel disease, ulcerative colitis, Crohn's diseases, and additional inflammatory conditions, infectious diseases, metabolic disorders, multiple sclerosis, Alzheimer's disease, Parkinson's disease, dementia, and other diseases of the central nervous system, thalassemia and other blood disorders, colorectal cancer, diarrhea and related diseases effecting gut motility, Type I diabetes, and autism spectrum disorders, among others. This list is not  
30 exhaustive, and those skilled in the art can readily treat additional indications that have been shown to have therapeutic effect of SCFAs.

Pharmaceutical preparations can be formulated from the composition of the invention by drug formulation methods known to those skilled in the art. Formulations are prepared using a pharmaceutically acceptable "carrier" composed of materials that are considered safe

and effective, without causing undesirable biological side effects or unwanted interactions. Suitable carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The composition can be adapted for the mode of administration and can be in the form of, e.g., a pill, tablet, capsule, spray, powder, or liquid.

5 In some embodiments, the pharmaceutical composition contains one or more pharmaceutically acceptable additives suitable for the selected route and mode of administration, such as coatings, fillers, binders, lubricant, disintegrants, stabilizers, or surfactants. These compositions may be administered by, without limitation, any parenteral route, including intravenous, intra-arterial, intramuscular, subcutaneous, intradermal,  
10 intraperitoneal, intrathecal, as well as topically, orally, and by mucosal routes of delivery such as intranasal, inhalation, rectal, vaginal, buccal, and sublingual. In some embodiments, the pharmaceutical compositions of the invention are prepared for administration to vertebrate (e.g. mammalian) subjects in the form of liquids, including sterile, non-pyrogenic liquids for injection, emulsions, powders, aerosols, tablets, capsules, enteric coated tablets, or  
15 suppositories.

## EXAMPLES

*Example 1: Preparation of N-(2-hydroxyethyl) methacrylamide monomers with pharmaceutically-active small molecules*

20 Ethanolamine (3.70 mL, 61.4 mmol, 2.0 eq), triethylamine (4.72 mL, 33.8 mmol, 1.1 eq) and 50 mL DCM were added into a 250 mL flask. After the system was cooled down by an ice bath, (1) methacryloyl chloride (3.00 mL, 30.7 mmol, 1.0 eq) was added dropwise under the protection of nitrogen. The reaction was allowed to warm up to room temperature and reacted overnight. Then the reaction mixture was concentrated by rotary evaporation and  
25 purified on a silica column using DCM/MeOH. The product N-(2-hydroxyethyl) methacrylamide (2) was obtained as colorless oil (3.42 g, 86.3%) and analyzed by <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.93 (s, 3H, C(CH<sub>2</sub>)-CH<sub>3</sub>), 3.43 (m, 2H, NH-CH<sub>2</sub>), 3.71 (m, 2H, O-CH<sub>2</sub>), 5.32 (s, 1H, CCH<sub>2</sub>), 5.70 (s, 1H, CCH<sub>2</sub>), 6.44 (br s, 1H, NH).

N-(2-hydroxyethyl) methacrylamide (3.30 mL, 25.6 mmol, 1.0 eq), triethylamine  
30 (7.15 mL, 51.2 mmol, 2.0 eq) and 50 mL DCM were added into a 250 mL flask. After the reaction system was cooled down by an ice bath, butyric anhydride (5.00 mL, 30.7 mmol, 1.2 eq) was added dropwise under the protection of nitrogen. The system was allowed to react overnight. The reaction mixture was filtered and washed by NH<sub>4</sub>Cl solution, NaHCO<sub>3</sub> solution, and water. After dried by anhydrous MgSO<sub>4</sub>, the organic layer was concentrated by

rotary evaporation and purified on a silica column using DCM/MeOH. The product N-butanoyloxyethyl methacrylamide (**3**) was obtained as pale yellow oil (4.56 g, 89.6%) and analyzed by <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 0.95 (t, 3H, CH<sub>2</sub>CH<sub>2</sub>-CH<sub>3</sub>), 1.66 (m, 2H, CH<sub>2</sub>-CH<sub>2</sub>), 1.97 (s, 3H, C(CH<sub>2</sub>)-CH<sub>3</sub>), 2.32 (t, 2H, CO-CH<sub>2</sub>), 3.59 (dt, 2H, NH-CH<sub>2</sub>), 4.23 (t, 2H, O-CH<sub>2</sub>), 5.35 (s, 1H, CCH<sub>2</sub>), 5.71 (s, 1H, CCH<sub>2</sub>), 6.19 (br s, 1H, NH). This example demonstrates the feasibility of attaching pharmaceutically-active small molecules to a monomeric unit of the block copolymer. Additional SCFAs can be attached to the monomeric unit in a similar manner.

10 *Example 2A: Preparation of block copolymers of hydrophilic polymers with N-(2-hydroxyethyl methacrylamide*

pHPMA was prepared using 2-cyano-2-propyl benzodithioate as the RAFT chain transfer agent and 2,2'-Azobis(2-methylpropionitrile) (AIBN) as the initiator. Briefly, HPMA (**4**) (1.0 g, 7.0 mmol, 1.0 eq), 2-cyano-2-propyl benzodithioate (15 mg, 0.07 mmol, 1/100 eq), and AIBN (2.9 mg, 0.017 mmol, 1/400 eq) were dissolved in 2.0 mL MeOH in a Schlenk tube. The reaction mixture was subjected to four freeze-pump-thaw cycles. The polymerization was conducted at 70°C for 15 h. The polymer was precipitated in petroleum ether for five times and dried in the vacuum chamber overnight. The product poly(HPMA) (**5**) was obtained as light pink solid (0.79 g, 79%) and analyzed by <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>), δ<sub>H</sub> ppm 0.8-1.2 (m, 6H, CH(OH)-CH<sub>3</sub> and backbone CH<sub>3</sub>), 1.5-1.8 (m, 2H, backbone CH<sub>2</sub>), 2.91 (m, 2H, NH-CH<sub>2</sub>), 3.68 (m, 1H, C(OH)-H), 4.70 (m, 1H, CH-OH), 7.18 (m, 1H, NH).

The block copolymer was prepared using pHPMA as the macro-RAFT chain transfer agent and N-butanoyloxyethyl methacrylamide (**3**) as the monomer of the second RAFT polymerization. Briefly, pHPMA (0.71 g, 1.0 eq), N-butanoyloxyethyl methacrylamide (2.0 g, 10.0 mmol, 100 eq), and AIBN (4.1 mg, 0.025 mmol, 0.25 eq) were dissolved in 4.0 mL MeOH in a Schlenk tube. The reaction mixture was subjected to four freeze-pump-thaw cycles. The polymerization was conducted at 70 °C for 15 h. The polymer was precipitated in petroleum ether for five times and dried in the vacuum chamber overnight. The product was obtained as light pink solid (1.54 g, 75%) and analyzed by <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ<sub>H</sub> ppm 0.80-1.1 (m, 9H, CH(OH)-CH<sub>3</sub> (HPMA), CH<sub>2</sub>-CH<sub>3</sub> (BMA), and backbone CH<sub>3</sub>), 1.55 (m, 4H, CH<sub>2</sub>-CH<sub>2</sub> (BMA) and backbone CH<sub>2</sub>), 2.28 (m, 2H, CO-CH<sub>2</sub> (BMA)), 2.91 (m, 2H, NH-CH<sub>2</sub> (HPMA)), 3.16 (m, 2H, NH-CH<sub>2</sub> (BMA)), 3.67 (m, 1H, CH(OH)-H), 3.98 (m,



2H, O-CH<sub>2</sub> (BMA)), 4.71 (m, 1H, CH-OH (HPMA)), 7.19 (m, 1H, NH), 7.44 (m, 1H, NH).

Extending from this example, molecular weights varying from 5 kD and 14 kD for each block respectively can be readily obtained, leading to a variety of ratios of inert and active blocks in the polymer. In this way, the range of pharmaceutically-active ingredient thereby

5 incorporated can be about 10-80% of the total polymer weight.

*Example 2B: Preparation of 2-(4-hydroxybenzoyloxy)ethyl methacrylate monomers with pharmaceutically-active small molecules*

2-Bromoethanol (4.36 mL, 61.5 mmol, 1.5 eq), triethylamine (9.15 mL, 65.6 mmol,  
10 1.6 eq) and 60 mL DCM were added into a 250 mL flask. After the system was cooled down by an ice bath, (1) methacryloyl chloride (4.00 mL, 41.0 mmol, 1.0 eq) was added dropwise under the protection of nitrogen. The reaction was allowed to warm up to room temperature and reacted overnight. Then the reaction mixture was concentrated by rotary evaporation and purified on a silica column using DCM/MeOH. The product 2-bromoethyl methacrylate (7)  
15 was obtained as colorless oil (6.62 g, 84.1%) and analyzed by <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.95 (s, 3H, C(CH<sub>2</sub>)-CH<sub>3</sub>), 3.57 (m, 2H, Br-CH<sub>2</sub>), 4.45 (m, 2H, O-CH<sub>2</sub>), 5.62 (s, 1H, CCH<sub>2</sub>), 6.18 (s, 1H, CCH<sub>2</sub>).

4-Hydroxybenzoic acid (2.00 g, 14.5 mmol, 1.2 eq), sodium bicarbonate (2.94 g, 35.0 mmol, 2.9 eq) and 20 mL DMF were added into a two-armed flask. The mixture was  
20 remained at 70 °C for 1 h. Then, 2-bromoethyl methacrylate (2.32 g, 12.1 mmol, 1.0 eq) was dissolved in 10 mL DMF and added into the flask dropwise. The reaction was conducted at 70 °C overnight. The reaction mixture was cooled, poured into 100 mL water and extracted three times with 100 mL of a 50:50 hexane/ethyl acetate mixture. The organic phases were washed twice with water (100 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and purified on a silica column using  
25 DCM/MeOH. The product 2-(4-hydroxybenzoyloxy)ethyl methacrylate (8) was obtained as viscous oil (0.87 g, 58%) and analyzed by <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.95 (s, 3H, C(CH<sub>2</sub>)-CH<sub>3</sub>), 4.48 (m, 2H, O-CH<sub>2</sub>), 4.54 (m, 2H, O-CH<sub>2</sub>), 5.62 (s, 1H, CCH<sub>2</sub>), 6.14 (s, 1H, CCH<sub>2</sub>), 6.86 (d, 2H, ArH), 7.96 (d, 2H, ArH).

2-(4-hydroxybenzoyloxy)ethyl methacrylate (0.87 g, 3.48 mmol, 1.0 eq),  
30 triethylamine (1.46 mL, 10.44 mmol, 3.0 eq) and 30 mL DCM were added into a 100 mL flask. After the reaction system was cooled down by an ice bath, butyric anhydride (1.14 mL, 6.96 mmol, 2.0 eq) was added dropwise under the protection of nitrogen. The system was allowed to react overnight. The reaction mixture was filtered and washed by NH<sub>4</sub>Cl solution, NaHCO<sub>3</sub> solution, and water. After dried by anhydrous MgSO<sub>4</sub>, the organic layer was

concentrated by rotary evaporation and purified on a silica column using DCM/MeOH. The product 2-(4-butanoyloxybenzoyloxy)ethyl methacrylate (**9**) was obtained as pale oil (1.02 g, 92%) and analyzed by <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> ppm 1.06 (t, 3H, CH<sub>2</sub>CH<sub>2</sub>-CH<sub>3</sub>), 1.72 (m, 2H, CH<sub>2</sub>-CH<sub>2</sub>), 1.94 (s, 3H, C(CH<sub>2</sub>)-CH<sub>3</sub>), 2.54 (t, 2H, CO-CH<sub>2</sub>), 4.48 (m, 2H, O-CH<sub>2</sub>), 4.56 (m, 2H, O-CH<sub>2</sub>), 5.59 (s, 1H, CCH<sub>2</sub>), 6.14 (s, 1H, CCH<sub>2</sub>), 7.16 (d, 2H, ArH), 8.06 (d, 2H, ArH).

*Example 3: Preparation of block copolymers of hydrophilic polymers with N-(2-hydroxyethyl methacrylamide*

10 pHPMA was prepared using 2-cyano-2-propyl benzodithioate as the RAFT chain transfer agent and 2,2'-Azobis(2-methylpropionitrile) (AIBN) as the initiator. Briefly, HPMA (**4**) (1.0 g, 7.0 mmol, 1.0 eq), 2-cyano-2-propyl benzodithioate (15 mg, 0.07 mmol, 1/100 eq), and AIBN (2.9 mg, 0.017 mmol, 1/400 eq) were dissolved in 2.0 mL MeOH in a Schlenk tube. The reaction mixture was subjected to four freeze-pump-thaw cycles. The  
15 polymerization was conducted at 70°C for 15 h. The polymer was precipitated in petroleum ether for five times and dried in the vacuum chamber overnight. The product poly(HPMA) (**5**) was obtained as light pink solid (0.79 g, 79%) and analyzed by <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ<sub>H</sub> ppm 0.8-1.2 (m, 6H, CH(OH)-CH<sub>3</sub> and backbone CH<sub>3</sub>), 1.5-1.8 (m, 2H, backbone CH<sub>2</sub>), 2.91 (m, 2H, NH-CH<sub>2</sub>), 3.68 (m, 1H, C(OH)-H), 4.70 (m, 1H, CH-OH), 7.18  
20 (m, 1H, NH)

The block copolymer (pHPMA-b-pBMA) was prepared using pHPMA as the macro-RAFT chain transfer agent and N-butanoyloxyethyl methacrylamide (**3**) as the monomer of the second RAFT polymerization. Briefly, pHPMA (0.71 g, 1.0 eq), N-butanoyloxyethyl methacrylamide (2.0 g, 10.0 mmol, 100 eq), and AIBN (4.1 mg, 0.025 mmol, 0.25 eq) were  
25 dissolved in 4.0 mL MeOH in a Schlenk tube. The reaction mixture was subjected to four freeze-pump-thaw cycles. The polymerization was conducted at 70 °C for 15 h. The polymer was precipitated in petroleum ether for five times and dried in the vacuum chamber overnight. The product was obtained as light pink solid (1.54 g, 75%) and analyzed by <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ<sub>H</sub> ppm 0.80-1.1 (m, 9H, CH(OH)-CH<sub>3</sub> (HPMA), CH<sub>2</sub>-CH<sub>3</sub>  
30 (BMA), and backbone CH<sub>3</sub>), 1.55 (m, 4H, CH<sub>2</sub>-CH<sub>2</sub> (BMA) and backbone CH<sub>2</sub>), 2.28 (m, 2H, CO-CH<sub>2</sub> (BMA)), 2.91 (m, 2H, NH-CH<sub>2</sub> (HPMA)), 3.16 (m, 2H, NH-CH<sub>2</sub> (BMA)), 3.67 (m, 1H, CH(OH)-H), 3.98 (m, 2H, O-CH<sub>2</sub> (BMA)), 4.71 (m, 1H, CH-OH (HPMA)), 7.19 (m, 1H, NH), 7.44 (m, 1H, NH). Extending from this example, molecular weights varying from 5

kD and 14 kD for each block respectively can be readily obtained, leading to a variety of ratios of inert and active blocks in the polymer. In this way, the range of pharmaceutically-active ingredient thereby incorporated can be about 10-80% of the total polymer weight.

5 *Example 4: Preparation of block copolymers of hydrophilic polymers with 2-(4-hydroxybenzoyloxy)ethyl methacrylate*

The block copolymer (pHPMA-b-pBBOMA) was prepared using pHPMA as the macro-RAFT chain transfer agent and 2-(4-butanoyloxybenzoyloxy)ethyl methacrylate (**10**) as the monomer of the second RAFT polymerization. Briefly, pHPMA (0.50 g, 1.0 eq), 2-(4-  
10 butanoyloxybenzoyloxy)ethyl methacrylate (1.12 g, 3.5 mmol, 50 eq), and AIBN (3.0 mg, 0.018 mmol, 0.005 eq) were dissolved in 4.0 mL MeOH in a Schlenk tube. The reaction mixture was subjected to four freeze-pump-thaw cycles. The polymerization was conducted at 70 °C for 15 h. The polymer was precipitated in petroleum ether for five times and dried in the vacuum chamber overnight. The product was obtained as light pink solid (1.34 g, 83%)  
15 and analyzed by <sup>1</sup>H-NMR (500 MHz, DMSO-d<sub>6</sub>) δ<sub>H</sub> ppm 0.80-1.1 (m, 9H, CH(OH)-CH<sub>3</sub> (HPMA), CH<sub>2</sub>-CH<sub>3</sub> (BBOMA), and backbone CH<sub>3</sub>), 1.57 (m, 4H, CH<sub>2</sub>-CH<sub>2</sub> (BBOMA) and backbone CH<sub>2</sub>), 2.46 (m, 2H, CO-CH<sub>2</sub> (BBOMA)), 2.90 (m, 2H, NH-CH<sub>2</sub> (HPMA)), 3.67 (m, 1H, CH(OH)-H), 4.00-4.40 (m, 4H, O-CH<sub>2</sub> (BBOMA)), 4.71 (m, 1H, CH-OH (HPMA)), 7.17 (m, 2H, ArH (BBOMA)), 7.92 (m, 2H, ArH (BBOMA)).

