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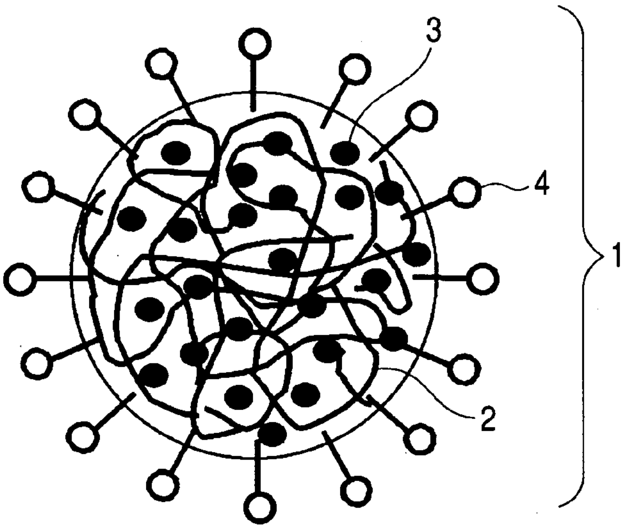
[Continued on next page]

(54) Title: POLYMER NANOPARTICLE AND CONTRAST AGENT FOR OPTICAL MOLECULAR IMAGING

FIG. 1



(57) Abstract: Provided are polymer nanoparticles each having a fluorescent dye dispersed in the matrix of a fluorescent polymer, characterized in that the surface of the nanoparticles is protected by a surfactant and fluorescence is emitted via FRET between the fluorescent polymer and the fluorescent dye, and a contrast agent for optical molecular imaging using the polymer nanoparticles.

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DESCRIPTION

POLYMER NANOPARTICLE AND CONTRAST AGENT FOR OPTICAL MOLECULAR IMAGING

TECHNICAL FIELD

The present invention relates to polymer nanoparticles emitting fluorescence via fluorescence resonance energy transfer, and a contrast agent for optical molecular imaging using the polymer nanoparticles.

BACKGROUND ART

Recently, research and development on polymer nanoparticles have been aggressively made with a view toward applying the nanoparticles in a wide variety of industrial fields. Of them, applications in the medical care/diagnosis fields including medicaments and diagnostic drugs have been increased more than ever. Particularly in recent years, application of polymer nanoparticles to a contrast agent for optical molecular imaging via fluorescence resonance energy transfer (hereinafter referred to as "FRET") has drawn attention.

As to the polymer nanoparticles emitting fluorescence via FRET, "Macromol. Rapid. Commun.", 2006, 27, pp. 200-202 (hereinafter referred to as "Non-Patent Document 1") discloses a technique for electrostatically immobilizing a cationic fluorescent dye on the surface of polyfluorene nanoparticles whose surface is protected by SDS (sodium dodecyl sulfate). The document reports that when polyfluorene (PF2/6) is excited with light, fluorescence is emitted from the cationic fluorescent dye (rhodamine 6G) via FRET.

On the other hand, "Nature Materials", 2003, 2, pp. 408-412 (hereinafter referred to as "Non-Patent Document 2") discloses a technique for obtaining a polymer-blended nanoparticles, including two types of polymers, by mixing chloroform dissolving the two types of fluorescent polymer (PF2/6 and m-LPPP) with an aqueous SDS solution, applying ultrasonic wave to the resultant solution to form an emulsion, and heating the emulsion to remove chloroform. The document reports that when PF2/6 is excited with light, fluorescence is emitted from m-LPPP via FRET.

Furthermore, "J. Phys. Chem. C, 2008, 112, pp. 1772-1781" (hereinafter referred to as "Non-Patent Document 3") discloses a technique for obtaining polymer nanoparticles each having a fluorescent dye dispersed in the matrix of a fluorescent polymer by supplying a THF (tetrahydrofuran) solution dissolving a fluorescent polymer (PDHF) and a fluorescent dye (coumarin 6) to desalted water while applying ultrasonic wave, filtrating the resultant suspension through a 0.2 μm membrane filter and thereafter removing THF under vacuum. The document reports that when PDHF is excited with light, fluorescence is emitted from coumarin 6 via FRET.

U.S. Patent No. 5,763,189 discloses a technique for obtaining polymer nanoparticles, which are polystyrene latex particles each having an organic dye(s) dispersed therein, by swelling the polystyrene latex particles having sulfonic acid (or carboxylic acid) on the surface with THF (or DMF) to incorporate a single or a plurality of types of organic dyes.

Non-Patent Document 1 reports that when polyfluorene (PF2/6) is excited by light, fluorescence is emitted from a cationic fluorescent dye (rhodamine 6G) via FRET; however, the efficiency of FRET is not high.

In addition, in the polymer nanoparticles of Non-Patent Document 1, the cationic fluorescent dye electrostatically adsorbed to the surface of the nanoparticles may desorb in an aqueous solution containing a salt such as physiological saline. As a result, the FRET efficiency may conceivably decrease.

On the other hand, Non-Patent Document 2 reports that when PF2/6 is excited with light, fluorescence is emitted from m-LPPP via FRET; however, FRET efficiency is not clearly described.

Furthermore, in the polymer nanoparticles of Non-Patent Document 2, two types of fluorescent polymers are contained within a nanoparticle. When different types of polymers are used, compatibility is a problem. More specifically, phase separation may occur, with the result that the FRET efficiency may conceivably decrease.

On the other hand, in Non-Patent Document 3, since the surface of polymer nanoparticles is not protected with a surfactant, coagulation of the polymer nanoparticles may occur in an aqueous solution containing a salt such as physiological saline. This is a matter of concern.

