Claims

1. Monodisperse superparamagnetic beads, having a core-shell structure, said bead comprising:

a core portion formed from a polystyrene polymeric matrix material, where the polystyrene polymeric matrix material encapsulates a first batch of superparamagnetic Fe_3O_4 nanoparticles;

a first shell portion located directly on top of the core portion and formed from a crosslinked polymeric matrix material that is formed from a styrene monomer, a crosslinking monomer and a first functional monomer that has functional groups, where the copolymeric matrix material encapsulates a second batch of superparamagnetic Fe_3O_4 nanoparticles; and

a second shell portion located directly on top of the first shell portion, which is formed as a first layer, a second layer and a third layer,

the first layer comprises superparamagnetic Fe_3O_4 nanoparticles and a first layer polymeric matrix material that comprises a conjugating monomer and a bulk monomer;

a second layer extending beyond the first layer, which is formed from a second layer polymeric material that comprises a bulk monomer; and

a third layer on top of the second layer, which is formed from a third layer polymeric material that comprises a bulk monomer and a second functional monomer that has functional groups, wherein:

the superparamagnetic Fe_3O_4 nanoparticles in the first layer are directly bound to the functional groups present on an outer surface of the first shell portion;

the first layer polymeric matrix material surrounds the superparamagnetic Fe_3O_4 nanoparticles in the first layer; and

the second layer polymeric matrix material extends from the first layer polymeric matrix material and forms an outer surface of each monodisperse superparamagnetic bead.

2. The beads according to Claim 1, wherein the second and third layer polymeric matrix materials function to prevent the superparamagnetic Fe_3O_4 nanoparticles from leaching out when the beads are placed into a solvent.

3. The beads according to Claim 1 or Claim 2, wherein the functional groups on the functional monomer in the first shell portion and the third layer of the second shell portion are independently selected from one or more of amino, carboxyl, epoxy, and hydroxyl, optionally wherein the functional groups on the functional monomer in the first shell portion and the

second layer of the second shell portion are independently selected from a combination of hydroxyl and carboxyl groups or a combination of amino and carboxyl groups.

4. The beads according to any one of the preceding claims, wherein one or both of the following apply:
(a) the beads have a coefficient of variation based on their diameter of less than 15%, such as less than 10%, such as less than 5%, optionally wherein the beads have a coefficient of variation based on their diameter of less than or equal to 2%; and
(b) the beads have an average diameter of from 0.2 to 5.0 μm, such as from 0.5 to 4.0 μm.

5. The beads according to any one of the preceding claims, wherein the polystyrene polymeric matrix material is formed from one or more of the group consisting of styrene, a styrene derivative, and copolymers thereof, optionally wherein the styrene derivative is selected from one or more of the group consisting of 4-methylstyrene, 3-methylstyrene, and 4-tertbutylstyrene.

6. The beads according to any one of the preceding claims, wherein all of the superparamagnetic Fe_3O_4 nanoparticles in a bead account for from 10 to 80 wt%, such as from 20 to 70 wt%, such as from 30 to 50 wt% of the entire weight of each bead, optionally wherein:
(a) the first batch of superparamagnetic Fe_3O_4 nanoparticles represents from 0.1 to 5 wt% of the entire weight of each bead; and/or
(b) the second batch of superparamagnetic Fe_3O_4 nanoparticles represents from 0.5 to 10 wt% of the entire weight of each bead; and/or
(c) the superparamagnetic Fe_3O_4 nanoparticles in the first layer of the second shell portion represent from 9.4 to 79.4 wt%, such as from 19.4 to 69.4 wt%, such as from 29.4 to 49.4 wt% of the entire weight of each bead.