20

*Example 5: Preparation of polymer solutions*

The polymer solutions were prepared using cosolvent evaporation method. 80 mg pHPMA-b-pBMA was dissolved in 1.0 mL ethanol in a beaker. Then, 1.0 mL 1X phosphate buffered saline (PBS) was added into the beaker. The solution was stirred vigorously for 6  
25 hours to let ethanol evaporate. The polymeric solution was obtained at 80 mg/mL in 1X PBS and was stored at 4 °C. The size of the polymeric formulation was measured by dynamic light scattering. This example demonstrates the assembly into particles in solution. Particles can be prepared using other solutions and additives in solutions as embodied in this example. Particle sizes from 10-1000nm are attainable as embodied in this example.

30

*Example 6: Administration of the polymer solutions via oral gavage and increase in fecal SCFA*

Mice were orally gavaged using the polymer solutions prepared. 125  $\mu$ L of a 80 mg/mL solution was administered by oral gavage into the stomach of a mouse using a needle. Mouse fecal samples were obtained every 4 hours and the fecal samples processed for gas chromatography. Gas chromatography samples were prepared as adapted from Kaur, 2012 by adding 0.4 mL of phosphate buffered saline to 0.1 g of feces and homogenizing by vortex. 0.1 mL of an internal standard mixture comprising 0.5 mM 4-methylvaleric acid, 5% meta-phosphoric acid, and 1.56 mg/mL copper sulfate was added, the sample vortexed, and then centrifuged at 13000 rpm for 10 min. 10  $\mu$ L of the supernatant was injected into the gas chromatograph for analysis and concentrations of fecal SCFA were determined using a calibration curve. This example demonstrates that oral administration of the block copolymer increases the fecal concentration of the SCFA attached to the block polymer

*Example 7: Administration of the polymer solutions via oral gavage and decrease in physiological markers of allergic reaction and anaphylaxis*

An allergy to peanuts was induced in mice by sensitization via intragastric gavage with 5 mg peanut protein + 10  $\mu$ g cholera toxin as adjuvant (“allergic positive control” or “allergic” in this example and figures 13, 14, 15, and 16) or 10  $\mu$ g cholera toxin adjuvant only (“non-allergic negative control” in this example and figures 13, 14, 15, and 16) at weaning and once weekly for 5 weeks total following a procedure previously reported and known to those skilled in the art. Data is reported for male mice only to control for subject weight and dosing. 80 mg/mL stock solutions of polymer were prepared, then diluted to 13.3 mg/mL (for a 2 mg daily dose of polymer) and 53.3 mg/mL (for a 8 mg daily dose of polymer) using phosphate buffered saline. 150  $\mu$ L of the polymer solution was administered daily by intragastric gavage into the stomach of “allergic” mice using a needle. For “allergic” mice dosed with 2 mg daily (N=6), the 13.3 mg/mL solution was used; For “allergic” mice dosed with 8 mg daily (N=5), the 53.3 mg/mL solution was used; for “allergic” mice receiving no dose of polymer (N=6), 150  $\mu$ L of 1X PBS was used as control. For “non-allergic negative control” mice (N=4), 150  $\mu$ L of 1X PBS was dosed daily as control. One week after the 5th sensitization (day 35 of the experiment), the mice were given 5 mg of peanut protein via intraperitoneal injection. Core body temperature was monitored rectally for 90 minutes after injection and mouse serum was collected 90 minutes and 24 hours after injection. Serum was processed for ELISA and peanut-specific Immunoglobulin E (IgE), peanut-specific Immunoglobulin G1 (IgG1), and mouse mast cell protease-1 (mMCP-1) was determined.

This example demonstrates that oral administration of the block copolymer reduces the physiological markers and symptoms of an allergic reaction when dosed daily

5 *Example 8: Administration of sodium butyrate solutions via oral gavage and inferiority of sodium butyrate compared to polymer in decreasing physiological markers of allergic reaction and anaphylaxis*

An allergy to peanuts was induced in mice by sensitization via oral gavage with 6 mg peanut protein + 10 µg cholera toxin (“allergic positive control” or “allergic”; N=19) at weaning and once weekly for 5 weeks total following a procedure previously reported and  
10 known to those skilled in the art. Mice received a dose of 2.83 mg sodium butyrate per g of mouse body weight daily by oral gavage of sodium butyrate dissolved in phosphate buffered saline into the stomach of a “allergic” mice using a needle. This dose is equivalent to the dose administered by polymer at 0.8 mg polymer per g of mouse body weight. Mice were weighed  
15 once weekly and the amount of solution adjusted based on the new weight. One week after the 5th sensitization (day 35 of the experiment), the mice were given 5 mg of peanut protein via intraperitoneal injection. Core body temperature was monitored rectally for 90 minutes after injection and mouse serum was collected 90 minutes and 24 hours after injection. Serum was processed for ELISA and peanut-specific Immunoglobulin E (IgE), peanut-specific Immunoglobulin G1 (IgG1), and mouse mast cell protease-1 (mMCP-1) was determined.  
20 These results demonstrate that oral administration of sodium butyrate at the same dose as an equivalent block copolymer is not effective at reducing the physiological markers and symptoms of an allergic reaction when dosed daily (Figure 17).

25 *Example 9: Administration of different formulations of polymer via oral gavage do not lead to equivalent effectiveness at decreasing physiological markers of allergic reaction and anaphylaxis*

Two formulations of polymer were prepared using the methods described in Examples 3 and 5. One polymer was characterized and demonstrated to have a ratio of HPMA:BMA block in the copolymer of 1.01 and a particle diameter of 329 nm by dynamic  
30 light scattering (“Formulation 2”). The other polymer was characterized and demonstrated to have a ratio of HPMA:BMA block in the copolymer of 0.75 and a particle diameter of 64.6 nm by dynamic light scattering (“Formulation 1”) (Figure 18).

An allergy to peanuts was induced in mice by sensitization via oral gavage with 6 mg peanut protein + 10 µg cholera toxin (“allergic positive control” or “allergic”) or 10 µg

cholera toxin alone (“non-allergic negative control”; N=4) at weaning and once weekly for 5 weeks total following a procedure previously reported and known to those skilled in the art. 80 mg/mL stock solutions of Formulation 2 polymer were prepared as in Example 3, then diluted to 53.3 mg/mL. 0.8 mg of polymer per g of mouse body weight was administered daily by oral gavage into the stomach of a “allergic” mice using a needle. Another group of “allergic” mice received 2.83 mg of sodium butyrate identically to Example 7. Another group of “allergic” mice received a 0.8 mg per g of mouse body weight daily dose of a control polymer that could not release butyric acid. For “allergic” mice receiving no dose of polymer, volume-matched solution of 1X PBS was used as control. For “non-allergic negative control” mice, 0.8 mg of control polymer per g of mouse body weight was dosed daily as control. One week after the 5th sensitization (day 35 of the experiment), the mice were given 5 mg of peanut protein via intraperitoneal injection. Core body temperature was monitored rectally for 90 minutes after injection and mouse serum was collected 90 minutes and 24 hours after injection (Figure 19). Serum was processed for ELISA and peanut-specific Immunoglobulin E (IgE), peanut-specific Immunoglobulin G1 (IgG1), and mouse mast cell protease-1 (mMCP-1) was determined. These results demonstrate that increase in fecal butyrate is not sufficient to predict efficacy, and that molecular and morphological characteristics of the polymer non-obviously result in differences in therapeutic effect. Specifically, Formulation 2, which differs from Formulation 1 only by molecular weight and block ratio, does not reduce the physiological markers and symptoms of an allergic reaction when dosed daily.

*Example 10: Administration of formulations of polymer via oral gavage leads to equivalent changes to fecal butyrate concentration, but does not lead to equivalent effectiveness at decreasing physiological markers of allergic reaction and anaphylaxis. Equivalent effectiveness requires strict control of certain polymer molecular characteristics.*

Three formulations of polymer were prepared using the methods described in Examples 3 and 5 (Figure 20). One polymer was characterized and demonstrated to have a ratio of HPMA:BMA block in the copolymer of 0.75, a molecular weight of about 40,000 g/mol, and a particle diameter of 60-70 nm by dynamic light scattering (“Formulation 1”). A second polymer was characterized and demonstrated to have a ratio of HPMA:BMA block in the copolymer of 0.71, a molecular weight of about 52,000 g/mol, and a particle diameter of 120-130 nm by dynamic light scattering (“Formulation 3”). A third polymer was characterized and demonstrated to have a ratio of HPMA:BMA block in the copolymer of

0.77, a molecular weight of 39,000 g/mol, and a particle diameter of 75-82 nm by dynamic light scattering (“Formulation 4”).

Analysis of the three formulations by GPC, DLS, and NMR are depicted in Figures 21-24.

5           8 mg of Formulation 1 and Formulation 3 were administered to groups of mice (N=8 per group) in a single dose oral administration via intragastric gavage and the fecal material from each mouse collected and combined over a period of 24 hours. The samples were processed for gas chromatography and the concentration of free butyrate was determined using the method described in Example 6. The ratio of free butyrate to theoretically released  
10 butyrate was determined and converted to percent release (Figure 26).

These three formulations were compared using the induced allergy model procedure described in Examples 5 and 7 (Figure 27). After the mice were given 5 mg of peanut protein via intraperitoneal injection, core body temperature was monitored rectally for 90 minutes after injection and mouse serum was collected 90 minutes and 24 hours after injection. Serum  
15 was processed for ELISA and peanut-specific Immunoglobulin E (IgE), peanut-specific Immunoglobulin G1 (IgG1), and mouse mast cell protease-1 (mMCP-1) was determined. These results demonstrate that molecular weight differences, and not block ratio differences, lead to differences in therapeutic effect. Specifically, Formulation 2, which does not differ significantly in block ratio from Formulations 1 and 3, but does differ in molecular weight  
20 and morphology does not reduce the physiological markers and symptoms of an allergic reaction when dosed daily. Formulations 1 and 3, which differ only slightly in molecular and morphological characteristics, do reduce the physiological markers and symptoms of an allergic reaction when dosed daily.

## 25 REFERENCES

The following references, some of which are cited above, are herein incorporated by reference in their entireties.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross,  
30 J.R., Pfeffer, K., Coffey, P.J., and Rudensky, A.Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504, 451–455.

- Berni Canani, R., Gilbert, J.A., and Nagler, C.R. (2015). The role of the commensal microbiota in the regulation of tolerance to dietary allergens. *Curr Opin Allergy Clin Immunol*, *15*, 243–249.
- 5 Berni Canani, R., Sangwan, N., Stefka, A.T., Nocerino, R., Papro, L., Aitoro, R., Calignano, A., Kahn, A.A., Gilbert, J.A., and Nagler, C.R. (2016). *Lactobacillus rhamnosus* GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. *ISME J*, *10*, 742–750.
- 10 Furusawa, Y., Obata, Y., Fukuda, S., Endo, T.A., Nakato, G., Takahashi, D., Nakanishi, Y., Uetake, C., Kato, K., Kato, T., Takahashi, M., Fukuda, N.N., Murakami, S., Miyauchi, E., Hino, S., Atarashi, K., Onawa, S., Fujimura, Y., Lockett, T., Clarke, J.M., Topping, D.L., Tomita, M., Hori, S., Ohara, O., Morita, T., Koseki, H., Kikuchi, J., Honda, K., Hase, K., and Ohno, H., (2013). Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* *504*, 446-450.
- 15 Haghikia, A., Jörg, S., Duscha, A., Berg, J., Arndt, M., Waschbisch, A., Hammer, A., Lee, D.-H., May, C., Wilck, N., Balogh, A., Ostermann, A.I., Schebb, N.H., Akkad, D.A., Grohme, D.A., Kleinewietfeld, M., Kempa, S., Thöne, J., Demir, S., Müller, D.N., Gold, R., and Linker, R.A. (2015). Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. *Immunity*, *43*, 817-829.
- 20 Kaur, A., (2012) Modulation of gut microbiota and its environment using starch-entrapped microspheres and cereal arabinoxylans. Purdue.
- 25 MacFabe, D.F. (2015) Enteric short-chain fatty acids: microbial messengers of metabolism, mitochondria, and mind: implications in autism spectrum disorders. *Microb Ecol Health Dis*, *26*, 28177.
- 30 Meijer, K. de Vos, P., and Priebe, M.G. (2010). Butyrate and other short-chain fatty acids as modulators of immunity: what relevance for health? *Curr Opin Clin Nutr Metab Care*. *13*, 715-721.



Nylund, L., Nermes, M., Isolauri, E., Salminen, S., de Vos, W.M., and Satokari, R. (2015). Severity of atopic disease inversely correlates with intestinal microbiota diversity and butyrate-producing bacteria. *Allergy*, *70*, 241-244.

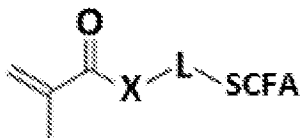
- 5 Sandin, A., Bråbäck, L., Norin, E., and Bengt Björkstén, B. (2009). Faecal short chain fatty acid pattern and allergy in early childhood. *Acta Paediatrica*, *98*, 823-827.

- Smith, P.M., Howitt, M.R., Panikov, N., Michaud, M., Gallini, C.A., Bohlooly-Y, M., Glickman, J.N., and Garrett, W.S. (2013). The microbial metabolites, short chain fatty acids, regulate colonic Treg cell homeostasis. *Science* *341*, 569–573.
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## CLAIMS

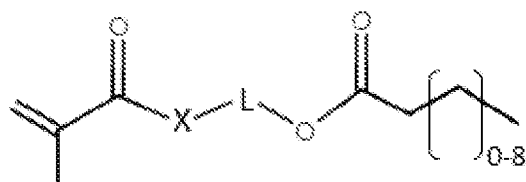
What is claimed is:

1. A composition comprising a copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (II):

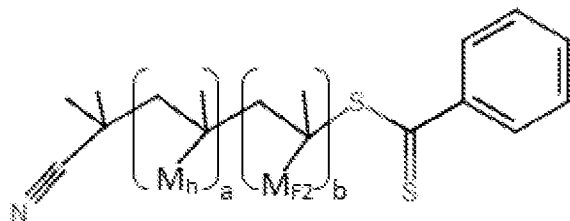


, wherein X is O, NH, or S; wherein L is a linker selected from an alkyl chain, a heteroalkyl chain, a substituted alkyl chain, or a substituted heteroalkyl chain; and wherein SCFA is a short-chain fatty acid.

2. The composition of claim 1, wherein the hydrophilic monomer forms a polymer selected from the group consisting of poly(ethylene oxide) (“PEG”), poly(ethylene oxide)-co-poly(propylene oxide) random, di- or multiblock copolymers, poly(vinyl alcohol), poly(ethylene-co-vinyl alcohol), poly(N-vinyl pyrrolidone), poly(acrylic acid), poly(methyloxazoline) (“PMOXA”), poly(ethyloxazoline), poly(alkylacrylates), poly(acrylamide), poly(N-alkylacrylamides), hydrophilic polypeptide, polysaccharide, poly(N,N-dialkylacrylamides), hyaluronic acid, alginate, cyclodextrin, poly(N-acryloylmorpholine), and poly(N-(2-hydroxypropyl)methacrylamide) (“polyHPMA”).
3. The composition of claim 2, wherein the hydrophilic monomer comprises a methacrylamide or methacrylate terminus.
4. The composition of claim 3, wherein the hydrophilic monomer comprises HPMA.
5. The composition of claim 1, wherein L is  $(\text{CH}_2)_n$ , wherein n is 1-16.
6. The composition of claim 1, wherein L is  $(\text{CH}_2)_n\text{O}(\text{CO})$ -benzene.
7. The composition of claim 1, wherein the the monomer of formula (II) comprises formula (III):

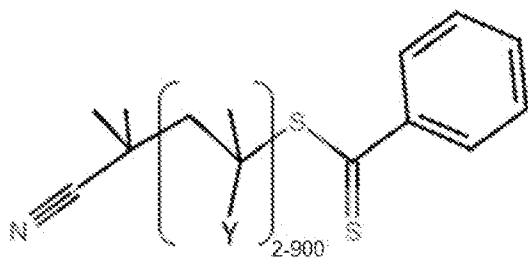


8. The composition of claim 1, wherein the SCFA is selected from the group consisting of acetic acid, propionic acid, isopropionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid, caprylic acid, capric acid, lauric acid, branched versions thereof, and derivatives thereof
9. The composition of claim 8, wherein the SCFA is butyric acid.
10. The composition of claim 9, wherein the composition is 10-80 wt% butyric acid.
11. The composition of claim 1, wherein the copolymer is a block copolymer comprising a hydrophilic block and a block of formula (II).
12. The composition of claim 11, comprising the formula (IV)



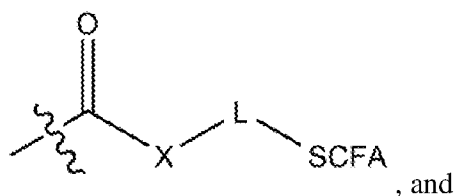
- ; wherein  $M_h$  is the hydrophilic monomer,  $M_{F2}$  is the monomer of Formula (II),  $a$  is 1-1000 and  $b$  is 1-1000.