On the other hand, in U.S. Patent No. 5,763,189, it is difficult to incorporate a donor dye and an acceptor dye into the nanoparticles according to a desired ratio. As a result, the ratio of dyes incorporated is likely to vary and FRET efficiency and fluorescence intensity may conceivably decrease.

DISCLOSURE OF THE INVENTION

The present invention was made in view of problems of the background art as described above. The object of the present invention is to provide polymer nanoparticles excellent in FRET efficiency by dispersing a fluorescent dye in the matrix of a fluorescent polymer and excellent in dispersibility by protecting the surface of the nanoparticles with a surfactant.

Another object of the present invention is to provide polymer nanoparticles having excellent FRET efficiency by dispersing two types of fluorescent dyes in the matrix of a polymer and having excellent dispersibility by protecting the surface of nanoparticles by a surfactant.

Furthermore, another object of the present invention is to provide a contrast agent for optical molecular imaging using the polymer nanoparticles.

The present inventors conducted intensive studies with a view to solving the aforementioned problems. As a result, they found polymer nanoparticles excellent in FRET efficiency and dispersibility. Based on the finding, they arrived at the present invention.

More specifically, the polymer nanoparticles of the present invention are polymer nanoparticles each having a fluorescent dye dispersed in the matrix of a fluorescent polymer, characterized in that the surface of the nanoparticles is protected by a surfactant and fluorescence is emitted via FRET between the fluorescent polymer and the fluorescent dye.

Furthermore, the polymer nanoparticles of the present invention are characterized in that the fluorescent polymer is a conjugated polymer.

Furthermore, the polymer nanoparticles according to another aspect of the present invention are polymer nanoparticles each having two types of fluorescent dyes dispersed in the matrix of a polymer, characterized in that the surface of the nanoparticles is protected by a surfactant and fluorescence is emitted via FRET between the two types of fluorescent dyes.

Furthermore, the polymer nanoparticles of the present invention are characterized in that the fluorescence emitted via FRET has a near-infrared wavelength of 600 nm or more and 1000 nm or less excellent in penetration through a living body.

Furthermore, the polymer nanoparticles of the present invention are characterized in that the surfactant is a nonionic surfactant.

Furthermore, the polymer nanoparticles of the present invention are characterized in that the nanoparticles have an average particle size of 10 nm or more and 200 nm or less.

Moreover, the present invention is directed to a contrast agent for optical molecular imaging characterized by containing the polymer nanoparticles.

Further features of the present invention will become apparent from the following description of exemplary embodiments with reference to the attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic view illustrating the structure of a polymer nanoparticle according to the first invention.

FIG. 2 is a schematic view illustrating the structure of a polymer nanoparticle according to the second invention.

FIG. 3 is a chart exemplifying the steps for manufacturing the polymer nanoparticles according to the first invention.

FIG. 4 is a chart exemplifying the steps for manufacturing the polymer nanoparticles according to the second invention.

FIG. 5 is a graph illustrating FRET characteristics of polymer nanoparticles 1 of Example 1.

FIG. 6 is a graph illustrating FRET characteristics of polymer nanoparticles 2 of Example 2.

FIG. 7 is a graph illustrating FRET characteristics of polymer nanoparticles 8 of Example 8.

FIG. 8 is a graph illustrating FRET characteristics of polymer nanoparticles 12 of Example 12.

FIG. 9 is a graph illustrating FRET characteristics of polymer nanoparticles 18 of Example 18.

FIG. 10 illustrates a TEM photograph of polymer nanoparticles 19 of Example 19.

FIG. 11 illustrates a fluorescent image of polymer nanoparticles 37 of Example 37.

FIG. 12 illustrates a fluorescent image of QD565 of Example 37.

FIG. 13 illustrates brightness histograms of polymer nanoparticles 37 and QD565 of Example 37.

FIG. 14 illustrates a TEM photograph of polymer nanoparticles 38 of Example 38.

FIG. 15 illustrates a fluorescent image of polymer nanoparticles 38 of Example 38.

FIG. 16 illustrates a fluorescent image of QD565 of Example 38.

FIG. 17 illustrates brightness histograms of polymer nanoparticles 38 and QD565 of Example 38.

FIG. 18 illustrates graphs illustrating particle size distributions of polymer nanoparticles 39, 40 and 41 in an aqueous sodium chloride solution.

FIG. 19 illustrates graphs illustrating particle size distributions of polymer nanoparticles 18 in PBS and FBS.

FIG. 20 illustrates graphs illustrating particle size distributions of polymer nanoparticles 40 in PBS and FBS.

FIG. 21 illustrates graphs illustrating particle size distributions of polymer nanoparticles 41 in PBS and FBS.

FIG. 22 is a TEM photograph of polymer nanoparticles 42 of Example 40.

FIG. 23 is a graph illustrating FRET characteristics of polymer nanoparticles 42 of Example 40.

FIG. 24 is a fluorescent image of polymer nanoparticles 42 of Example 40.

FIG. 25 is a fluorescent image of QD 800 of Example 40.

FIG. 26 illustrates brightness histograms of polymer nanoparticles 42 and QD800 of Example 40.

FIG. 27 illustrates graphs illustrating particle size distributions of polymer nanoparticles 43 in PBS and FBS;

FIG. 28 is a fluorescent image of a nude mouse to which polymer nanoparticles 42 of Example 40 is administered.

FIG. 29 is a graph illustrating normalized fluorescence spectra of polymer nanoparticles synthesized according to U.S. Patent No. 5,763,189.