7. The beads according to any one of the preceding claims, wherein one or more of the following apply:
(a) the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, butanediol dimethacrylate, tricyclodecane dimethanol diacrylate, pentaerythritol triacrylate, tripropylene glycol diacrylate, propoxylated neopentyl diacrylate, pentaerythritol triacrylate, ditrimethylolpropane tetraacrylate, dipentaerythritol pentaacrylate, dipentaerythritol penta/hexa-acrylate, tripropylene diacrylate, trimethylol propane ethoxylate triacrylate,

trimethylol propane propoxylate triacrylate, di(trimethylolpropane) tetraacrylate, glycerol propoxylate triacrylate, pentaerythritol propoxylate triacrylate, poly(ethylene glycol) diacrylate, poly(propylene glycol) diacrylate, and tri(propylene glycol) diacrylate, optionally wherein the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, and N,N′-methylenebis(acrylamide);

(b) the first functional monomer is selected from one or more of the group consisting of acrylic acid, methacrylic acid, 2-carboxyethyl acrylate, acrylamide, methacrylamide, allylamine, (hydroxyethyl)methacrylate, hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, glycidyl methacrylate, allyl glycidyl ether, 1,2-epoxy-5-hexene, maleic anhydride, 2-hydroxyethyl methacrylate, and 2-carboxyethyl acrylate oligomers;

(c) the styrene monomer is selected from one or more of the group consisting of styrene, and a styrene derivative, optionally wherein the styrene derivative is selected from one or more of the group consisting of 4-methylstyrene, 3-methylstyrene, and 4-tertbutylstyrene;

(d) the first shell further comprises a first layer of a first polymeric matrix composition and a second layer of a second polymeric matrix composition, where:

the first layer is formed from a copolymer of a styrene monomer, a crosslinking monomer; and

the second layer is formed from a copolymer a styrene monomer, a crosslinking monomer and a functional monomer;

(e) the superparamagnetic Fe_3O_4 nanoparticles have an average diameter of from 5 to 15 nm;

(f) the weight to weight ratio of styrene groups to crosslinking groups in the core portion and first shell portion is from 20:1 to 1:2, such as from 10:1 to 1:1; and

(g) the weight to weight ratio of styrene groups to functional groups in the core portion and first shell portion is from 20:1 to 1:2, such as from 10:1 to 1:1.

8. The beads according to any one of the preceding claims, wherein one or more of the following apply:

(a) the Fe_3O_4 nanoparticles further comprise Co_3O_4 and/or Mn_3O_4 nanoparticles;

(b) the conjugating monomer is selected from one or more of the group consisting of methacryloyl chloride, 3-ethoxy-acryloyl chloride, acryloyl chloride, 4-pentenoyl chloride, methacrylic anhydride, 4-pentenoic anhydride, crotonic anhydride, valeric anhydride, 10-undecenoyl chloride, glycidol methacrylate, glycidol, and allyl glycidyl ether;

(c) the bulk monomer is selected from one or more of a polyether monomer, a polyester monomer, a polyacrylamide monomer, and a polyacid monomer, optionally wherein the bulk

monomer is selected from one or more of the group consisting of methyl methacrylate, acrylic acid, methacrylic acid, 2-carboxyethyl acrylate, 2-carboxyethyl acrylate oligomers, acrylamide, methacrylamide, allylamine, (hydroxyethyl)methacrylate, hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, glycidyl methacrylate, allyl glycidyl ether, 1,2-epoxy-5-hexene, maleic anhydride, optionally wherein the bulk monomer is selected from methacrylic acid and/or 2-hydroxyethyl methacrylate;

(d) the second functional monomer is selected from one or more of the group consisting of one or more of the group consisting of acrylic acid, methacrylic acid, 2-carboxyethyl acrylate, acrylamide, methacrylamide, allylamine, (hydroxyethyl)methacrylate, hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, glycidyl methacrylate, allyl glycidyl ether, 1,2-epoxy-5-hexene, maleic anhydride, 2-hydroxyethyl methacrylate, and 2-carboxyethyl acrylate oligomers;

(e) the second shell portion further comprises a crosslinking monomer in the first and/or second and/or third layer, optionally wherein the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, N,N′-methylenebis(acrylamide), bisphenol A epoxy acrylate, bisphenol diacrylate, tripropylene glycol diacrylate, propoxylated neopentyl diacrylate, propoxylated glycerol diacrylate, pentaerythritol propoxylate triacrylate, butandiol dimethacrylate, tricyclodecane dimethanol diacrylate, pentaerythritol triacrylate, ditrimethylolpropane tetraacrylate, dipentaerythritol penta/hexa-acrylate, tripropylene diacrylate, trimethylol propane ethoxylate triacrylate, trimethylol propane propoxylate triacrylate, di(trimethylolpropane) tetraacrylate, glycerol propoxylate triacrylate, poly(ethylene glycol) diacrylate, poly(propylene glycol) diacrylate, and tri(propylene glycol) diacrylate, for example the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, and N,N′-methylenebis(acrylamide); and

(f) the combined weight of the second and third layer of the second shell accounts for from 1-30 wt%, preferably 2-20 wt% of the entire weight of each bead.