13. The composition of claim 1, wherein the copolymer is a random copolymer.
14. The composition of claim 13, comprising the formula (V):

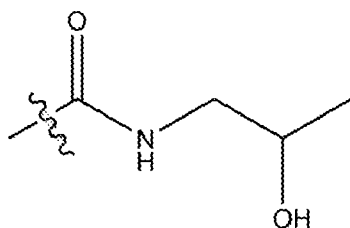


; wherein each Y is independently

selected from the side chain of formula (II):



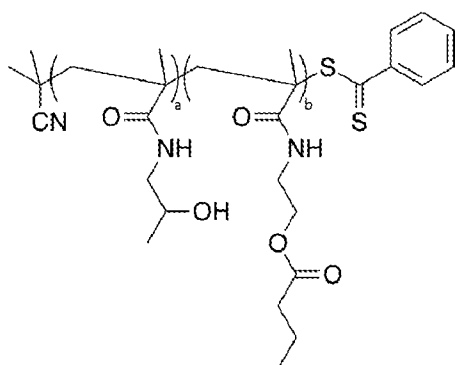
the side chain of polyHPMA:



15. The composition of claim 1, wherein the monomer of formula (II) comprises N-butanoyloxyalkyl methacrylamide.

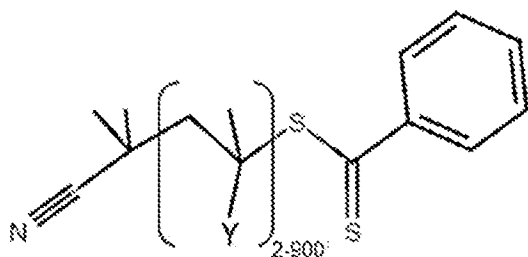
16. The composition of claim 15, wherein the N-butanoyloxyalkyl methacrylamide monomer is 2-butanoyloxyethyl methacrylamide.

17. The composition of claim 16, wherein the copolymer is a block copolymer and comprises formula (VI):



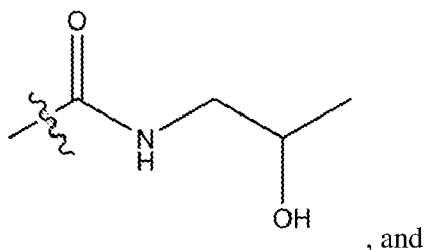
; wherein a and b are independently 1-1000.

18. The composition of claim 16, wherein the copolymer is a random copolymer and comprises formula (V):



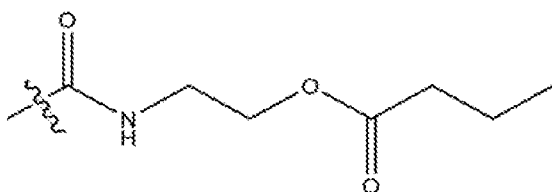
; wherein each Y is independently

selected from (i) the side chain of polyHPMA:



, and

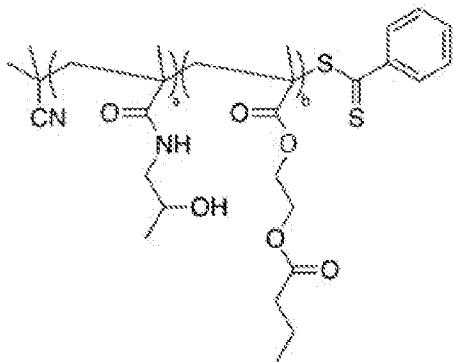
(ii) the side chain of poly(2-butanoyloxyethyl methacrylamide):



19. The composition of claim 1, wherein the monomer of formula (II) comprises an N-butanoyloxyalkyl methacrylate.

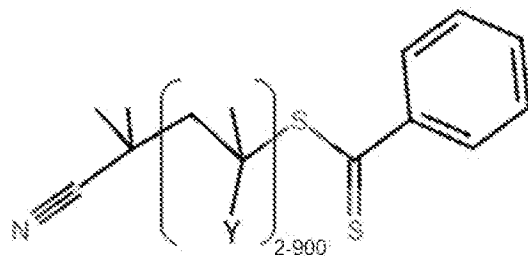
20. The composition of claim 19, wherein the a N-butanoyloxyalkyl methacrylate monomer is a 2-butanoyloxyethyl methacrylate.

21. The composition of claim 20, wherein the copolymer is a block copolymer and comprises formula (VII):



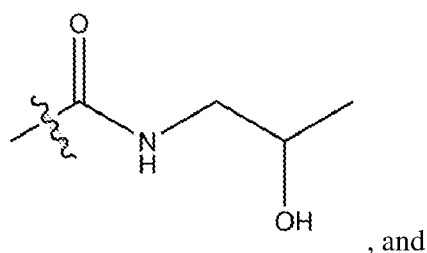
; wherein a and b are independently 1-1000.

22. The composition of claim 20, wherein the copolymer is a random copolymer and comprises formula (V):



; wherein each Y is independently

selected from (i) the sidechain of polyHPMA:

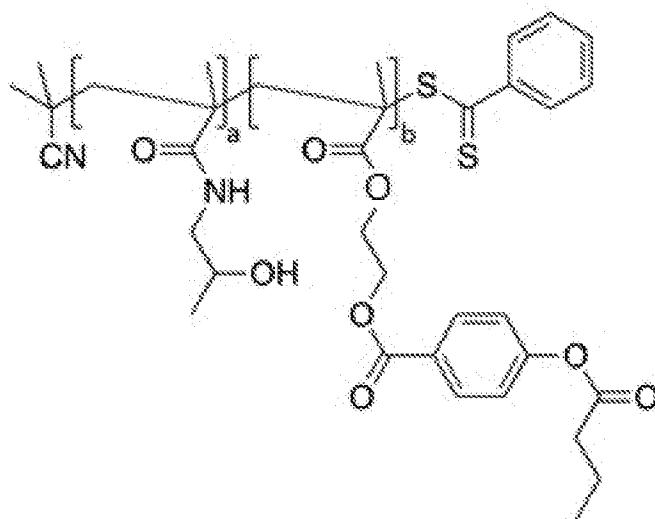


(ii) an 2-butanoyloxyethyl methacrylate.

23. The composition of claim 1, wherein the monomer of formula (II) comprises an N-(4-butanoyloxybenzoyloxy)alkyl methacrylate monomer.

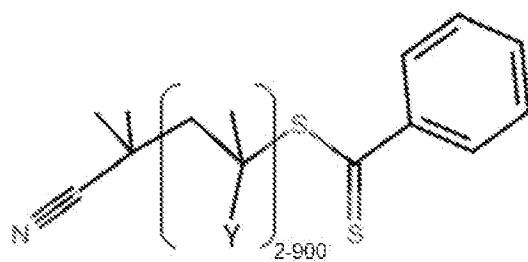
24. The composition of claim 23, wherein the N-(4-butanoyloxybenzoyloxy)alkyl methacrylate monomer is 2-(4-butanoyloxybenzoyloxy)ethyl methacrylate.

25. The composition of claim 24, wherein the copolymer is a block copolymer and comprises formula (VIII):

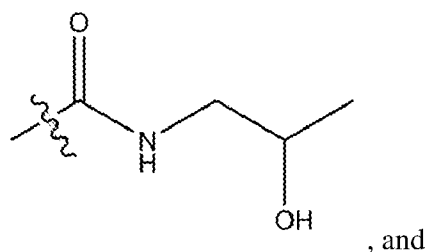


; wherein a and b are independently 1-1000.

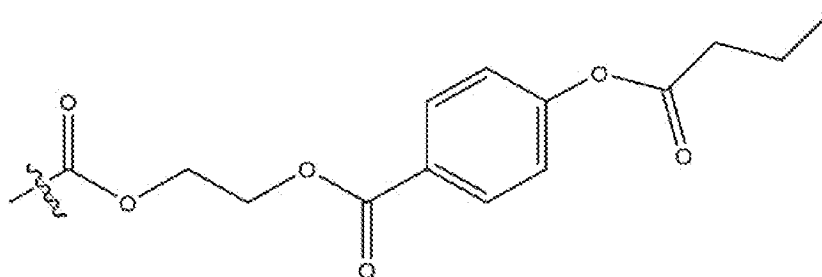
26. The composition of claim 24, wherein the copolymer is a random copolymer and comprises formula (V):



; wherein each Y is independently selected from (i) the side chain of polyHPMA:



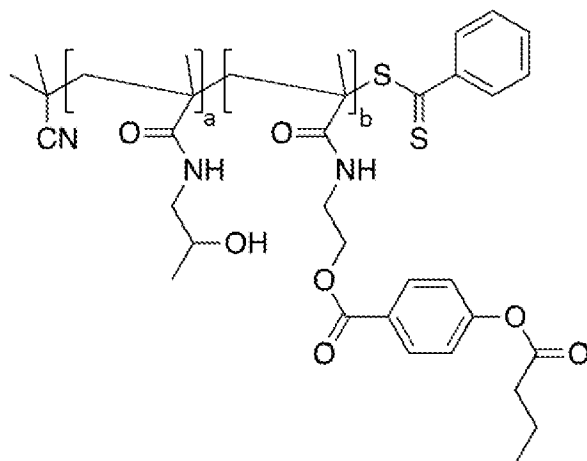
(ii) the side chain of poly(2-(4-butanoyloxybenzoyloxy)ethyl methacrylate):



27. The composition of claim 1, wherein the monomer of formula (II) comprises an N-(4-butanoyloxybenzoyloxy)alkyl methacrylamide monomer.

28. The composition of claim 27, wherein the N-(4-butanoyloxybenzoyloxy)alkyl methacrylamide monomer is 2-(4-butanoyloxybenzoyloxy)ethyl methacrylamide.

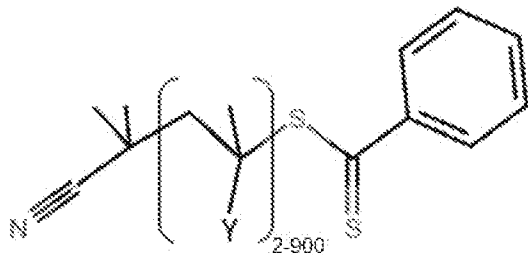
29. The composition of claim 28, wherein the copolymer is a block copolymer and comprises formula (IX):



; wherein a and b are independently 1-1000.

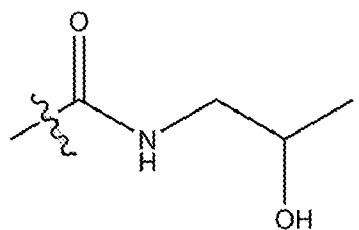


30. The composition of claim 28, wherein the copolymer is a random copolymer and comprises formula (V):



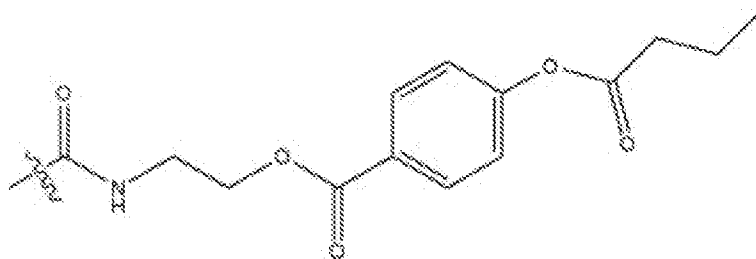
; wherein each Y is independently

selected from (i) the side chain of polyHPMA:



, and

(ii) the side chain of poly(2-(4-butanoyloxybenzoyloxy)ethyl methacrylamide):



31. A system comprising a supramolecular assembly of the compositions of one of claims 1-30.

32. The system of claim 31, wherein the supramolecular assembly is a micelle or nanoparticle.

33. A pharmaceutical composition comprising a system of claim 31 or 32 and a pharmaceutically-acceptable carrier.

34. A food or nutraceutical composition comprising a system of claim 31 or 32 and a pharmaceutically-acceptable carrier.
35. A method comprising administering to a subject a pharmaceutical composition of claim 33 or a food or nutraceutical composition of claim 34 to a subject.
36. The method of claim 35, wherein the method is performed to treat or prevent a disease or condition.
37. The method of claim 36, wherein the disease or condition is selected from the group consisting of autoimmune diseases, allergies, inflammatory conditions, infections, metabolic disorders, diseases of the central nervous system, colon cancer, diabetes, autism spectrum disorders.
38. A method of synthesizing the composition of one of claims 1-30.
39. Use of the composition of one of claims 1-30 for the treatment or prevention of a disease or condition.