FIG. 30 is a graph illustrating the fluorescence intensity of polymer nanoparticles 49 in comparison with that of polymer nanoparticles 50.

FIG. 31 is a fluorescent image of a nude mouse to which polymer nanoparticles 18 of Example 18 is administered.

BEST MODES FOR CARRYING OUT THE INVENTION

The present invention will be more specifically described below.

(Polymer nanoparticles)

The first invention is directed to polymer nanoparticles 1 each having a fluorescent dye (acceptor) 3 dispersed in the matrix of a fluorescent polymer 2, as shown in FIG. 1, and a surfactant 4 is present on the surface of the polymer nanoparticles 1.

Furthermore, the second invention of the present invention is directed to polymer nanoparticles 10 each having two types of fluorescent dyes 3 and 5 dispersed in the matrix of a polymer 6, as shown in FIG. 2, and a surfactant 4 is present on the surface of polymer nanoparticles 10.

Furthermore, according to a preferred embodiment of the present invention, the polymer nanoparticles are characterized by emitting fluorescence excellent in penetration through a living body via FRET caused by a combination of a fluorescent dye emitting fluorescence within a near-infrared wavelength region of 600 nm or more and 1000 nm or less and a fluorescent polymer.

Furthermore, according to another preferred embodiment of the present invention, the polymer nanoparticles are characterized by emitting fluorescence excellent in penetration through a living body via FRET caused by a combination of two types of fluorescent dyes (donor and acceptor) emitting fluorescence within a near-infrared wavelength region of 600 nm or more and 1000 nm or less.

The polymer nanoparticles of the present invention are characterized by having excellent dispersibility even in an aqueous solution containing a salt such as physiological saline since the surface of the nanoparticles is protected by a surfactant.

The polymer nanoparticles each have a fluorescent polymer, a fluorescent dye and a surfactant. The fluorescent dye is dispersed in the matrix of the fluorescent polymer and the surfactant is present on the surface of the polymer nanoparticles.

The polymer nanoparticles of the present invention are characterized in that the average particle size thereof can be controlled depending upon desired use and is 10 nm or more and 200 nm or less.

(FRET)

FRET(Fluorescence Resonance Energy Transfer) refers to the phenomenon below. When two types of fluorescent molecules, in the state where the fluorescence spectrum of a donor fluorescent molecule has an overlap with the absorption spectrum of an acceptor fluorescent molecule, are present in close contact with each other within the range of 1 to 10 nm, the optical energy of the donor fluorescent molecule excited is transferred to the acceptor fluorescent molecule and excites the acceptor fluorescent molecule. The intensity of FRET is inversely proportional to the sixth power of the distance between the two molecules and rapidly decreases with the distance. In the present invention, a fluorescent polymer and a fluorescent dye are used as the two types of fluorescent molecules.

(Fluorescent polymer)

The fluorescent polymer of the first invention is a polymer which emits fluorescence when the polymer excited by excitation light returns to the ground state. Furthermore, the polymer is not particularly limited as long as it has a fluorescence spectrum has an overlap with the absorption spectrum of a fluorescent dye. The fluorescent polymers may include conjugated polymers below as a part. Specific examples of the conjugated polymers include

F8BT: poly[2,1,3-benzothiadiazole-4,7-diyl(9,9-dioctyl-9H-fluorene-2,7-diyl)] represented by Formula 1,

MEH-PPV: poly(2-methoxy-5-(2'-ethyl-hexyloxy)-p-phenylene vinylene) represented by Formula 2,

P-1: copoly(2,3-diphenylthieno[3,4-b]pyrazine-alt-9,9-didecylfluorene) represented by Formula 3,

P-2: copoly(thieno[3,4-b]pyrazine-alt-9,9-didecylfluorene) represented by Formula 4,

PF11112: poly(9,9-bis(3,7,11-trimethyldodecyl)fluorene) represented by Formula 5,

m-LPPP: ladder-type poly(para-phenylene) represented by Formula 6,

PFB: poly(9,9-dioctylfluorene-co-N,N'-bis(4-butylphenyl)-N,N'-diphenyl-1,4-phenylenediamine) represented by Formula 7,

PF2/6: poly[9,9-bis(2-ethylhexyl)fluorene-2,7-diyl] represented by Formula 8,

ADS104RE: poly[(2-methoxy-5-(3,7-dimethyl-octyloxy)-1,4-phenylenevinylene) end capped with DMP represented by Formula 9,

ADS106RE: poly[9,9-di-(2-ethylhexyl)-fluorenyl-2,7-diyl] end capped with 2,5-diphenyl-1,2,4-oxadiazole~~,~~ represented by Formula 10,

ADS300RE: poly[2,5-bis(3,7-dimethyloctyloxy)-1,4-phenylene-vinylene] represented by Formula 11,

ADS129BE: poly(9,9-dioctyfluorenyl-2,7-diyl) end capped with dimethylphenyl~~,~~ represented by Formula 12,

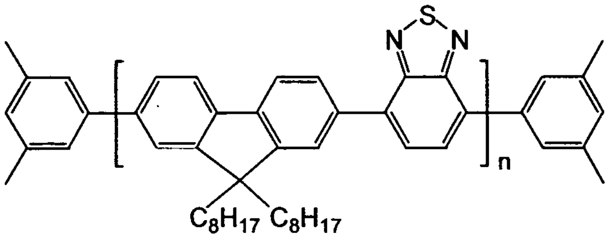
CDPDOF: copoly(2,3-diphenylthieno[3,4-b]pyrazine-alt-9,9-dioctylfluorene) represented by Formula 13,

COTTOF: copoly(thienothiadiazole-alt-9,9-dioctylfluorene) represented by Formula 14 (wherein x:y is 1:0 to 0.1:1.), and

COBBOF: copoly(benzobisthiadiazole-alt-9,9-dioctylfluorene) represented by Formula 15 (wherein x:y is 1:0 to 0.1:1).