9. A method of preparing monodisperse superparamagnetic beads, having a core-shell structure, the method comprising:

(a) providing monodisperse superparamagnetic precursor beads, which comprise

a core portion formed from a polystyrene polymeric matrix material, where the polystyrene polymeric matrix material encapsulates a first batch of superparamagnetic Fe_3O_4 nanoparticles;

a first shell portion located directly on top of the core portion and formed from a crosslinked polymeric matrix material that is formed from a styrene monomer, a crosslinking monomer and a first functional monomer that has functional groups, where the copolymeric matrix material encapsulates a second batch of superparamagnetic Fe_3O_4 nanoparticles; and

a second shell portion located directly on top of the first shell portion, which comprises superparamagnetic Fe_3O_4 nanoparticles and polymeric precursor anchor points, both which are bound to the functional groups on a surface of the first shell portion; and

(b) forming a functional coating layer on the monodisperse superparamagnetic precursor beads by a one-pot free radical polymerization, which:

(i) in a first step uses a bulk monomer; and

(ii) in a second step uses a second functional monomer that has functional groups to form the monodisperse superparamagnetic beads, wherein

the functional coating layer is bound to the first shell portion by way of the polymeric precursor anchor points.

10. The process according to Claim 9, wherein the bulk monomer is selected from one or more of the group consisting of a polyether monomer, a polyester monomer, a polyacrylamide monomer, and polyacid monomer.

11. The process according to Claim 10, wherein the bulk monomer is selected from one or more of the group consisting of methyl methacrylate, acrylic acid, methacrylic acid, 2-carboxyethyl acrylate, 2-carboxyethyl acrylate oligomers, acrylamide, methacrylamide, allylamine, (hydroxyethyl)methacrylate, hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, glycidyl methacrylate, allyl glycidyl ether, 1,2-epoxy-5-hexene, maleic anhydride, optionally wherein the bulk monomer is selected from methacrylic acid and/or 2-hydroxyethyl methacrylate.

12. The process according to any one of Claims 9 to 11, wherein the first step uses a mixture comprising the bulk monomer and an initiator, optionally wherein the initiator is selected from one or more of the groups consisting of tertiary-amyl hydroperoxide, potassium persulfate, sodium persulfate, ammonia persulfate, 4,4'-azobis(4-cyanovaleric acid), 2,2'-azobis[2-methyl-N-(2-hydroxyethyl)propionamide, 2,2'-azobis[N-(2-carboxyethyl)-2-methylpropionamidine]hydrate, 2,2′-azobis(2-methylpropionamidine) dihydrochloride, 2,2''-azobis[2-(2-imidazolin-2-yl)propane], and 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride, optionally wherein the mixture in the first step further comprises:

(i) a crosslinker, optionally wherein the crosslinker is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, N,N′-methylenebis(acrylamide), bisphenol A epoxy acrylate, bisphenol diacrylate, tripropylene glycol diacrylate, propoxylated neopentyl diacrylate, propoxylated glycerol diacrylate, pentaerythritol propoxylate triacrylate, butandiol dimethacrylate, tricyclodecane dimethanol diacrylate, pentaerythritol triacrylate, ditrimethylolpropane tetraacrylate, dipentaerythritol penta/hexa-acrylate, tripropylene diacrylate, trimethylol propane ethoxylate triacrylate, trimethylol propane propoxylate triacrylate, di(trimethylolpropane) tetraacrylate, glycerol propoxylate triacrylate, poly(ethylene glycol) diacrylate, poly(propylene glycol) diacrylate, and tri(propylene glycol) diacrylate, for example the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, and N,N′-methylenebis(acrylamide); and/or

(ii) a solvent, optionally wherein the solvent is selected from one or more of the group consisting of 1,4-dioxane, tetrahydrofuran, diglyme, ethyl acetate, butyl acetate, acetone, methyl ethyl ketone, and water.