FIG. 1

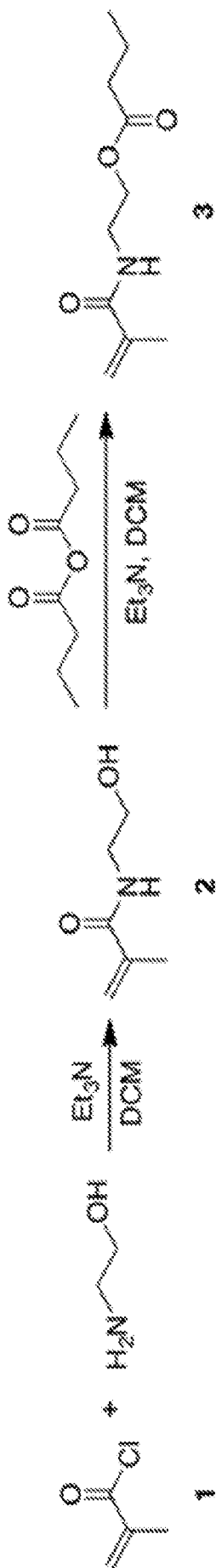


FIG. 2A

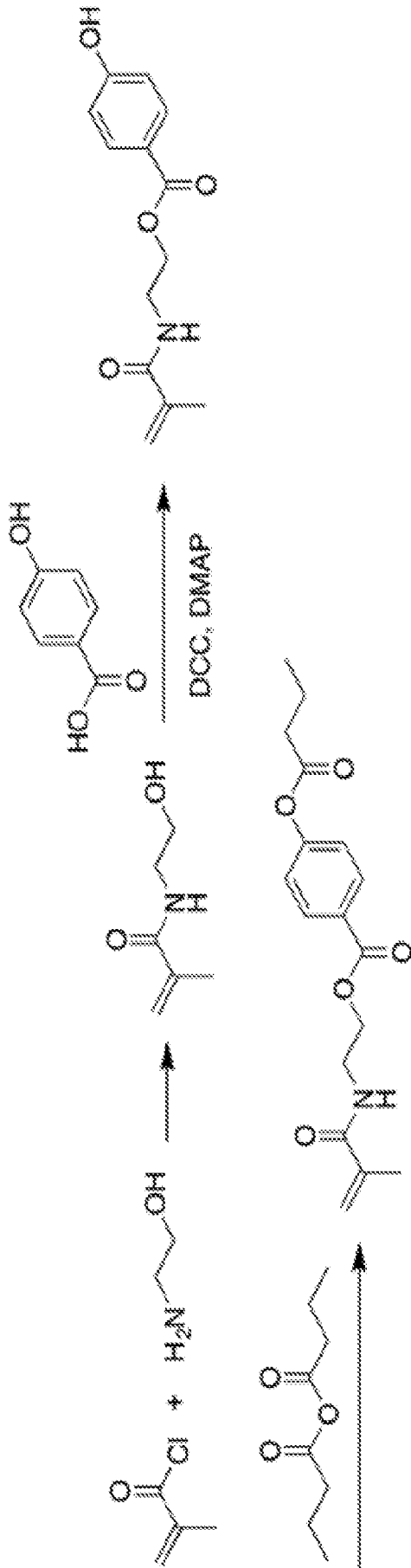




FIG. 3

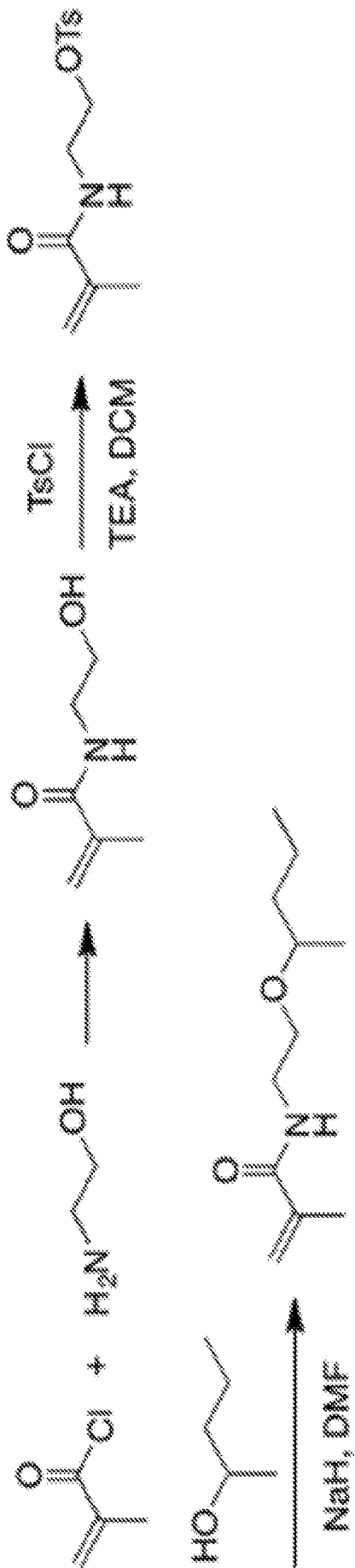




FIG. 5

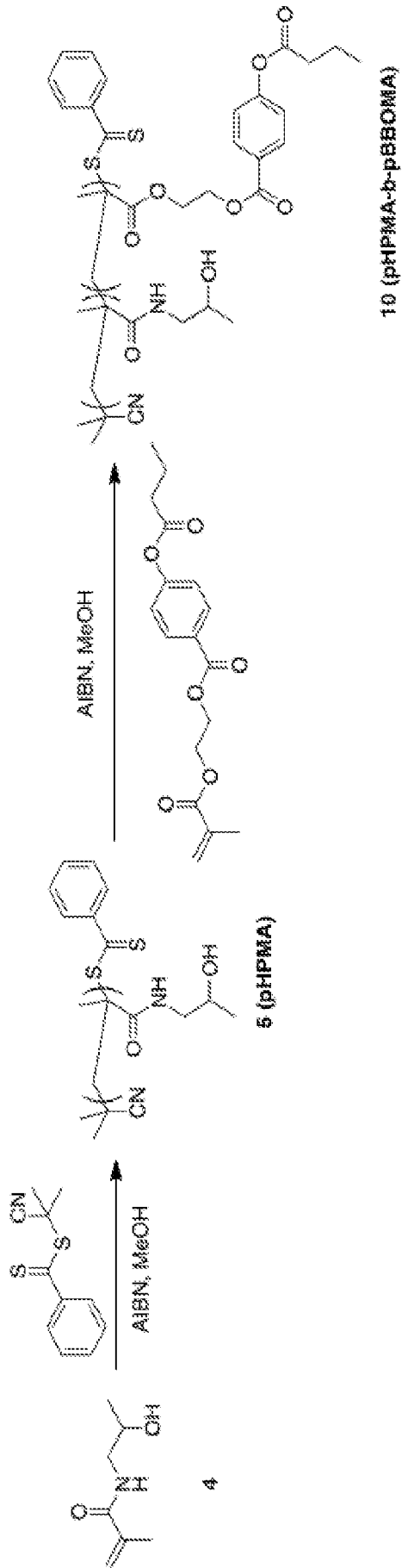






FIG. 7

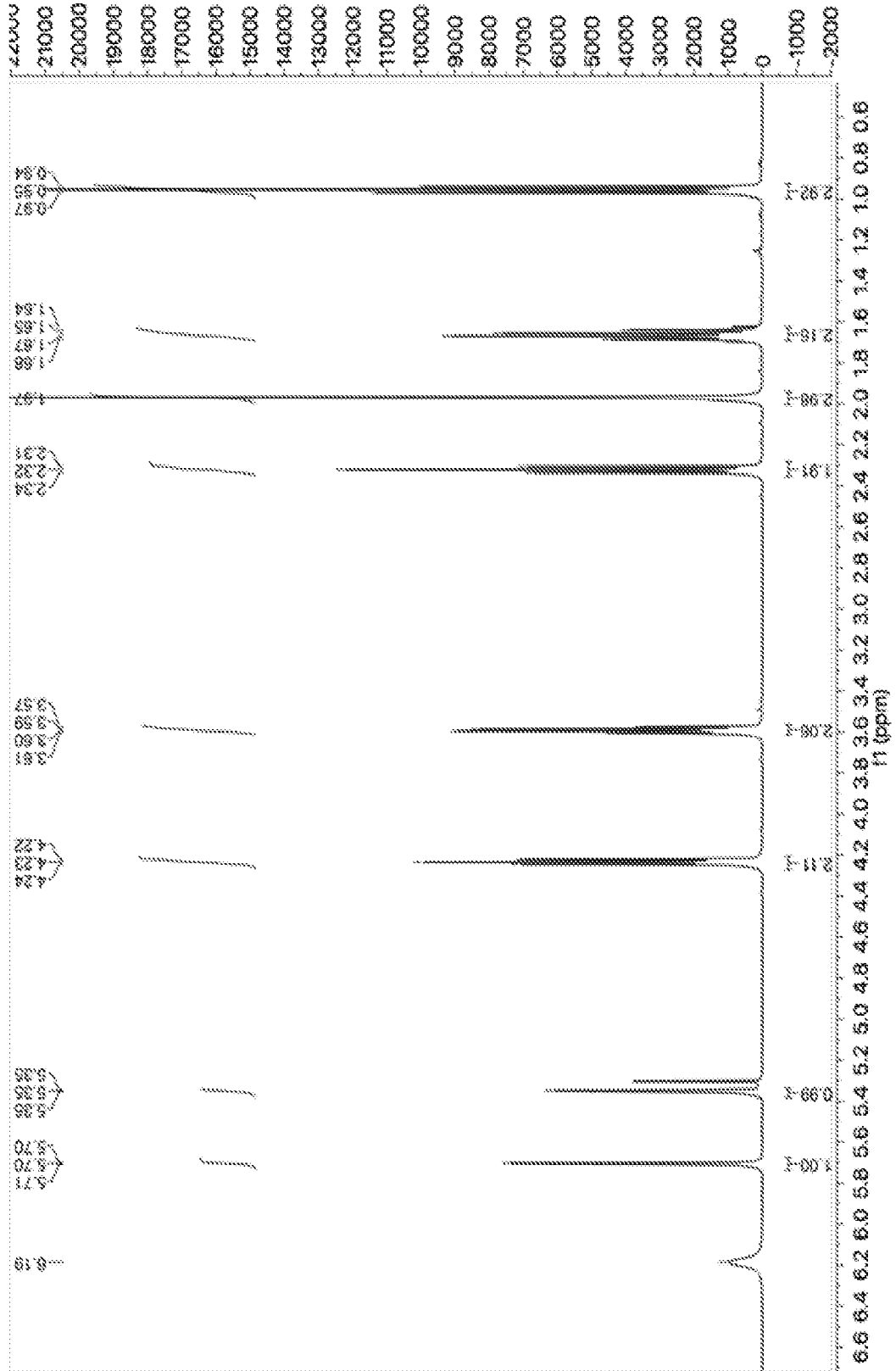


FIG. 8

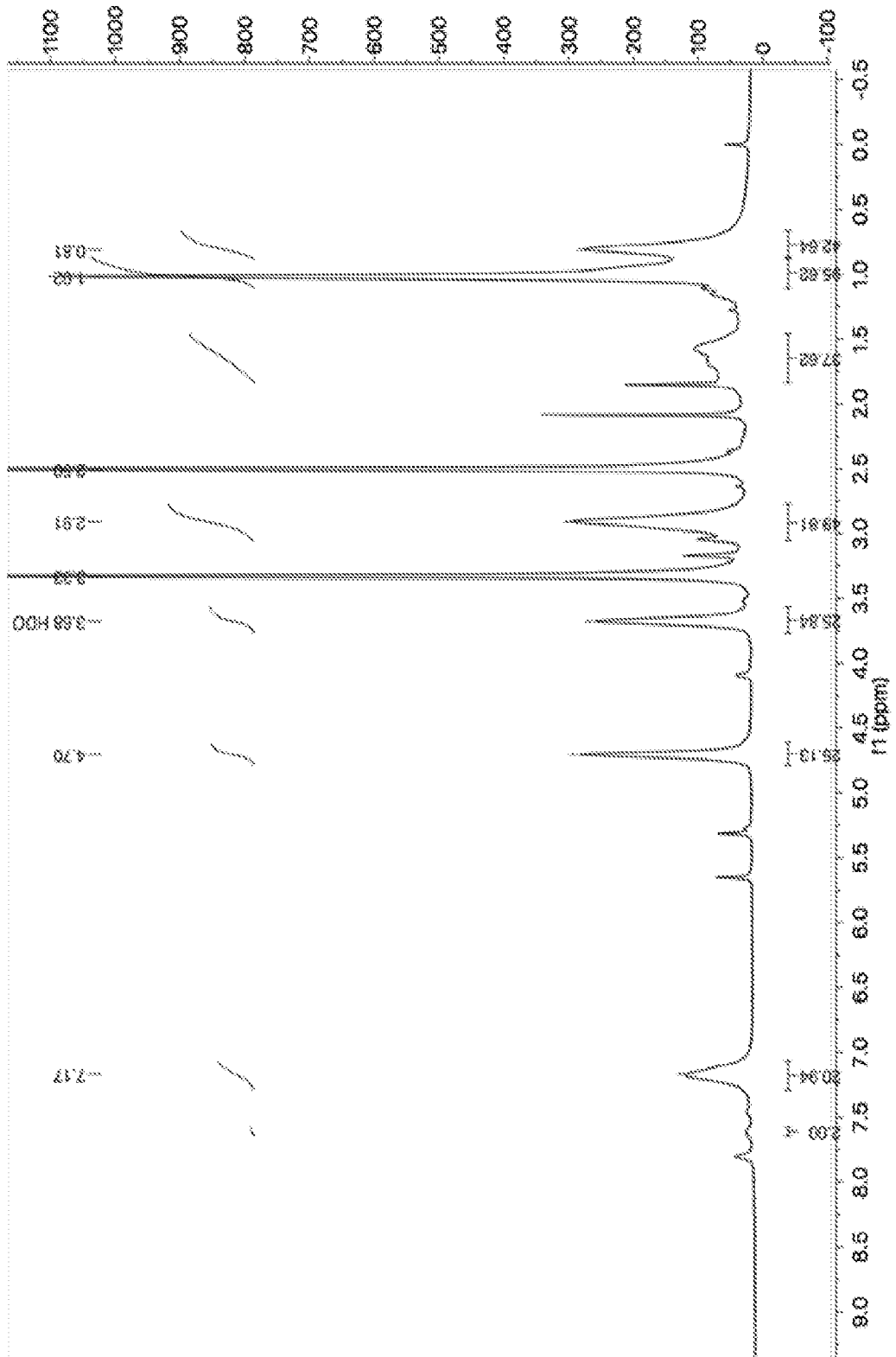


FIG. 9

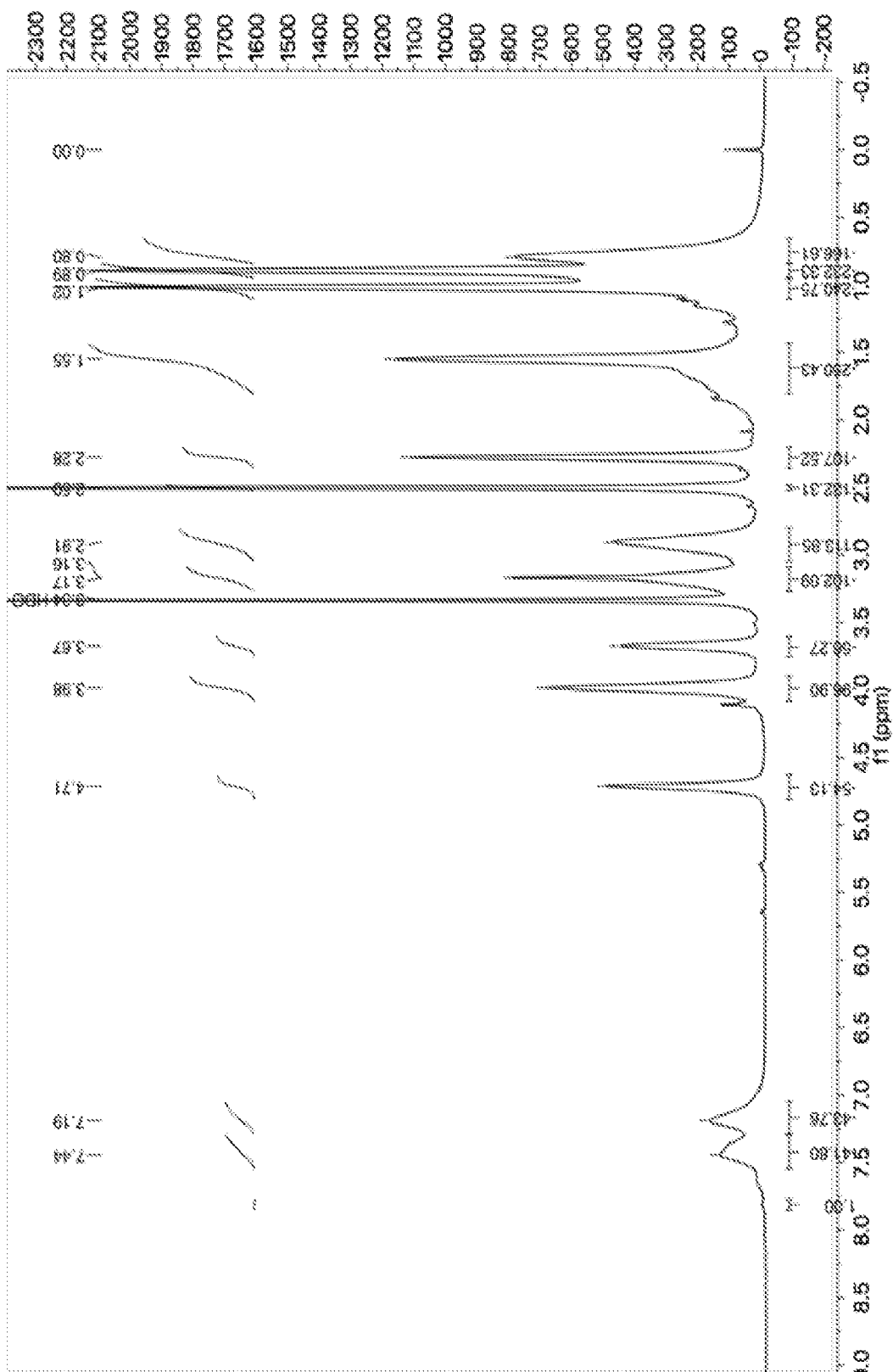


FIG. 10

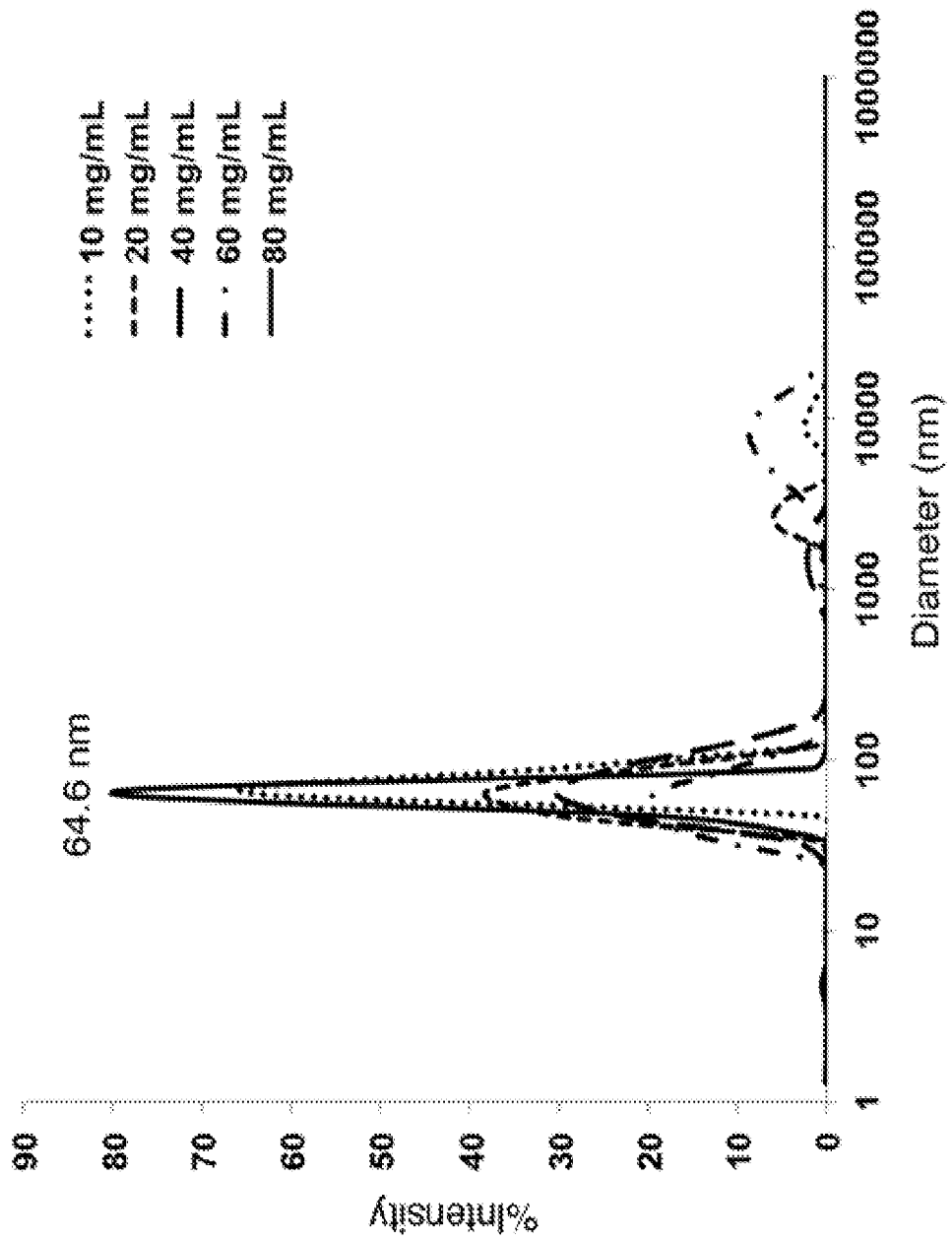


FIG. 11

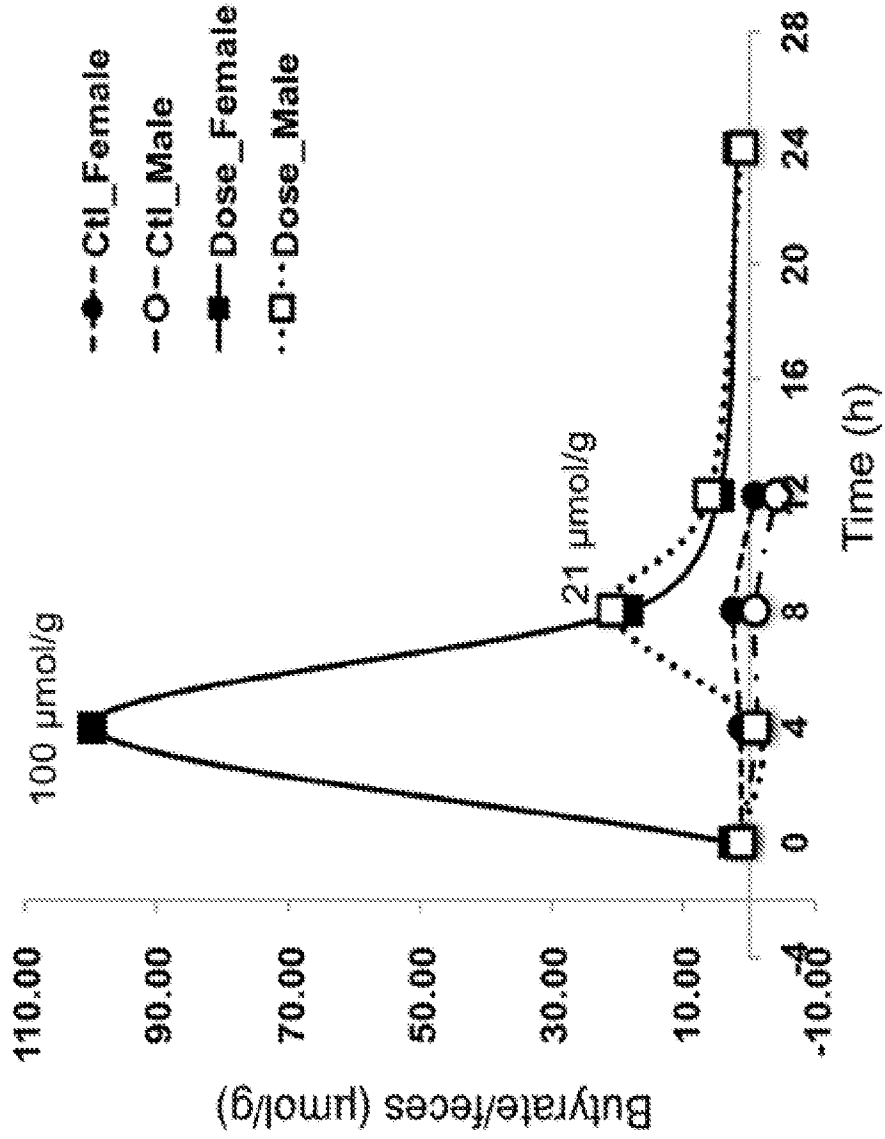