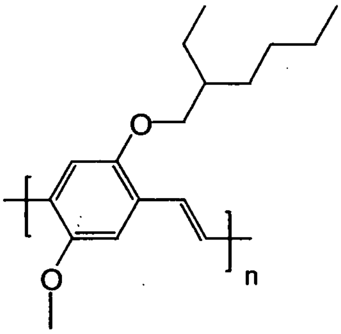
However, the fluorescent polymer of the present invention is not limited to those mentioned above.

These fluorescent polymers having an average molecular weight of 2000 to 1000000, and preferably 10000 to 600000 can be suitably used.



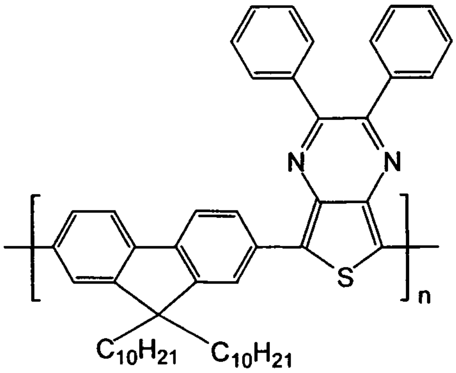
(1)

F8BT



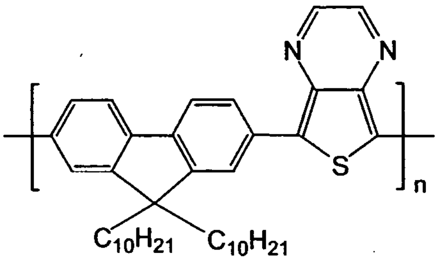
(2)

MEH-PPV



(3)

P-1



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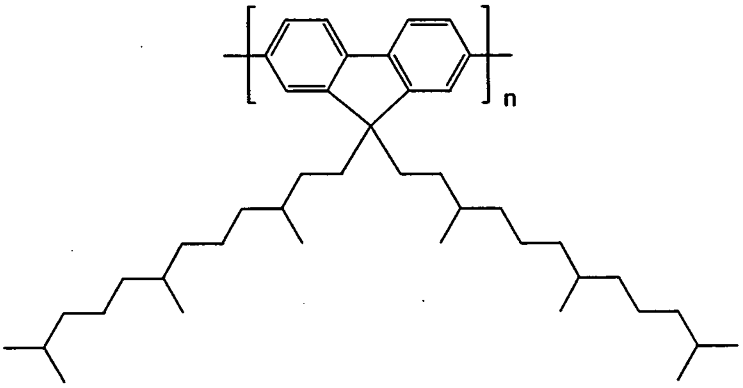
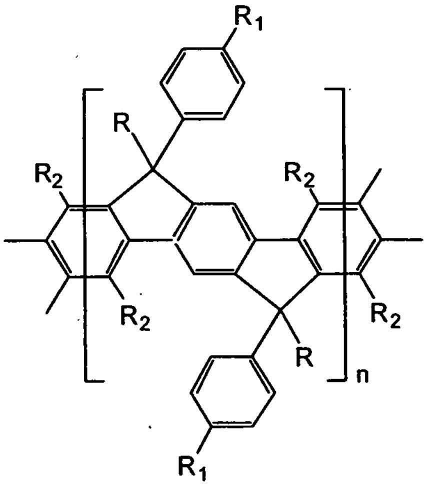
P-2



(5)

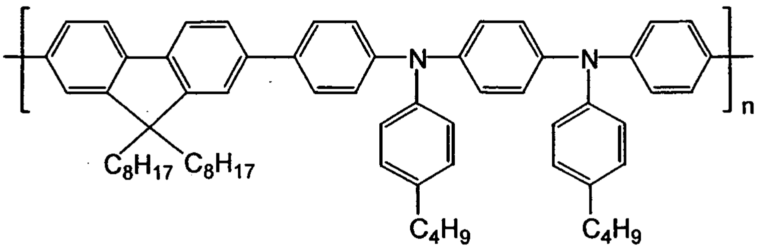
PF11112



R : methyl
R_1 : decyl
R_2 : hexyl

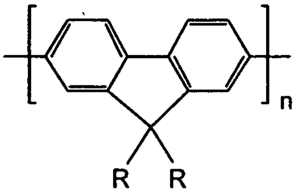
(6)

m-LPPP



(7)

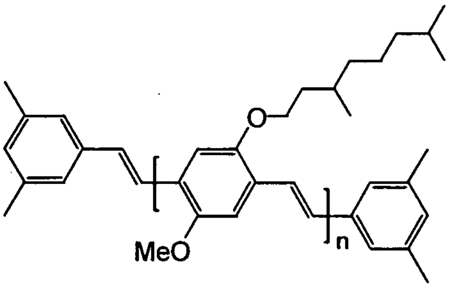
PFB



R : 2-ethylhexyl

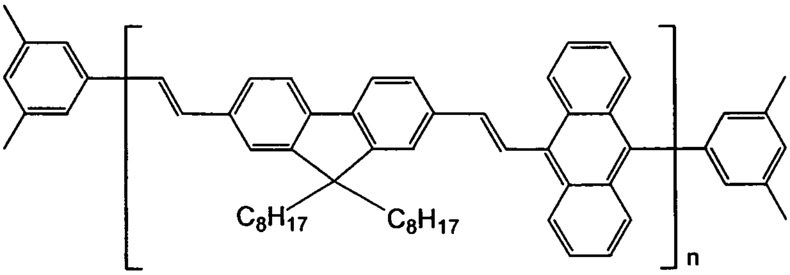
(8)