13. The process according to any one of Claims 9 to 12, wherein one or more of the following apply:

(a) the second functional monomer is selected from one or more of the group consisting of one or more of the group consisting of acrylic acid, methacrylic acid, 2-carboxyethyl acrylate, acrylamide, methacrylamide, allylamine, (hydroxyethyl)methacrylate, hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, glycidyl methacrylate, allyl glycidyl ether, 1,2-epoxy-5-hexene, maleic anhydride, 2-hydroxyethyl methacrylate, and 2-carboxyethyl acrylate oligomers;

(b) the polymerization of the first and/or second step is conducted at a temperature of from 30 to 80 °C, such as from 50 to 70 °C; and

(c) the total polymerization time for the first and second step is from 10 to 30 hours, such as from 16 to 24 hours.

14. The process according to any one of Claims 9 to 13, wherein the monodisperse superparamagnetic precursor beads are formed by a process that comprises:

(i) providing naked monodisperse superparamagnetic beads, which comprise

a core portion formed from a polystyrene polymeric matrix material, where the polystyrene polymeric matrix material encapsulates a first batch of superparamagnetic Fe_3O_4 nanoparticles;

a first shell portion located directly on top of the core portion and formed from a crosslinked polymeric matrix material that is formed from a styrene monomer, a crosslinking monomer and a first functional monomer that has functional groups, where the copolymeric matrix material encapsulates a second batch of superparamagnetic Fe_3O_4 nanoparticles; and

a second shell portion located directly on top of the first shell portion, which comprises superparamagnetic Fe_3O_4 nanoparticles which is bound to the functional groups on a surface of the first shell portion; and

(ii) forming the monodisperse superparamagnetic precursor beads by forming polymeric precursor anchor points on the first shell portion by reacting the naked monodisperse superparamagnetic beads with an anchoring material selected from one or more of the group consisting of methacryloyl chloride, 3-ethoxy-acryloyl chloride, acryloyl chloride, 4-pentenoyl chloride, methacrylic anhydride, 4-pentenoic anhydride, crotonic anhydride, valeric anhydride, 10-undecenoyl chloride, glycidol methacrylate, glycidol, and allyl glycidyl ether.

15. The process according to Claim 14, wherein the naked monodisperse superparamagnetic beads are formed by a process that comprises:

(ai) providing monodisperse beads, which comprise:

a core portion formed from a polystyrene polymeric matrix material; and

a first shell portion located directly on top of the core portion and formed from a crosslinked polymeric matrix material that is formed from a styrene monomer, a crosslinking monomer and a first functional monomer that has functional groups; and

(aii) forming the naked monodisperse superparamagnetic beads by placing the monodisperse beads into a solution that comprises a Fe(III) salt and a Fe(II) salt and adding a base, optionally wherein one or more of the following apply:

(a) the Fe(III) salt is selected from $FeCl_3$ and/or $Fe_2(SO_4)_3$;

(b) the Fe(II) salt is selected from one or more of the group consisting of $FeCl_2$, $FeSO_4$, $Fe(OAC)_2$;

(c) the base is selected from one or more of the group consisting of ammonia hydroxide, NaOH, KOH, and amine; and

(d) the solution further comprises $CoCl_2$ and/or $MnCl_2$.