FIG. 12

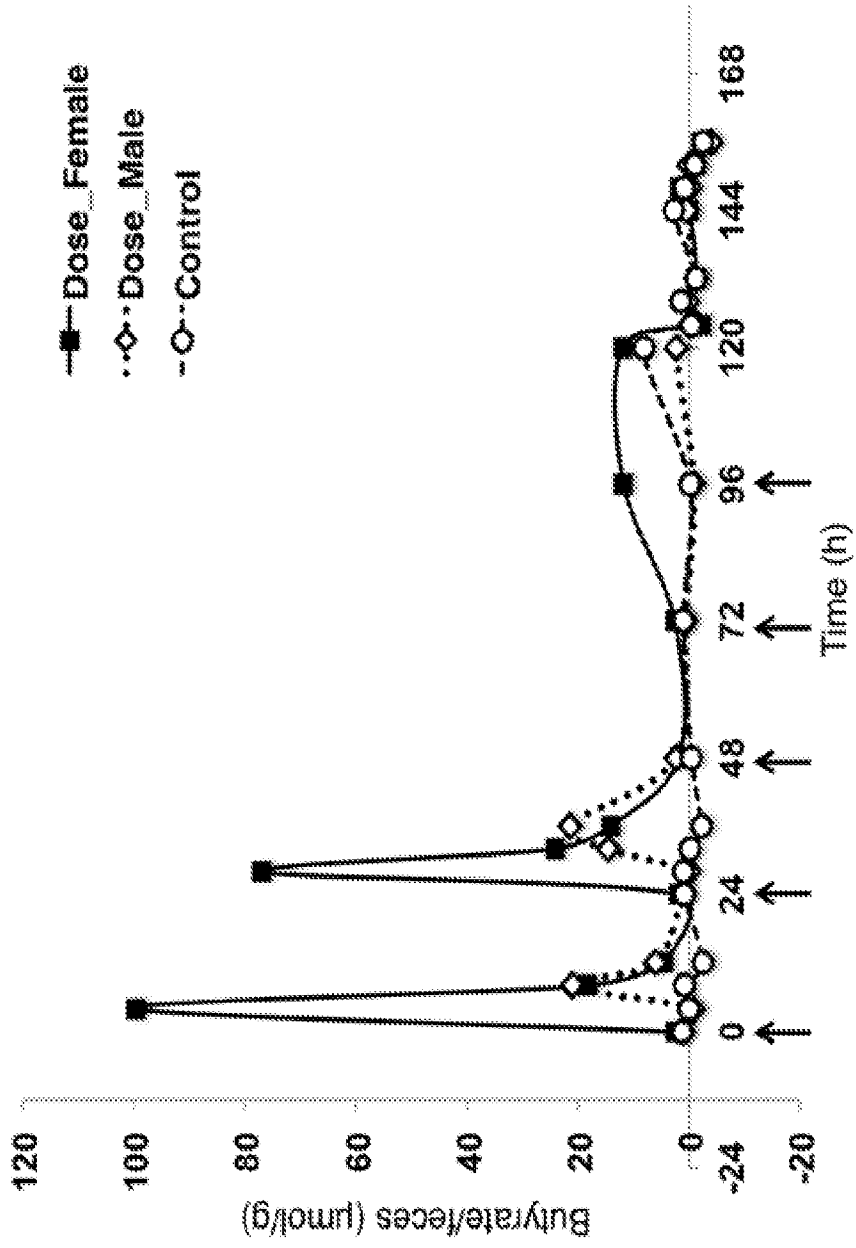


FIG. 13

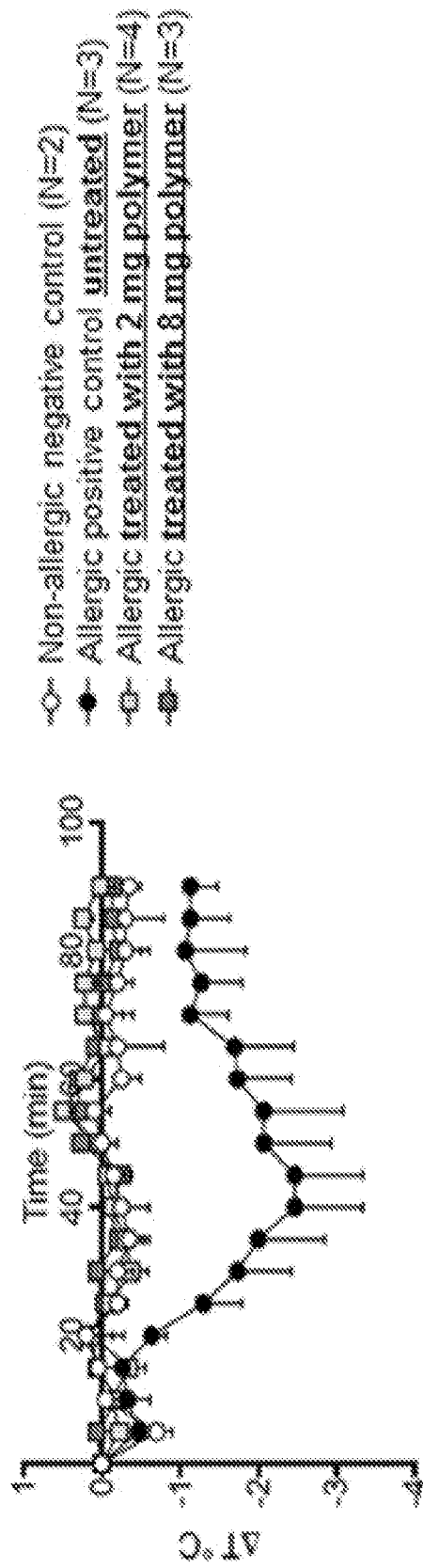




FIG. 14

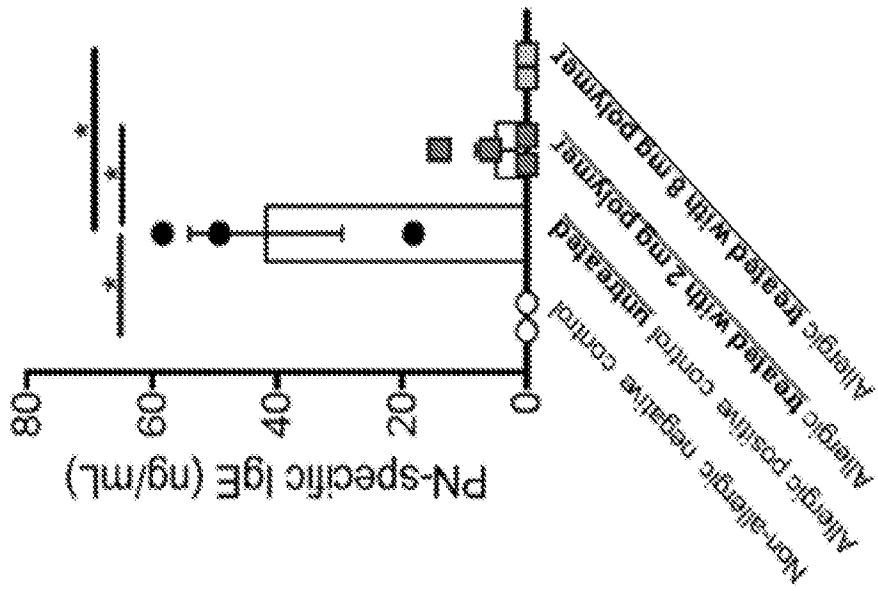


FIG. 15

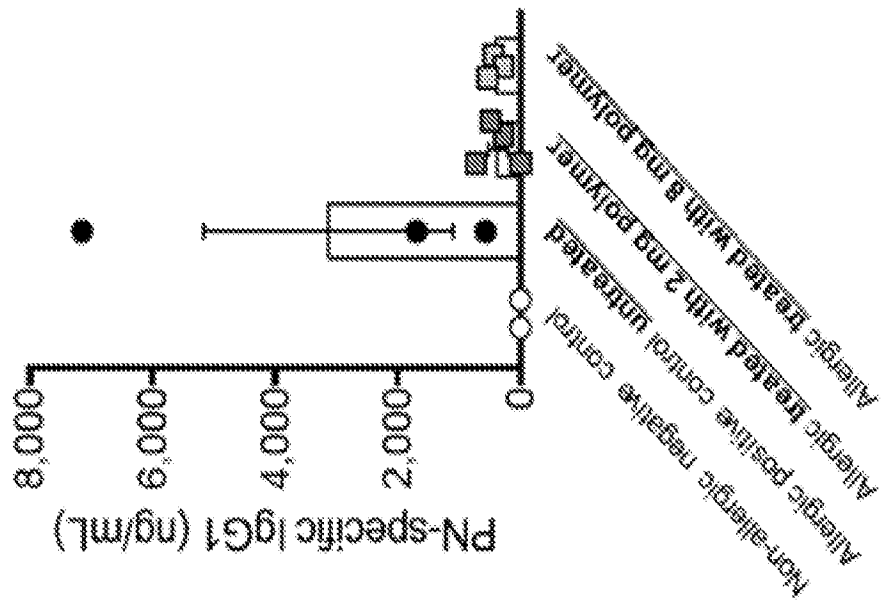


FIG. 16

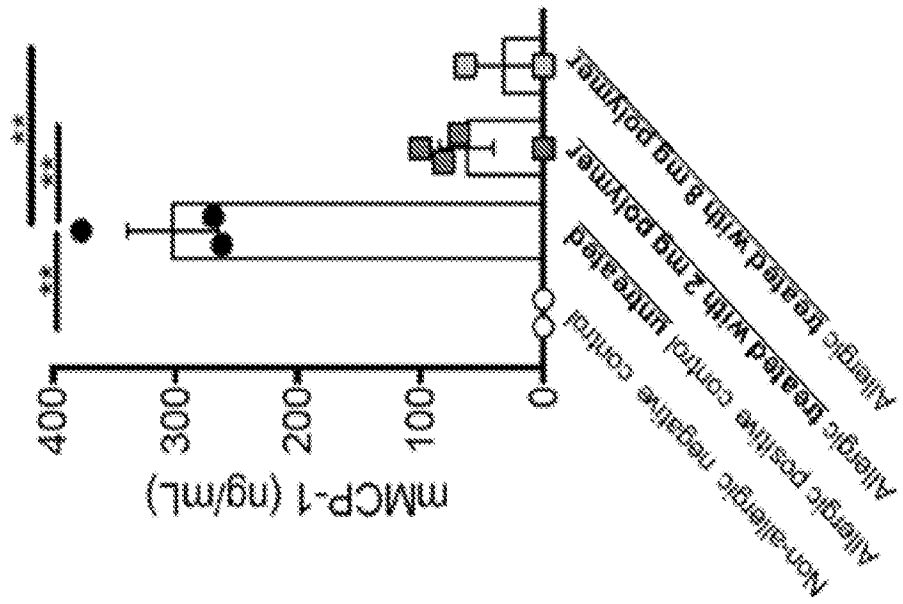


FIG. 17

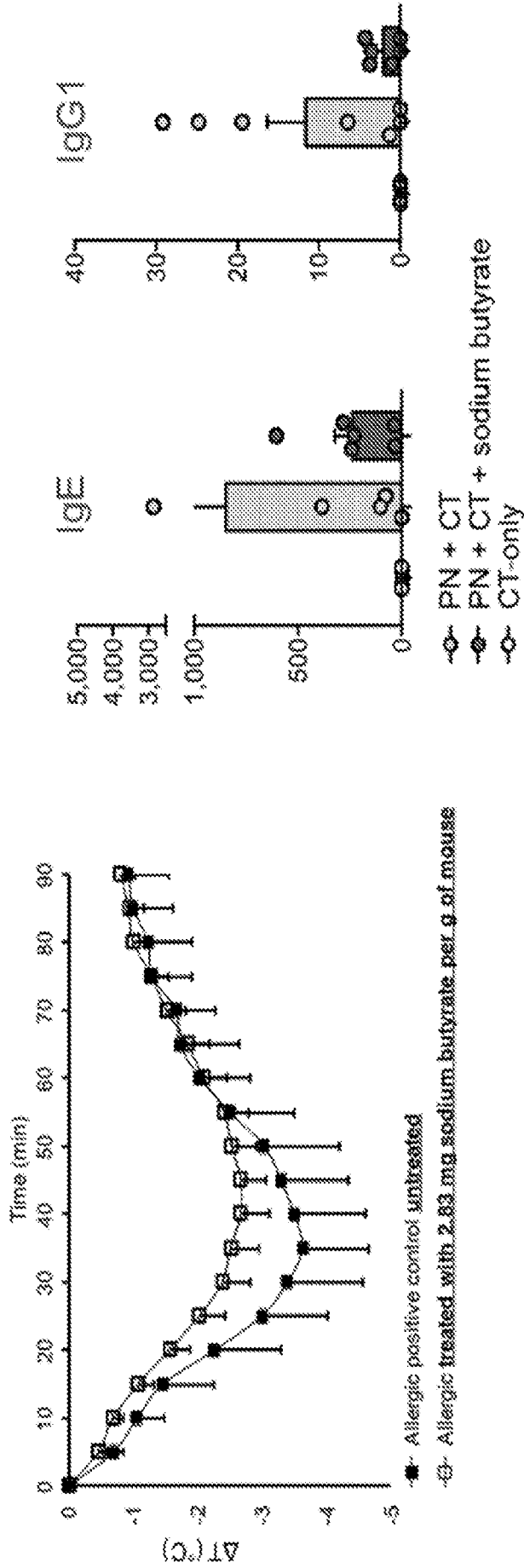


FIG. 18

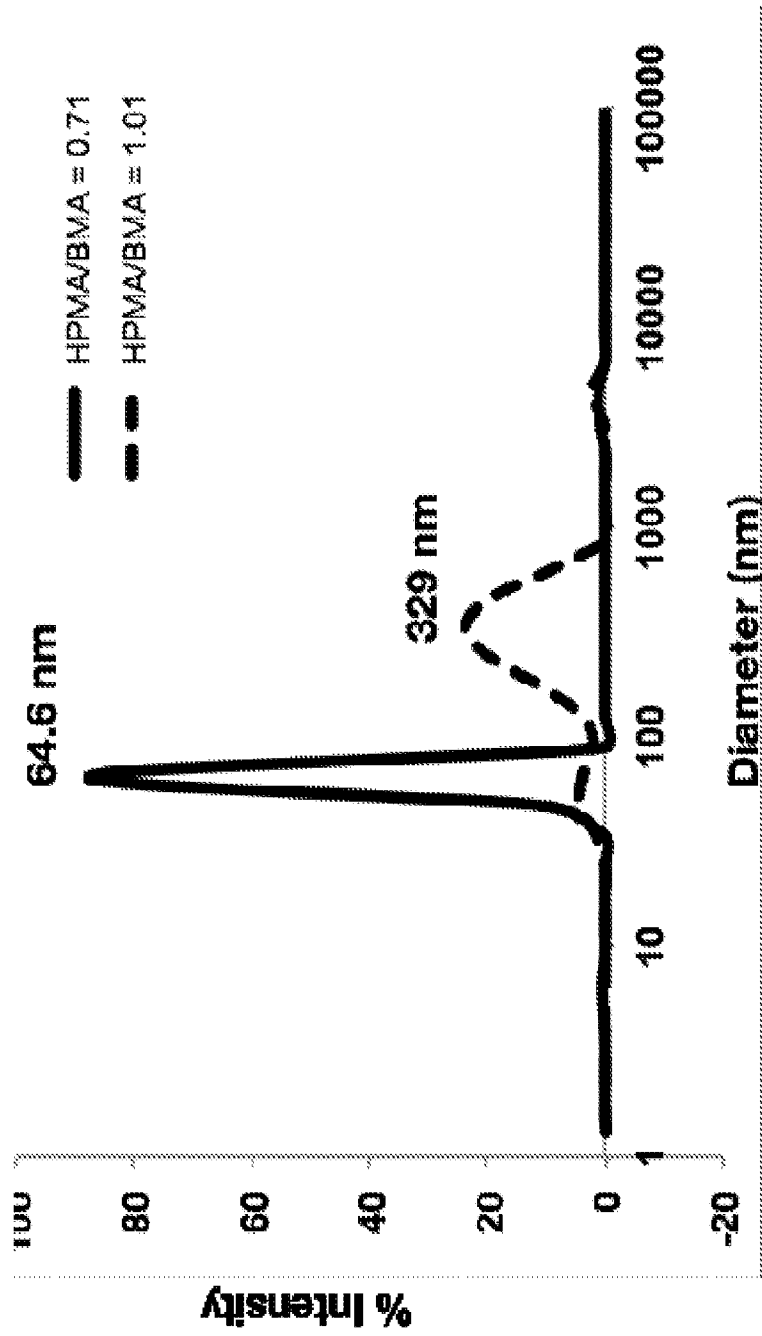


FIG. 19

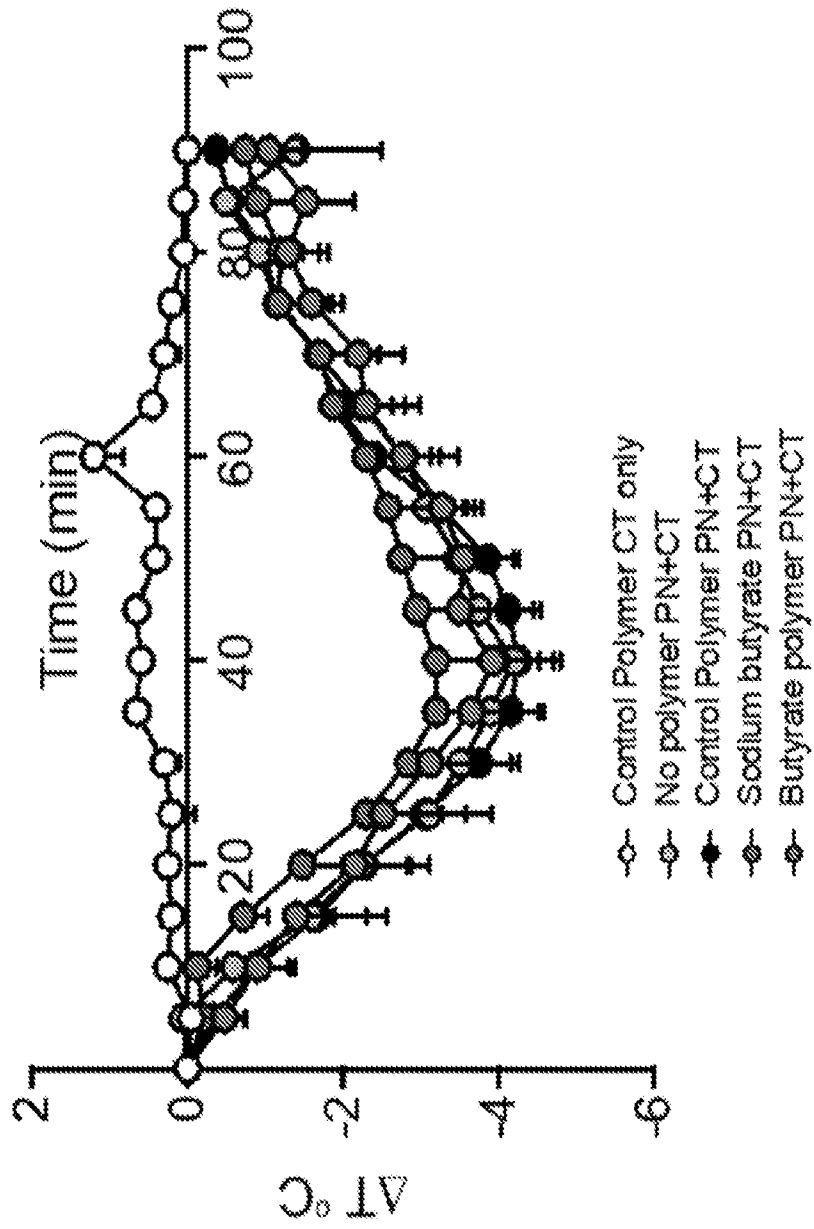