PF2/6



(9)

ADS104RE



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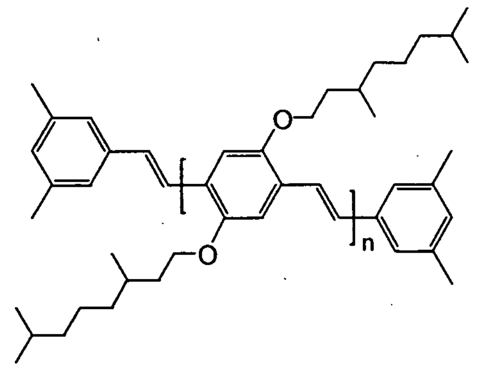
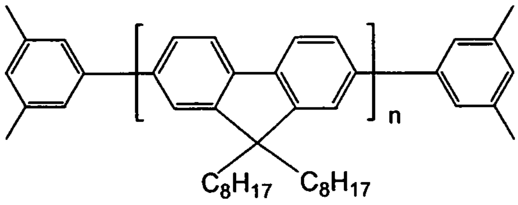
ADS106RE



(11)

ADS300RE



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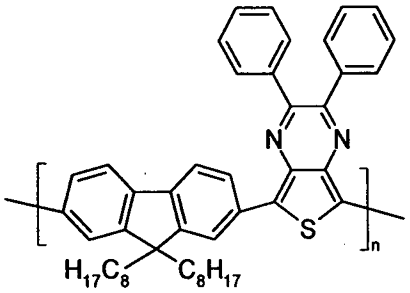
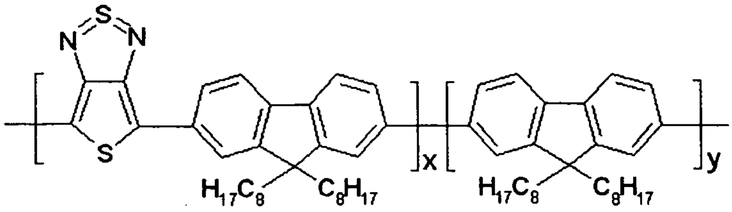
ADS129BE



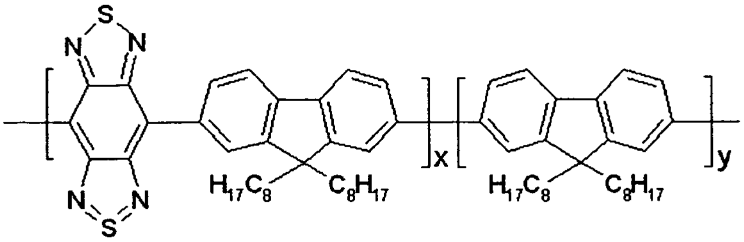
(13)

CDPDOF



(14)

COTTOF



(15)

COBBOF

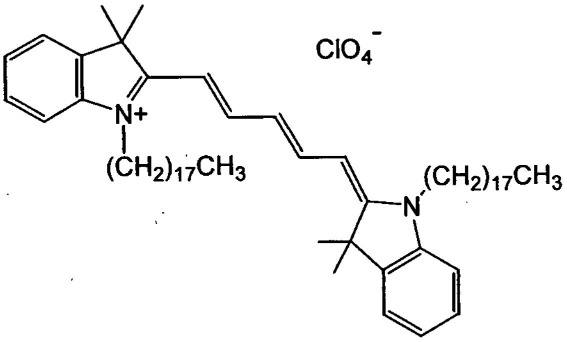
(Fluorescent dye)

The fluorescent dye of the first invention is not particularly limited as long as it has an absorption spectrum overlapped with the fluorescence spectrum of a fluorescent polymer. In view of penetration through a living body, a fluorescent dye is preferred to emit fluorescence within the near-infrared wavelength region of 600 nm or more and 1000 nm or less. The polymer nanoparticles according to the second present invention may contain three or more different types of fluorescent dyes.

The two types of fluorescent dyes according to the second invention are not particularly limited as long as an absorption spectrum of one of the fluorescent dye is overlapped with the fluorescence spectrum of the other fluorescent dye, in other words, as long as they are a combination of donor and acceptor fluorescent dyes. In view of penetration through a living body, the fluorescent dyes are preferred to emit fluorescence within the near-infrared wavelength region of 600 nm or more and 1000 nm or less.

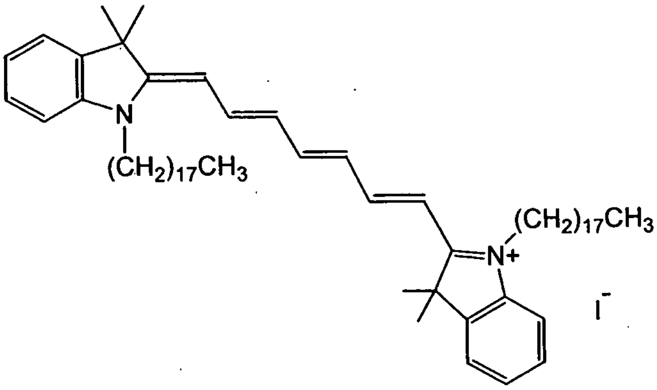
As these fluorescent dyes, the dyes as exemplified below may be mentioned.