16. The process according to Claim 15, wherein the monodisperse beads are formed by a “one-pot three-stage” continuous process, which process comprises:

(a) in a first stage, generating a polystyrene core by dispersion polymerization of a styrene monomer with an initiator and a polymer stabilizer in a mixture of alcohol with water to form nucleated polystyrene cores;

(b) in a second stage, adding a crosslinking monomer to mixture comprising nucleated polystyrene cores; and

(c) in a third stage adding a first functional monomer to the material obtained from the second stage to provide the monodisperse beads, optionally wherein the polystyrene beads are generated *in situ*, and the subsequent addition of crosslinker and functional monomers do not cause a second nucleation, thus leading to spherical monodisperse copolymer beads of (styrene / crosslinker / functional monomer) bearing functional groups on a surface, optionally wherein one or more of the following apply:

(i) the initiator is selected from an azo initiator, optionally wherein the azo initiator is selected from one or more of the group consisting of 2,2′ -azobis(2-methylpropionitrile) (AIBN), and 2,2′ -azobis(2-methylbutyronitrile) (AMBN);

(ii) the polymer stabilizer is selected from one or more of the group consisting of poly(vinylpyrrolidone) (PVP), polyethylenimine (PEI), polyacrylic acid (PAA), polyvinyl alcohol(PVA), hydroxypropyl methylcellulose (HPC), and chitosan;

(iii) the styrene monomer is selected from one or more of the group consisting of styrene, and a styrene derivative, optionally wherein the styrene derivative is selected from one or more of the group consisting of 4-methylstyrene, 3-methylstyrene, and 4-tertbutylstyrene;

(iv) the alcohol is selected from one or more of the group consisting of methanol, ethanol, isopropanol, or a mixture of them;

(v) the volume ratio of alcohol to water is from 1:1 to 40:1, such as from 2:1 to 20:1;

(vi) the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene, ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, butanediol dimethacrylate, tricyclodecane dimethanol diacrylate, pentaerythritol triacrylate, tripropylene glycol diacrylate, propoxylated neopentyl diacrylate, pentaerythritol triacrylate, ditrimethylolpropane tetraacrylate, dipentaerythritol pentaacrylate, dipentaerythritol penta/hexa-acrylate, tripropylene diacrylate, trimethylol propane ethoxylate triacrylate, trimethylol propane propoxylate triacrylate, di(trimethylolpropane) tetraacrylate, glycerol propoxylate triacrylate, pentaerythritol propoxylate triacrylate, poly(ethylene glycol) diacrylate, poly(propylene glycol) diacrylate, and tri(propylene glycol) diacrylate, optionally wherein the crosslinking monomer is selected from one or more of the group consisting of divinylbenzene,

ethylene glycol dimethylacrylate, bisphenol A dimethacrylate, and N,N ′ -methylenebis(acrylamide);

(vii) the functional groups on the first functional monomer are independently selected from one or more of amino, carboxyl, epoxy, and hydroxyl, optionally wherein the functional groups on the functional monomer in the first shell portion and the second layer of the second shell portion are independently selected from a combination of hydroxyl and carboxyl groups or a combination of amino and carboxyl groups, optionally wherein the first functional monomer is selected from one or more of the group consisting of acrylic acid, methacrylic acid, 2-carboxyethyl acrylate, acrylamide, methacrylamide, allylamine, (hydroxyethyl)methacrylate, hydroxypropyl methacrylate, 4-hydroxybutyl acrylate, glycidyl methacrylate, allyl glycidyl ether, 1,2-epoxy-5-hexene, maleic anhydride, 2-hydroxyethyl methacrylate, and 2-carboxyethyl acrylate oligomers; and

(viii) the weight ratio of the styrene monomer to cross-linker is from 20:1 to 1:2, such as from 10:1 to 1:1.

17. The process according to any one of Claim 16, wherein the weight ratio of the styrene monomer to first functional monomer is between 20:1 to 1:2, preferably between 10:1 to 1:1.

18. The process according to any one of Claims 16, wherein the as-synthesized monodisperse beads are washed with one or more solvents before use in a subsequent process step, optionally wherein the solvent is selected from one or more of the group consisting of water, methanol, ethanol, isopropanol, and THF.

19. The process according to any one of Claims 9 to 18, wherein the resulting monodisperse superparamagnetic beads have:

(a) a coefficient of variation based on their diameter of less than 15%, such as less than 10%, such as less than 5%, such as less than or equal to 2%; and/or

(b) an average diameter of from 0.2 to 5.0 microns, such as from 0.5 to 4.0 microns.

20. The superparamagnetic beads according to any one of Claims 1 to 8, wherein these beads are applied in IVD assays.