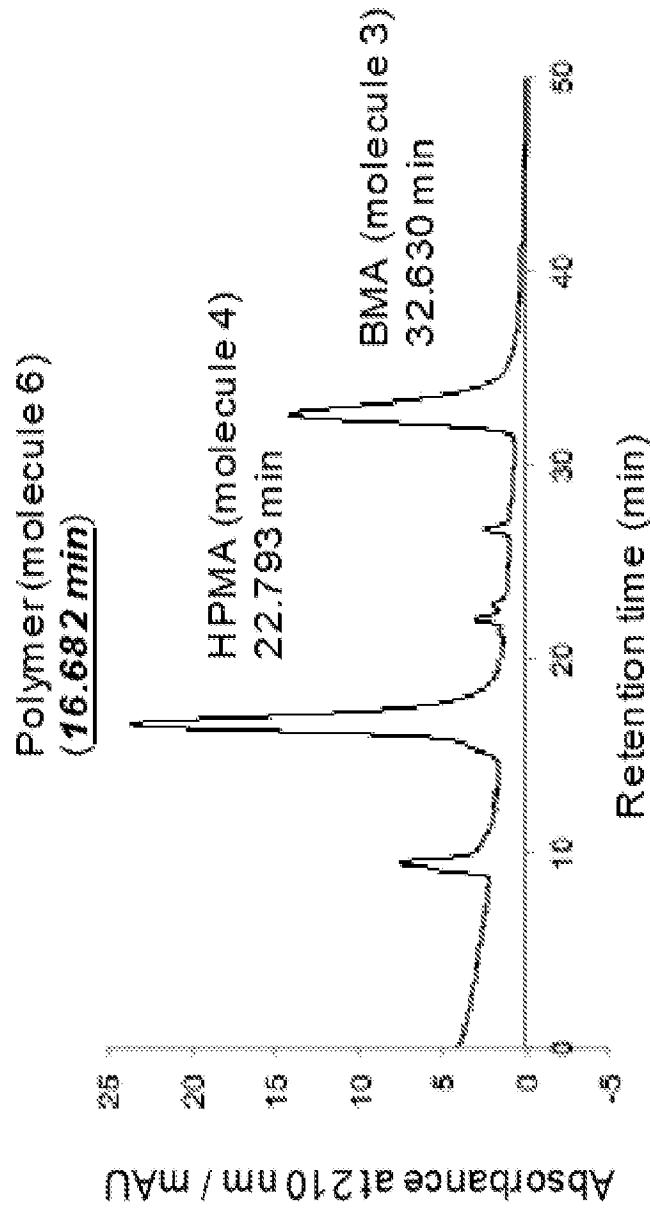


FIG. 20

	Formulation 1	Formulation 3	Formulation 4
Molecular weight	GPC 38,487 g/mol NMR 40,782 g/mol	57,412 g/mol 52,101 g/mol	37,895 g/mol 38,888 g/mol
Ratio of active:inactive	# inactive unit 100 # active unit 133 total units 233 active:inactive ratio 0.75	123 173 296 0.71	97 126 225 0.77
Particle size	60-70 nm	120-130 nm	75-82 nm
Appearance	Light pink solution	Cloudy, opaque, white solution	Light pink solution