Cyanine fluorescent dyes such as DiD: 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate represented by Formula 16, DiR: 1;1'-Dioctadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide represented by Formula 17, DiI: 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, and ICG (indocyanine green); phthalocyanine fluorescent dyes such as SiPcTHSO: silicon phthalocyanine bis(trihexyl-silyloxide) represented by Formula 18, silicon 2,3,9,10,16,17,23,24-octakis (octyloxy)-29H,31H-phthalocyanine dihydroxide, silicon 2,9,16,23-tetra-tert-butyl-29H,31H-phthalocyanine dihydroxide, and β-tetra (neopentyloxy)-29H,31H-phthalocyanine represented by Formula 19, 2,3,9,10,16,17,23,24-octa(4-tert-butylphenoxy)-29H,31H-phthalocyanine, β-tetra (thiobuthoxy)-29H,31H-phthalocyanine, and 1,4,8,11,15,18,22,25 octaethoxy-zinc phthalocyanine; naphthalocyanine fluorescent dyes such as silicon 2,3-naphthalocyanine dioctyloxide represented by Formula 20, silicon 2,3-naphthalocyanine dihydroxide and silicon 2,3-naphthalocyanine bis(trihexylsilyloxide); porphyrin fluorescent dyes such as hexapropyl-3,6-diphenyltetraazaporphyrin and 5,10,15,20-Tetraphenyl-22H,24H-porphyrin; porphyrazine fluorescent dyes such as β-tetra(tert-butyl)-tetrapyrazino porphyrazine; xanthene fluorescent dyes such as rhodamine 6G, rhodamine B, rhodamine X and tetramethyl rhodamine; thiazine fluorescent dyes such as methylene blue; coumarin fluorescent dyes such as coumarin 6; oxazine fluorescent dyes, polymethine fluorescent dyes, polyphenyl fluorescent dyes, stilbene fluorescent dyes, oxazole fluorescent dyes, oxadiazole fluorescent dyes, perylene fluorescent dyes, acridine fluorescent dyes and BODIPY fluorescent dyes. However, the fluorescent dye of the present invention is not limited to those mentioned above.



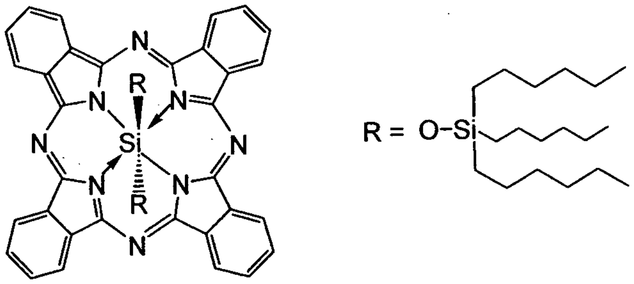
(16)

DiD



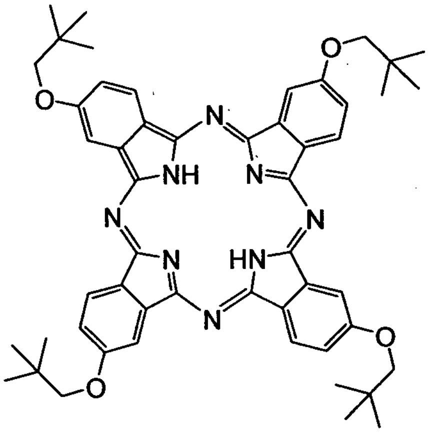
(17)

DiR



(18)

SiPcTHSO



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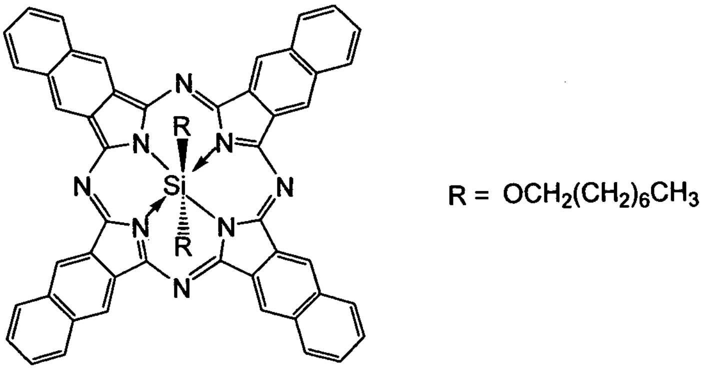


(20)

(Polymer)

The polymer according to the second invention is not particularly limited as long as it can dissolve in an organic solvent as described later. For example, polymers such as PS: polystyrene, PMMA: poly(methyl methacrylate), PBMA: poly(butyl methacrylate), P(BMA-co-MMA): poly(butyl methacrylate-co-methyl methacrylate), polylactic acid, polyglycolic acid, polyorthoester, poly-ε-caprolactone, a polyacid anhydride, a dextran derivative and a cellulose derivative may be mentioned.

(Method for manufacturing polymer nanoparticles)

The method for manufacturing the polymer nanoparticles according to the first invention will be described below.

The method for manufacturing the polymer nanoparticles according to the first invention has

a step of adding a first liquid containing a fluorescent polymer and a fluorescent dye to a second liquid containing a surfactant to obtain a solution mixture;

a step of obtaining an emulsion from the solution mixture; and

a step of distilling away the first liquid from dispersoid of the emulsion. In short, a mini-emulsion method may be mentioned as the method of obtaining the polymer nanoparticles of the present invention; however, the method of the invention is not limited to the aforementioned one.

