therefore desirable to provide a delivery system for antibodies and other antigen-binding molecules.

SUMMARY OF THE INVENTION

The present invention shows how to incorporate antigen-binding molecules such as antibodies into the polymeric matrix of nanospheres, while preserving their antigen-binding and biological activity. The thus encapsulated antigen-binding molecules are protected from enzymatic degradation and the surface of the nanospheres remains free for further modification such as by targeting molecules or molecules increasing the half-live of the nanospheres in the subject's body.

Thus, the invention provides a nanosphere comprising:

a) a polymeric matrix formed by one or more than one polymer comprising a main monomeric constituent selected from one or more than one of C_1-C_{10}-alkyl cyanoacrylates and C_1-C_6-alkoxy-C_1-C_{10}-alkyl cyanoacrylates; and

b) one or more than one antigen-binding molecule comprising at least one immunoglobulin light chain variable domain and at least one immunoglobulin heavy chain variable domain,

wherein the one or more than one antigen-binding molecule is esterase-releasably incorporated in the polymeric matrix.

The invention also provides a nanosphere comprising:

a) a polymeric matrix formed by one or more than one polymer comprising a main monomeric constituent selected from one or more than one of C_1-C_{10}-alkyl cyanoacrylates and C_1-C_6-alkoxy-C_1-C_{10}-alkyl cyanoacrylates; and

b) one or more than one antigen-binding molecule comprising at least one immunoglobulin light chain variable domain and at least one immunoglobulin heavy chain variable domain,

wherein the one or more than one antigen-binding molecule is dissolved or dispersed in the polymeric matrix.

The invention further provides a plurality of nanospheres as described herein having a polydispersity of 0.5 or less and an average diameter of 300 nm or less as determined by Photon Correlation Spectroscopy.

The invention also provides a method for preparing nanospheres, the method comprising:

i) providing a hydrophobic liquid phase comprising one or more than one polymerizable monomer selected from C_1-C_{10}-alkyl cyanoacrylates and C_1-C_6-alkoxy-C_1-C_{10}-alkyl cyanoacrylates;

ii) finely dispersing the hydrophobic liquid phase in a hydrophilic liquid phase so as to form an emulsion, the pH of the emulsion being 4.0 or less;

iii) increasing the pH of the emulsion to a value in the range of 4.0-6.0 so as to accelerate the polymerization of the polymerizable monomer(s);

CLAIMS

1. A nanosphere comprising:
   a) a polymeric matrix formed by one or more than one polymer comprising a main monomeric constituent selected from one or more than one of C_1-C_{10}-alkyl cyanoacrylates and C_1-C_6-alkoxy-C_1-C_{10}-alkyl cyanoacrylates; and
   b) one or more than one antigen-binding molecule comprising at least one immunoglobulin light chain variable domain and at least one immunoglobulin heavy chain variable domain,

   wherein the one or more than one antigen-binding molecule is dissolved or dispersed in the polymeric matrix.
2. The nanosphere of claim 1, wherein the one or more than one antigen-binding molecule is selected from gammaglobulins, antibody dimers, and Fab fragments and $F(ab)_2$ fragments.
3. The nanosphere of claim 1 or claim 2, wherein at least 20% of the antigen-binding molecule(s) is still capable of binding to its antigen after release from the nanosphere.
4. The nanosphere of any one of claims 1-3, wherein the antigen-binding molecules released from the nanosphere retain at least 20% their original biological activity as measured with a biological assay such as a cell assay.
5. The nanosphere of any one of claims 1-4, wherein the main monomeric constituent of the matrix-forming polymer(s) is selected from one or more than one of methyl 2-cyanoacrylate, 2-methoxyethyl 2-cyanoacrylate, ethyl 2-cyanoacrylate, n-butyl 2-cyanoacrylate, 2-octyl 2-cyanoacrylate and isobutyl 2-cyanoacrylate.
6. The nanosphere of any one of claims 1-5, wherein the one or more than one matrix-forming polymer is selected from poly(n-butyl 2-cyanoacrylate), poly(ethyl 2-cyanoacrylate), and mixtures thereof.
7. A plurality of nanospheres of any one of claims 1-6 having a polydispersity in the range of 0.5 or less as determined by cumulant analysis according to ISO13321 and ISO22412 and an average diameter in the range of 20-300 nm as determined by Photon Correlation Spectroscopy.
8. A method for preparing nanospheres, the method comprising:

i) providing a hydrophobic liquid phase comprising one or more than one polymerizable monomer selected from C_1-C_{10}-alkyl cyanoacrylates and C_1-C_6-alkoxy-C_1-C_{10}-alkyl cyanoacrylates;

ii) finely dispersing the hydrophobic liquid phase in a hydrophilic liquid phase so as to form an emulsion, the pH of the emulsion being 4.0 or less;

iii) increasing the pH of the emulsion to a value in the range of 4.0-6.0 so as to accelerate the polymerization of the polymerizable monomer(s);

iv) then, adding one or more than one antigen-binding molecule comprising at least one immunoglobulin light chain variable domain and at least one immunoglobulin heavy chain variable domain; and

v) finally, allowing the polymerization to continue by further increasing the pH to a value not exceeding pH 8.0;

thereby forming a suspension of nanospheres, wherein the one or more than one antigen-binding molecule is incorporated in a polymeric matrix formed by the polymerization of the polymerizable monomer(s).

9. The method of claim 8, wherein the nanospheres are as defined in any one of claims 3, 4, 6 and 7.

10. The method of claim 8 or claim 9, wherein step (ii) is carried out by homogenization under pressure and/or ultrasonically.

11. The method of any one of claims 8-10, wherein in step (iii) the pH is increased to a value in the range of 4.8-5.5.

12. The method of any one of claims 8-11, wherein the emulsion is incubated for 5-20 min at room temperature after addition of the antigen-binding molecule(s).

13. The method of any one of claims 8-12, wherein in step (v) the pH of the emulsion is increased to be in the range of 6.8-7.5.

14. The method of any one of claims 8-13, wherein the amount of the hydrophobic liquid phase is from 1-40 wt-% relative to the total weight of the hydrophilic and hydrophobic liquid phases.

15. The method of any one of claims 8-14, wherein the hydrophilic liquid phase or the hydrophobic liquid phase or both contain(s) one or more than one stabilizer.

16. The method of any one of claims 15, wherein the amount of the stabilizer(s) is from 5-25 wt-% relative to the total weight of the polymerizable monomers.

17. The method of claim 15 or claim 16, wherein the one or more than one stabilizer is selected from poloxamers, sodium n-C_{12}-C_{16}-alkyl sulfate, sorbitan fatty acid esters, polyoxyethylene sorbitan fatty acid esters, poloxamines,

poly(oxyethylene) ethers, poly(oxyethylene) esters, polyethylene glycols, and mixtures thereof.

18. The method of any one of claims 8-17, wherein the one or more than one polymerizable monomer is selected from methyl 2-cyanoacrylate, 2-methoxyethyl 2-cyanoacrylate, ethyl 2-cyanoacrylate, n-butyl 2-cyanoacrylate, 2-octyl 2-cyanoacrylate and isobutyl 2-cyanoacrylate.

19. The method of any one of claims 8-18, wherein the one or more than one antigen-binding molecule is selected from gammaglobulins, antibody dimers, and Fab fragments and $F(ab)_2$ fragments.

20. A pharmaceutical composition comprising a plurality of nanospheres according to any one of claims 1-7, and a pharmacologically acceptable carrier.