FIG. 21A





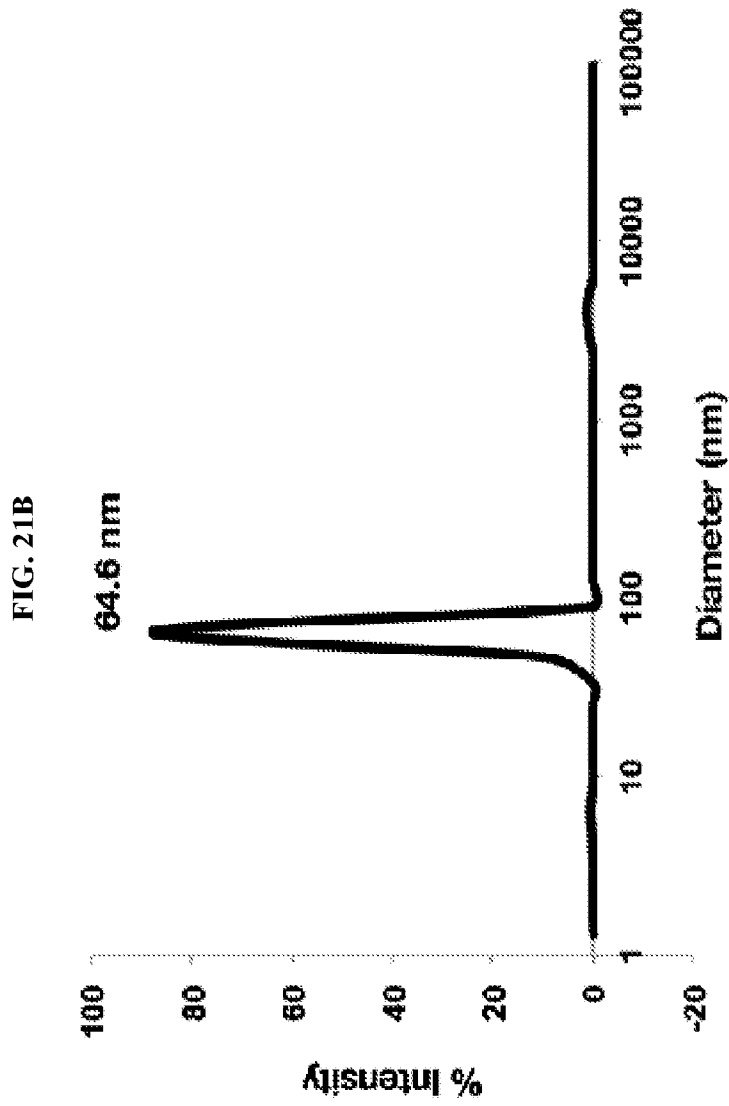




FIG. 22A

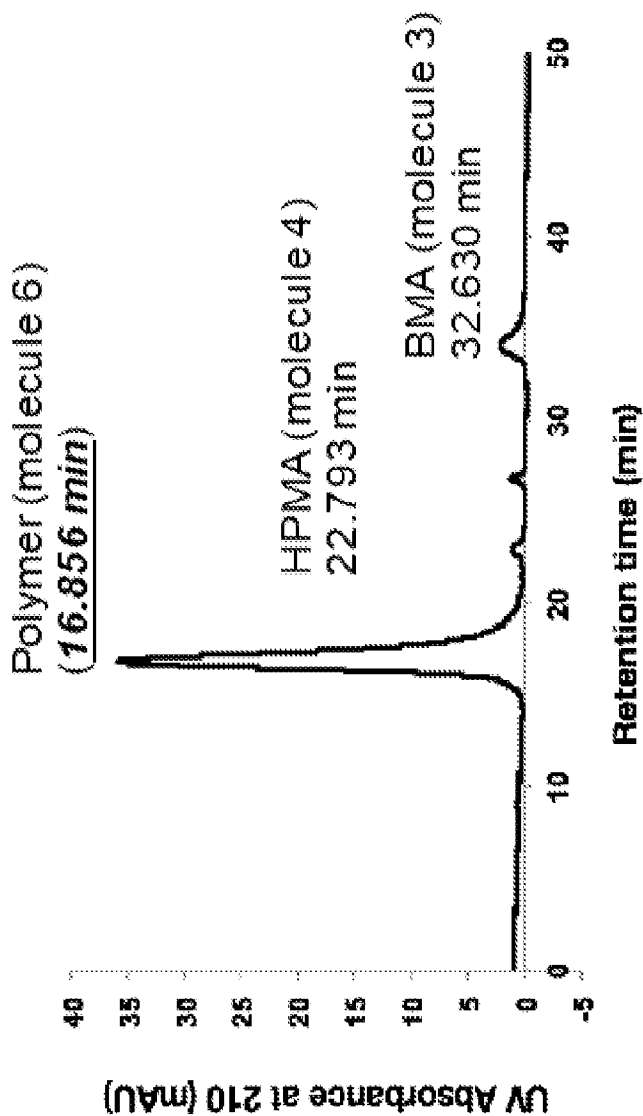


FIG. 22B

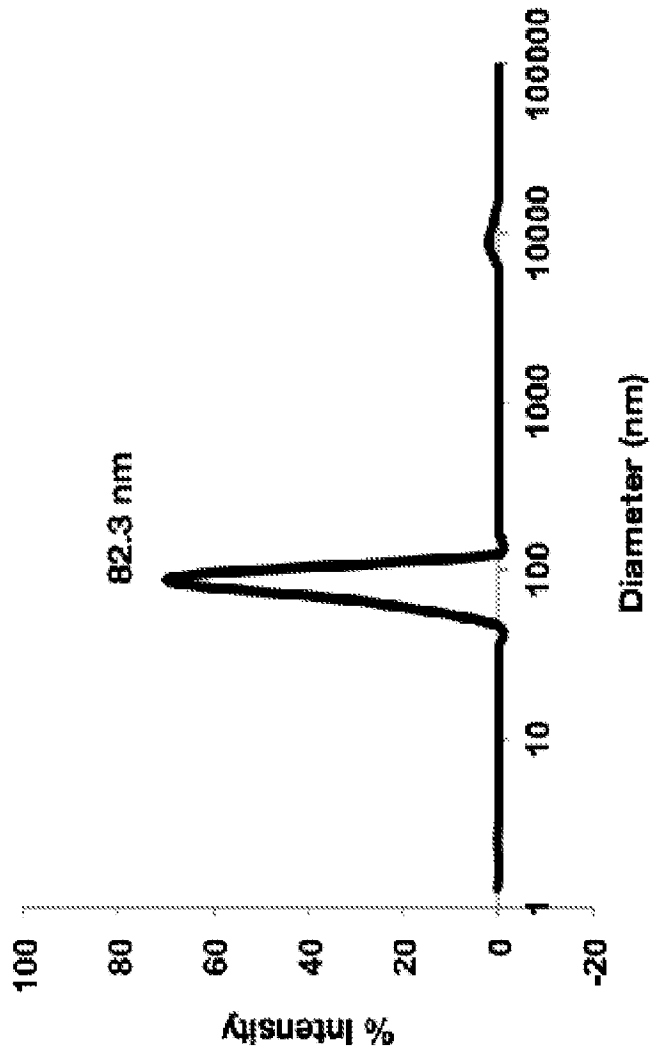


FIG. 22C

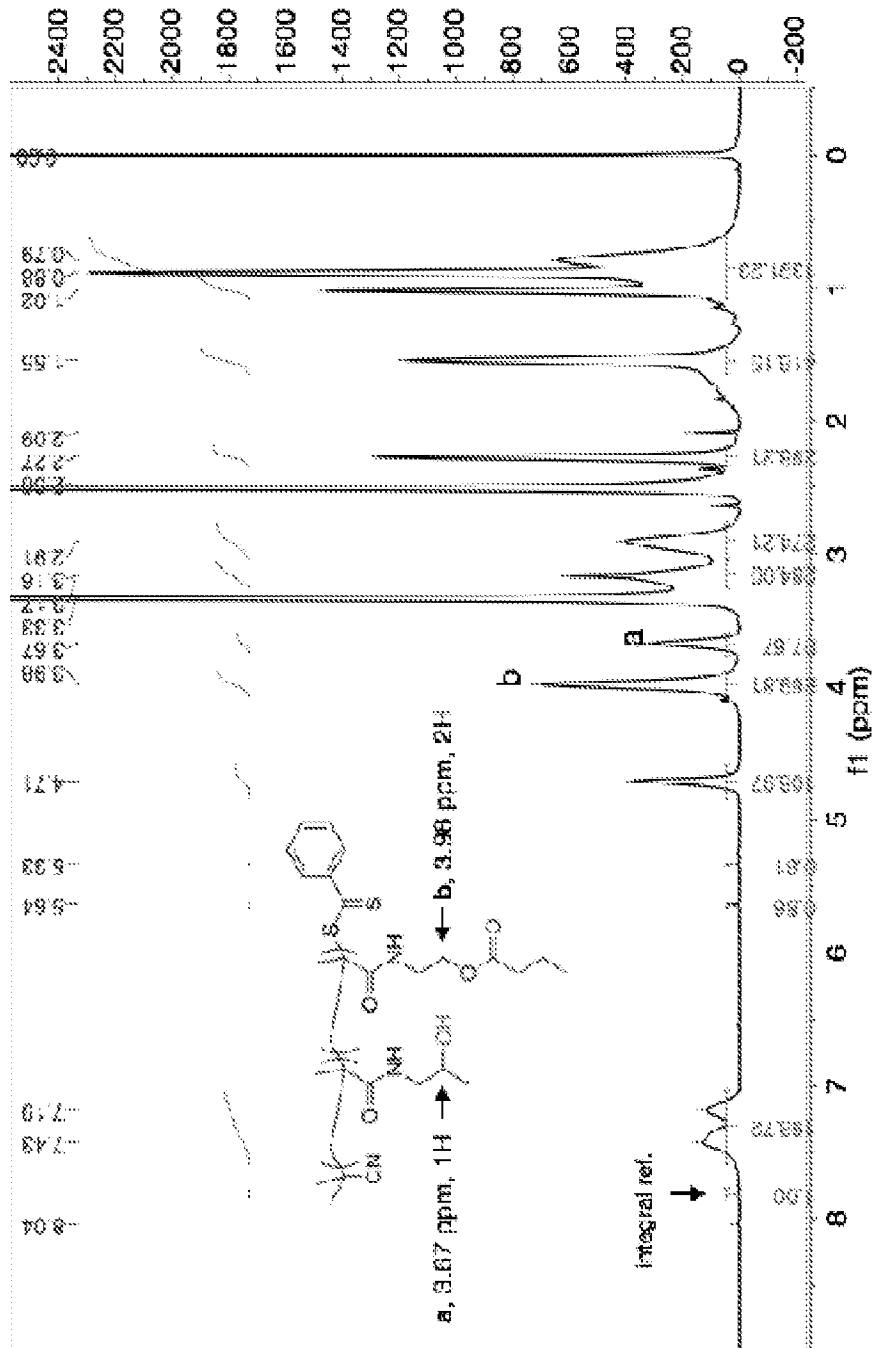


FIG. 23A

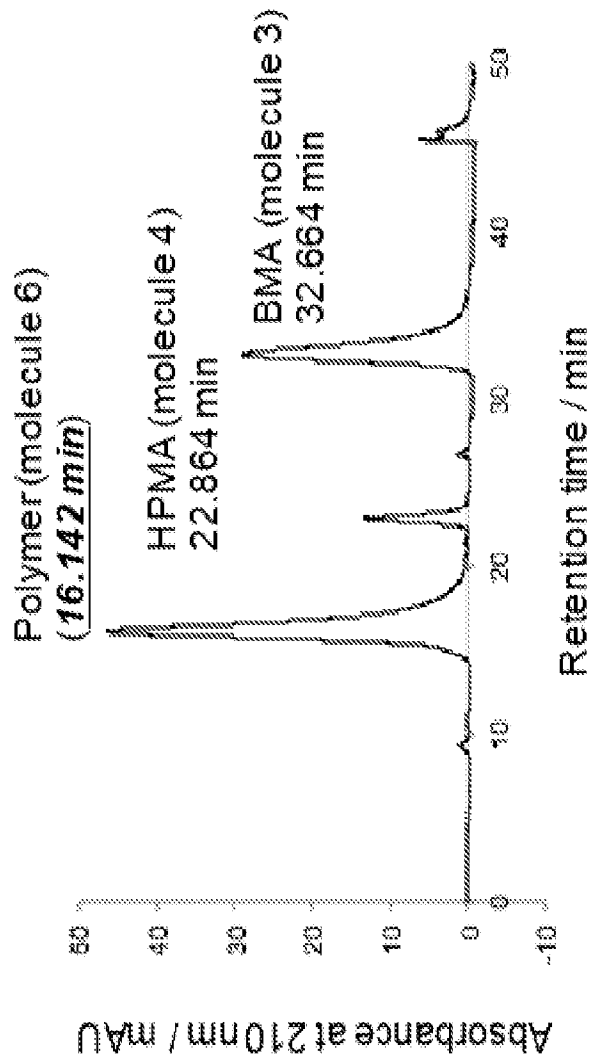


FIG. 23B

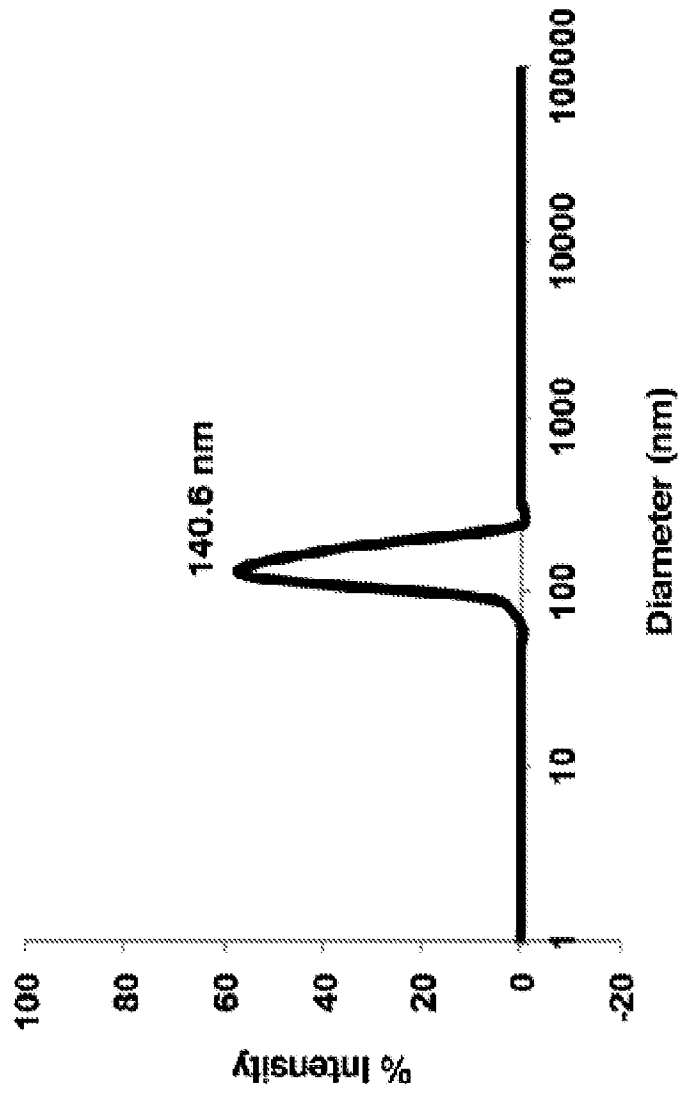


FIG. 23C

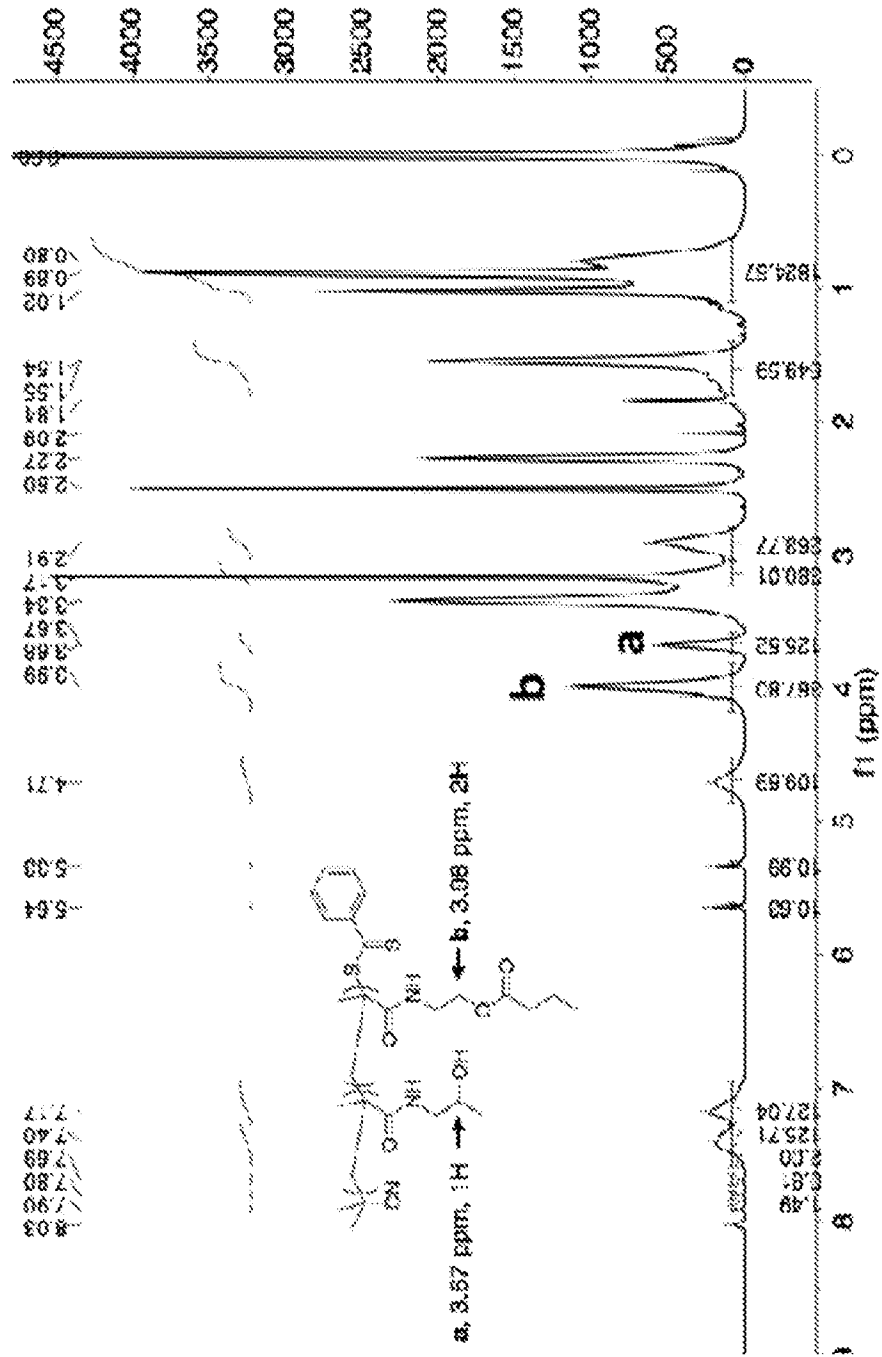
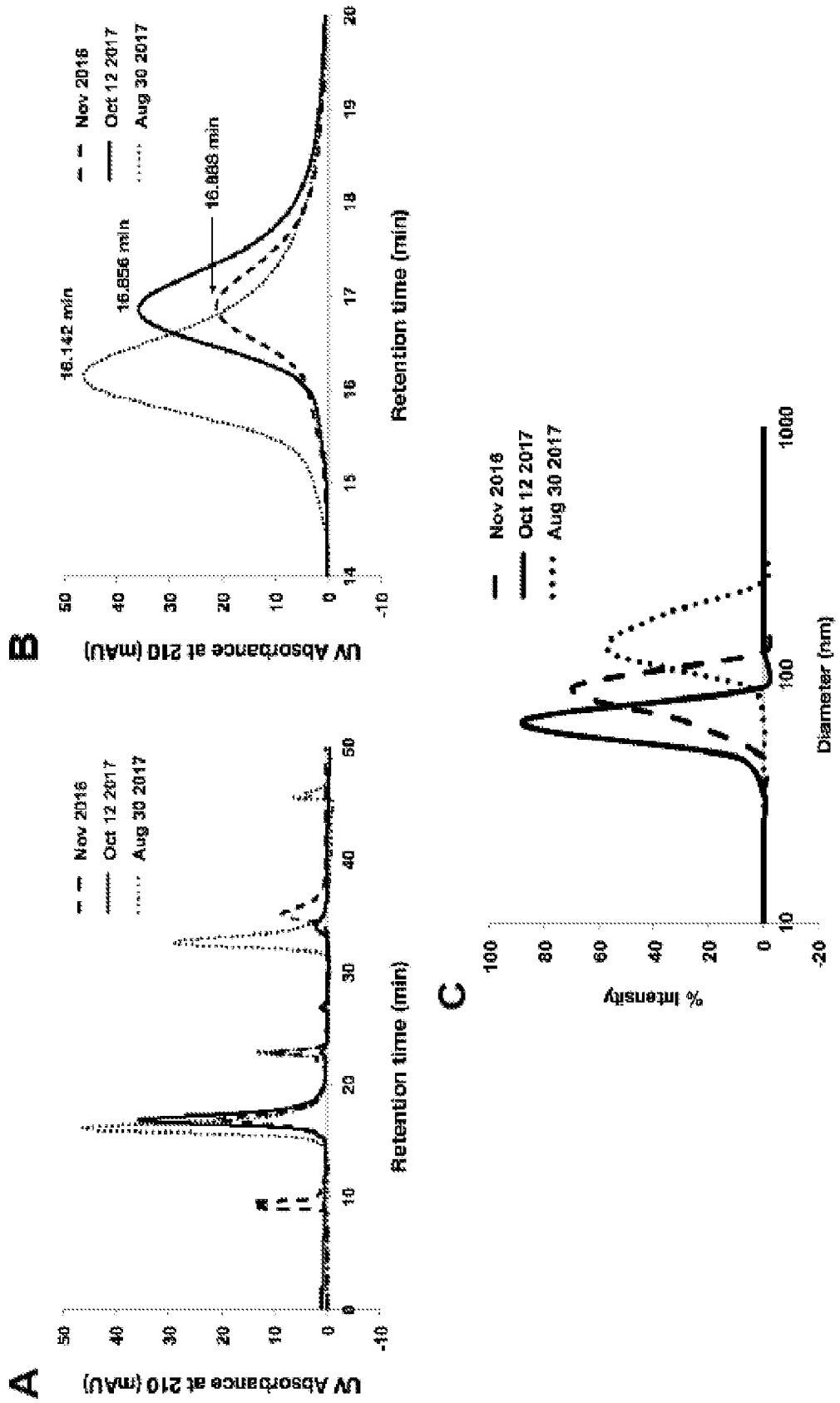




FIG. 24



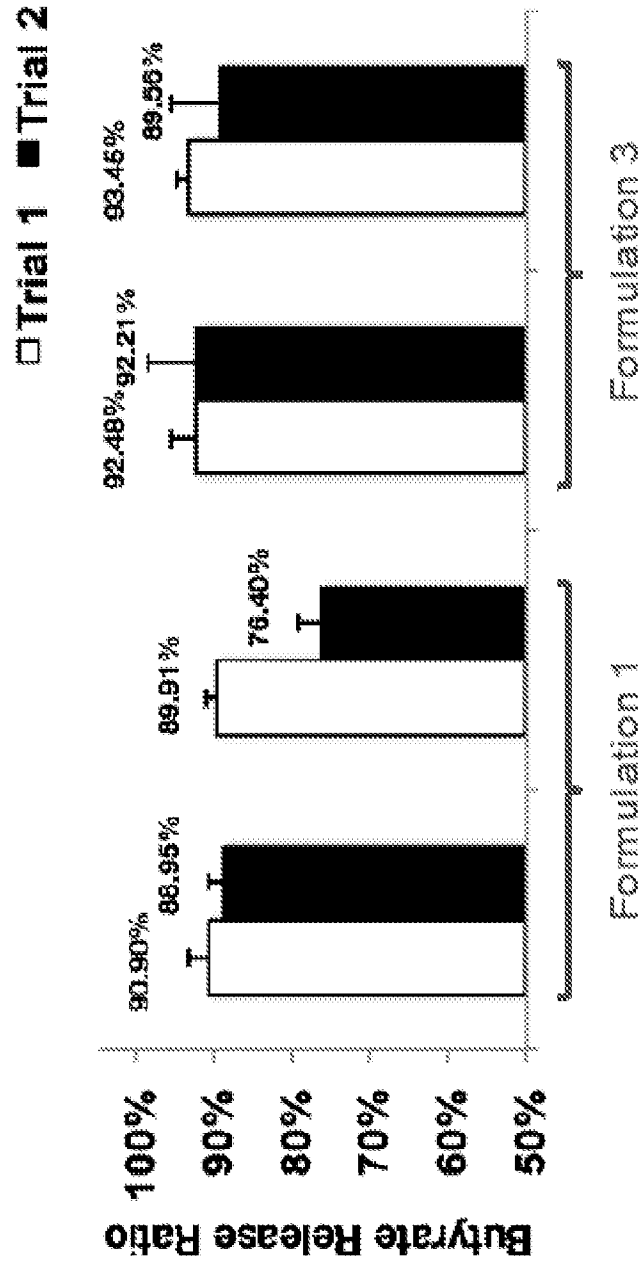
**FIG. 25**



From left to right:

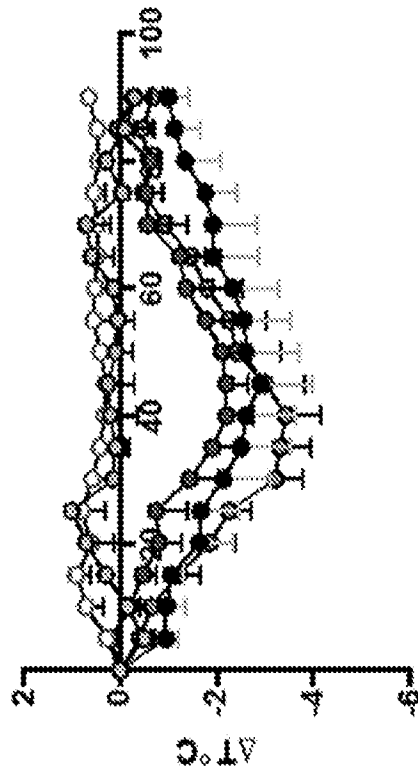
1. Formulation 4 (lot 1); light pink solution
2. Formulation 4 (lot 2); light pink solution
3. Formulation 1; light pink solution
4. Formulation 3; opaque, cloudy, white suspension