Next, the method for manufacturing the polymer nanoparticles of the second invention will be described.

The method for manufacturing the polymer nanoparticles of the second invention has

a step of adding a first liquid containing two types of fluorescent dyes and a polymer to a second liquid containing a surfactant to obtain a solution mixture;

a step of obtaining an emulsion from the solution mixture; and

a step of distilling away the first liquid from dispersoid of the emulsion. In short, a mini-emulsion method may be mentioned as the method of obtaining the polymer nanoparticles of the present invention; however, the method of the invention is not limited to the aforementioned one.

(First liquid)

The first liquid is an organic solvent. Any solvent may be applicable as long as it is insoluble or less soluble in water and can dissolve a fluorescent polymer, a polymer and a fluorescent dye. However, a volatile solvent is preferred.

Specific examples of the organic solvent may include halogenated hydrocarbons (e.g., dichloromethane, chloroform, chloroethane, dichloroethane, trichloroethane, carbon tetrachloride), ethers (e.g., ethyl ether, isobutyl ether), esters (e.g., ethyl acetate, butyl acetate) and aromatic hydrocarbons (e.g., benzene, toluene, xylene). These may be used alone or as a mixture of two or more types blended in an appropriate ratio. However, the organic solvent serving as the first liquid is not limited to the aforementioned ones.

The concentrations of a fluorescent polymer and a fluorescent dye in the first liquid are not particularly limited as long as they can be dissolved. As a preferable concentration of the fluorescent polymer, 0.5 to 100 mg/ml may be mentioned. As a preferable concentration of the fluorescent dye, 0.0005 to 1 mg/ml may be mentioned.

The weight ratio of a fluorescent polymer to a fluorescent dye contained in the first liquid preferably falls within the range of 1000:1 to 4:1.

Furthermore, the concentrations of a polymer and two types of fluorescent dyes in the first liquid are not particularly limited as long as they can be dissolved. As a preferable concentration of the polymer, 0.5 to 100 mg/ml may be mentioned. As preferable concentrations of the two types of fluorescent dyes, for example, 0.0005 to 5 mg/ml may be mentioned.

Furthermore, the weight ratio of a polymer to two types of fluorescent dyes contained in the first liquid preferably falls within the range of 1000:1 to 4:1. In two types of fluorescent dyes contained in the first liquid, the weight ratio of a donor fluorescent dyes to an acceptor fluorescent dye preferably falls within the range of 1000:1 to 0.1:1.

(Second liquid)

The second liquid is water or an aqueous solution.

In order to stabilize the emulsion formed by mixing it with the first liquid, it is preferred that a surfactant is previously added to the second liquid as a dispersant. However, as long as a surfactant is contained in a dispersion solution having the first and second liquids mixed therein, the addition method of the surfactant is not limited to the aforementioned method.

(Surfactant)

The following materials can be used as a surfactant.

Examples of a nonionic surfactant may include Tween 20, Tween 40, Tween 60, Tween 80, Tween 85, Brij 35, Brij 58, Brij 76, Brij 98, Triton X-100, Triton X-114, Triton X-305, Triton N-101, Nonidet P-40, Igepol CO530, Igepol CO630, Igepol CO720 and Igepol CO730.

Examples of an anionic surfactant may include sodium dodecyl sulfate, dodecylbenzene sulfonate, decylbenzene sulfonate, undecylbenzene sulfonate, tridecylbenzene sulfonate, nonylbenzene sulfonate and sodium, potassium and ammonium salts of these.

Examples of a cationic surfactant may include cetyltrimethyl ammonium bromide, hexadecylpyridinium chloride, dodecyltrimethylammonium chloride and hexadecyltrimethylammonium chloride.

Among these surfactant, a nonionic surfactant is preferably.

The concentration of a surfactant contained in the second liquid varies depending upon the mixing ratio with the first liquid and can be preferably, e.g., 0.1 mg/ml to 100 mg/ml.

(Emulsion)

As an emulsion, an emulsion having any physical properties may be used as long as the object of the present invention can be attained; preferably, a monodispersion emulsion having a particle size distribution with a single peak and an average particle size of 10 nm or more and 200 nm or less.

Such an emulsion can be prepared by an emulsification method known in the art. Example of the emulsification method known in the art may include an intermittent concussion method, a stirring method using a mixer such as a propeller-type stirrer or a turbine-type stirrer, a colloid mill method, a homogenizer method and a supersonic irradiation method. These methods may be used alone or in combination of a plurality of methods. The emulsion may be prepared by a one-step emulsification or by a multi-stage emulsification. However, emulsification is not limited to the aforementioned method as long as the object of the present invention can be attained.

The emulsion is an oil-in-water (O/W) type prepared from a solution mixture obtained by adding the first liquid to the second liquid. The mixing the first liquid and the second liquid refers to the state where the first liquid is in contact with the second liquid without spatially separating them. It is not necessary to intimately mix with each other.

The ratio of the first liquid to the second liquid in the solution mixture is not particularly limited as long as an oil-in-water (O/W) type emulsion can be prepared. Mixing is preferably performed such that the weight ratio of the first liquid to the second liquid falls within the range of 1:2.5 to 1:12.5.

(Distillation)

Distillation is an operation for removing the first liquid from the dispersoid of the emulsion. More specifically, distillation refers to removing an organic solvent from the dispersoid including a fluorescent polymer, a polymer, a fluorescent dye and the organic solvent.