FIG. 26



Filled = healthy male mice  
Unfilled = healthy female mice

FIG. 27A



- Non-allergic negative control (N=2)
- Allergic positive control untreated (N=2)
- Allergic treated with 0.8 mg of Formulation 3 per gram of mouse (N=5)
- Allergic treated with 0.283 mg of sodium butyrate per gram of mouse (N=3)
- ◇ Allergic treated with 0.283 mg of inactive polymer (non-butyrate containing) per gram of mouse (N=4)

FIG. 27B

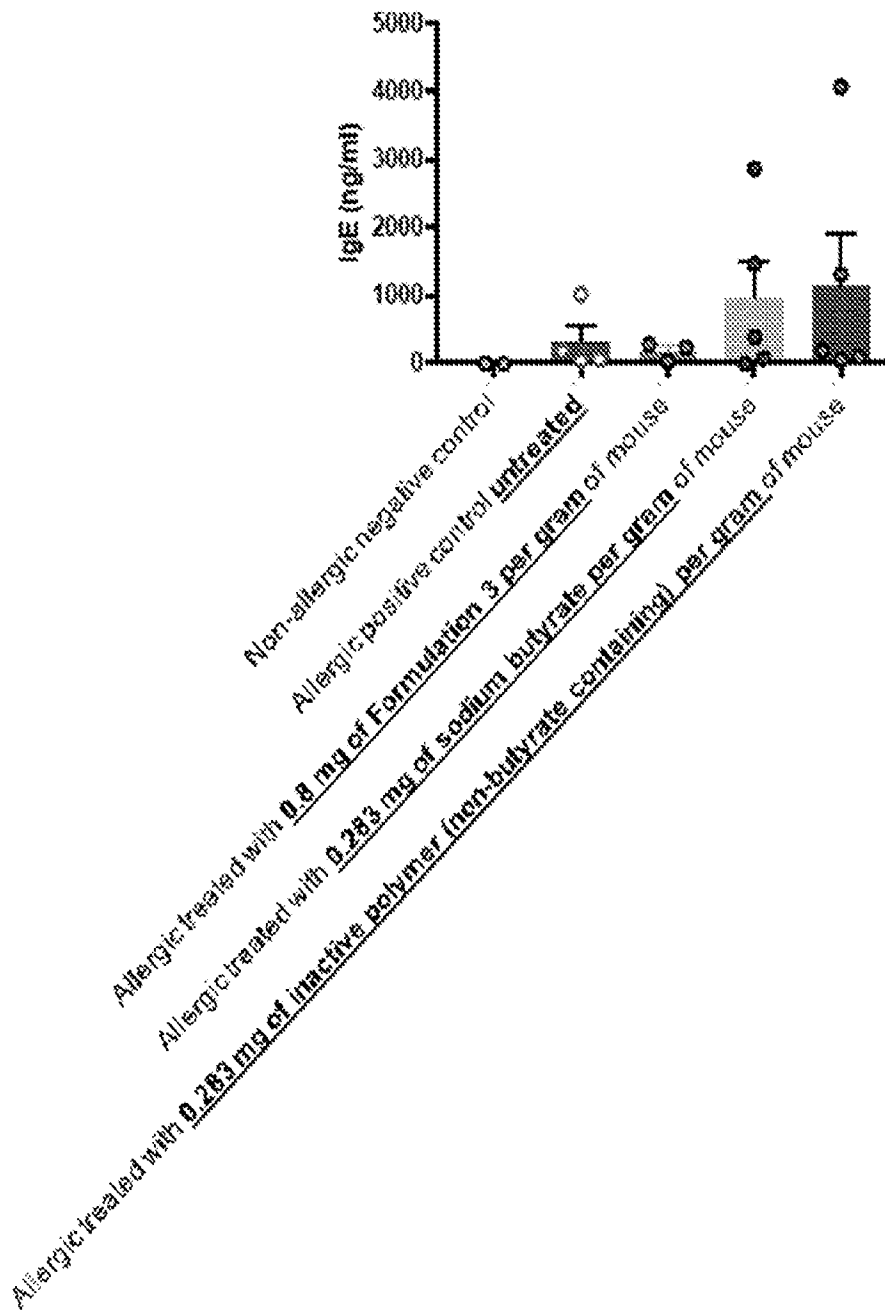


FIG. 27C

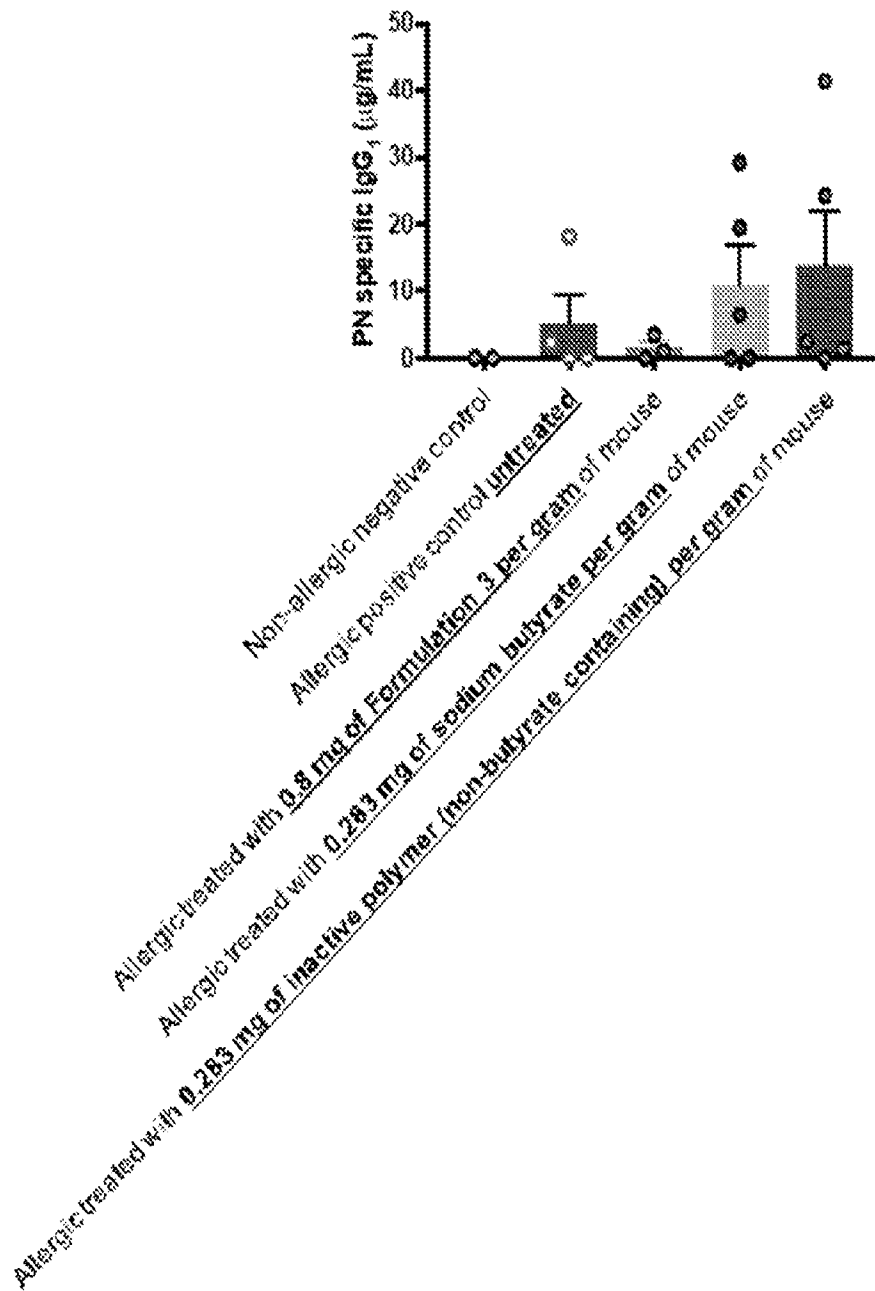
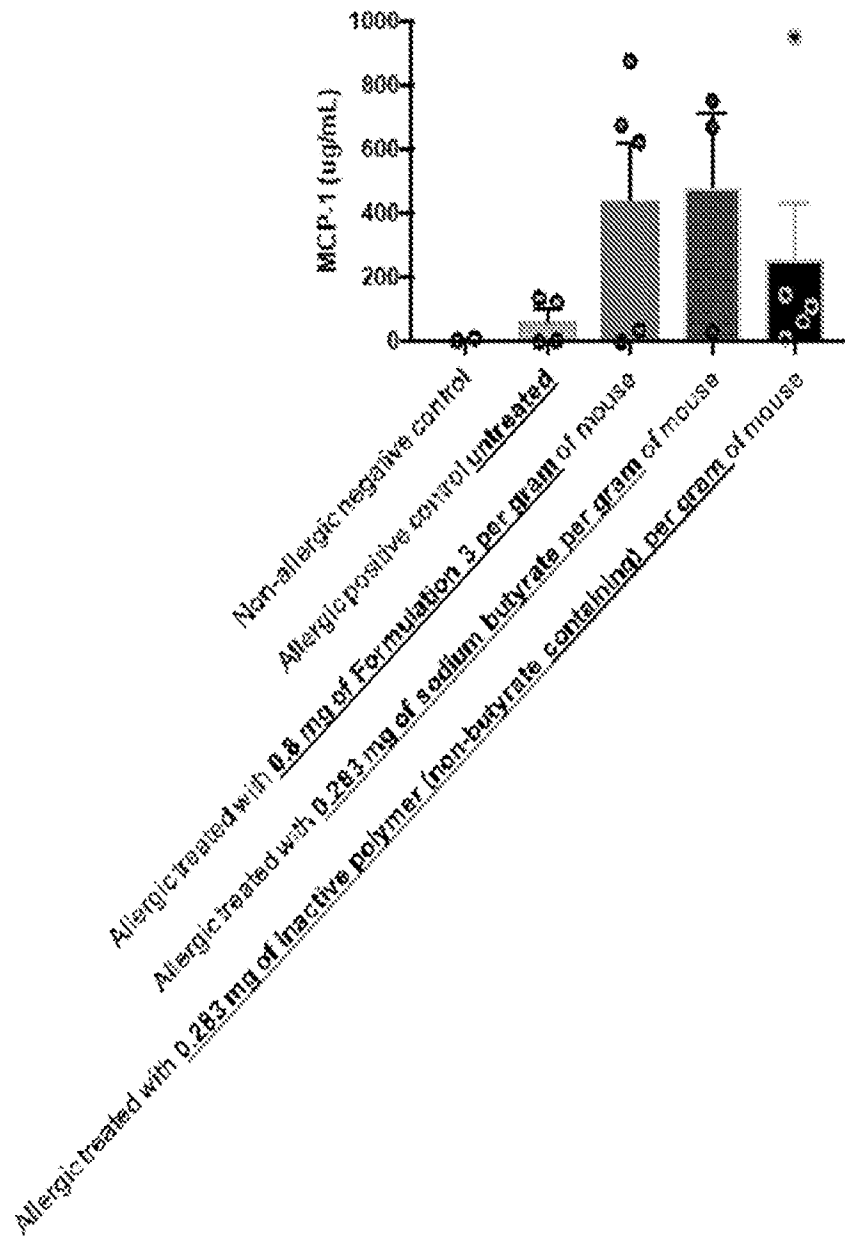


FIG. 27D



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/27955

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC(8) - C08F 8/00; A61K 47/58 (2018.01)  
 CPC - A61K 47/58; C08F 293/005; C08F 220/10; C08F 2438/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,054,722 A (YOSHIDA et al.) 18 October 1977 (18.10.1977) col 2, ln 18-54; col 4, ln 63 to col 5, ln 2; col 7, ln 31-66; col 8, ln 52-54	1-5, 7-10, 19-20 ----- 11-12, 21, (31-32)/(1-5,7-12,19-21)
Y	WO 2014/049363 A1 (THE UNIVERSITY OF SHEFFIELD) 03 April 2014 (03.04.2014) para [0001], [0025], Fig 9; para [0204]; Fig 16; pg 55	11-12, 21, (31-32)/(1-5,7-12,19-21)
A	WO 2016/025747 A1 (STAYTON et al.) 18 February 2016 (18.02.2016) Entire Document	1-5, 7-12, 19-21, (31-32)/(1-5,7-12,19-21)
A	US 5,698,611 A (OKADA et al.) 16 December 1997 (16.12.1997) Entire Document	1-5, 7-12, 19-21, (31-32)/(1-5,7-12,19-21)

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

27 August 2018

Date of mailing of the international search report

18 SEP 2018

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
 P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300  
 PCT OSP: 571-272-7774



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/27955

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3.  Claims Nos.: 33-37  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:  
Please see attached sheet--

1.  As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3.  As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 7-12, 19-21 and 31-32

**Remark on Protest**

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

## Attachment to Box.No.III:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: Claims 1-32 directed to a composition comprising a copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (II), wherein X is O, NH, or S; wherein L is a linker selected from an alkyl chain, an heteroalkyl chain, a substituted alkyl chain, or a substituted heteroalkyl chain; and wherein SCFA is a short-chain fatty acid. The copolymer composition will be searched to the extent that the copolymer encompasses a monomer of formula (II), wherein X is O; L is alkyl chain, which is unsubstituted and wherein the copolymer is a block copolymer of formula (IV). It is believed that claims 1-5, 7-12, 19-21 and 31-32 read on this first named invention, and thus these claims will be searched without fee to the extent that they encompass the first species of claim 1 described above. Applicant is invited to elect additional copolymer composition(s), wherein each additional composition elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Additionally, an exemplary election wherein different actual variables are selected is suggested. An exemplary election would be a composition comprising a block copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (II), wherein X is NH; L is unsubstituted alkyl chain (i.e., claims 1-5, 7-12, 15-17 and 31-32).

Group II: Claim 38, directed to a method of synthesizing a copolymer composition comprising a hydrophilic monomer and a monomer of formula (II).

Group III: Claim 39, directed to use of the copolymer composition comprising a hydrophilic monomer and a monomer of formula (II), for the treatment or prevention of a disease or condition.

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

## Special Technical Features:

Group I+ includes the technical feature of a unique copolymer, which is not required by any other invention of Group I+.  
Group II includes the technical feature of a method of synthesizing a copolymer, not required by Group I+ and III.  
Group III includes the technical feature of use of a copolymer for the treatment or prevention of a disease or condition, not required by Groups I+ and II.

## Common technical features:

The inventions of Group I+ share the technical feature of a composition comprising a copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (II).

Groups I+ and II-III, also share the technical feature of a composition comprising a copolymer of (i) a hydrophilic monomer and (ii) a monomer of formula (II).

This shared technical feature, however, does not provide a contribution over the prior art, as being anticipated by US 4,054,722 A to Yoshida et al. (hereinafter 'Yoshida'), which discloses a composition comprising a copolymer of (I) a hydrophilic monomer and (ii) a monomer of formula (II), wherein X is O; L is -CH<sub>2</sub>-CH<sub>2</sub>- and SCFA is acetic acid residue (col 7, ln 31-33; col 7, ln 45-46, 2-Hydroxyethyl acrylate/2-acetoxyethyl methacrylate copolymer (molar ratio 10.90); col 8, ln 64 to col 9, ln 6, dissolving them in solvents). As said copolymer composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions of Groups I+ or those of Groups I+-III.

The inventions of Group I+ further share the technical feature of a system comprising a supramolecular assembly of the copolymer composition, wherein the supramolecular assembly is a micelle or nanoparticle. This shared technical feature, however, does not provide a contribution over the prior art, as being obvious over Yoshida in view of WO 2016/025747 A1 to Stayton et al. published on 18 February 2016 (hereinafter 'Stayton'), as described below.

Yoshida discloses a copolymer composition as described above, but does not disclose a system comprising a supramolecular assembly of the copolymer composition. However, Stayton discloses an analogous copolymer composition comprising a block copolymer of (i) a hydrophilic monomer and (ii) a pH-responsive endosomal releasing block (pg 2, ln 15 to pg 3, ln 13), analogous in structure to formula (II), wherein a C<sub>4</sub>-alkyl replaces the -X-L-SCFA moiety of formula (II) (pg 4, ln 1-9, copolymer of butyl methacrylate (BMA) and diethylaminoethyl methacrylate (DEAMEA); Fig 1A), and further discloses a system comprising a supramolecular assembly of the copolymer composition, having therapeutic utility toward intracellular delivery of a biologically active moiety (pg 4, ln 1-13; Fig 1A-1C). Since both Yoshida and Stayton disclose copolymers having a hydrophilic monomer and a methacrylate fatty acid ester, it would have been obvious to one of ordinary skill in the art to produce a system comprising self-assembled nanoparticle assemblies as disclosed in Stayton, of the copolymer composition disclosed in Yoshida, through routine experimentation, in order to evaluate the capability of the resulting nanoparticles toward intracellular drug delivery.

As said system was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions of Groups I+.

The inventions of Group I+-III thus lack unity under PCT Rule 13.

Note reg. item 4: Claims 33-37 are unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a). These claims are, therefore, not included in the above analysis.