Distillation can be performed by any method known in the art. A removal method by heating or by using a vacuum unit such as an evaporator may be mentioned. In the removal method by heating, the heating temperature is not particularly limited as long as an O/W type emulsion can be maintained; however the temperature preferably falls within the range of 0°C to 80°C. However, distillation is not limited to the aforementioned methods as long as the object of the present invention can be attained.

The steps for manufacturing the polymer nanoparticles 1 in the first invention are exemplified in FIG. 3.

More specifically, a water dispersion solution of polymer nanoparticles can be obtained via the following steps (1) to (3).

(1) a step of adding an organic solvent 7 insoluble or less soluble in water and containing a fluorescent polymer 2 and a fluorescent dye (acceptor) 3 to an aqueous solution 8 containing a surfactant 4 to obtain a solution mixture,

(2) a step of emulsifying the solution mixture to obtain an O/W type emulsion 9, and

(3) distilling away the organic solvent from the dispersoid of the emulsion 9.

Furthermore, the steps for manufacturing the polymer nanoparticles 10 in the second invention are exemplified in FIG. 4. More specifically, a water dispersion solution of polymer nanoparticles can be obtained via the following steps (1) to (3).

(1) a step of adding an organic solvent insoluble or less soluble in water and containing a polymer 6 and two types of fluorescent dyes 3 and 5 to an aqueous solution 8 containing a surfactant 4 to obtain a solution mixture,

(2) a step of emulsifying the solution mixture to obtain an O/W type emulsion 11, and

(3) distilling away the organic solvent from the dispersoid of the emulsion 11.

(Contrast agent for optical molecular imaging)

The polymer nanoparticles of the present invention can be used as a contrast agent for optical molecular imaging via FRET between a fluorescent polymer and a fluorescent dye within a nanoparticle or FRET between two types of fluorescent dyes within a nanoparticle.

The polymer nanoparticles of the present invention can emit fluorescence excellent in penetration through a living body via FRET caused by a combination of a fluorescent dye emitting fluorescence within the near-infrared wavelength region of 600 nm or more and 1000 nm or less and a fluorescent polymer. Therefore, the polymer nanoparticles are suitable as a contrast agent for optical molecular imaging.

Furthermore, the polymer nanoparticles of the present invention can emit fluorescence excellent in penetration through a living body via FRET caused by a combination of two types of fluorescent dyes emitting fluorescence within the near-infrared wavelength region of 600 nm or more and 1000 nm or less. Therefore, the polymer nanoparticles are suitable as a contrast agent for optical molecular imaging.

The contrast agent for optical molecular imaging according to the present invention can be used for visualizing a tumor site by delivering the polymer nanoparticles to the tumor site by use of the EPR (enhanced penetration and retention) effect, exciting the polymer nanoparticles by light and detecting fluorescence emission via FRET.

The polymer nanoparticles of the present invention are protected with a surfactant at the surface. Therefore, when the polymer nanoparticles are used as a contrast agent for optical molecular imaging, the polymer nanoparticles dispersed in water can be suitably used.

Examples

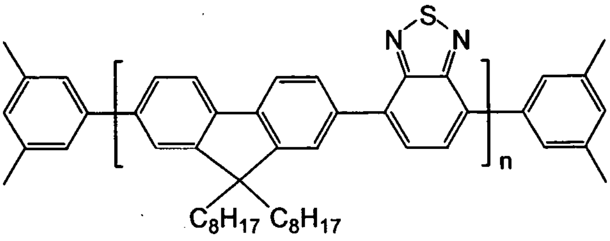
The present invention will be more specifically described by way of Examples below; however, the present invention is not limited to these Examples. Materials, composition conditions, reaction conditions and others may be freely varied as long as polymer nanoparticles having the same function and effect can be obtained.

First, the first invention will be more specifically described.

<Example 1>

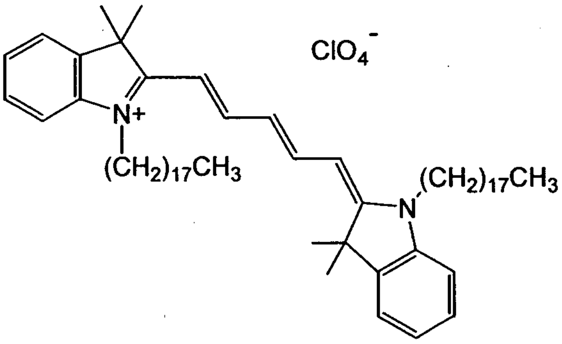
(Synthesis of polymer nanoparticles 1)

F8BT (0.4 mg, average molecular weight of from 10000 to 30000 manufactured by ADS) represented by Formula 1 and DiD (0.087 mg manufactured by Biotium, Inc.) represented by Formula 16 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.



(1)

F8BT



(16)

DiD

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1% by weight, 10 g) dissolving Tween 20 (manufactured by TCI) to prepare a solution mixture. After the solution mixture was stirred and treated by an ultrasonic dispersion machine (Microson XL2000 manufactured by Misonix, Inc.) for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for one hour to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 1 was obtained whose surface was protected by Tween 20 and having DiD dispersed in F8BT.

The particle size of polymer nanoparticles 1 thus obtained was analyzed by a dynamic light scattering analyzer (ELS-Z manufactured by Otsuka Electronics Co., Ltd). As a result, the average particle size thereof was 87.4 nm.

(Evaluation of FRET characteristics)

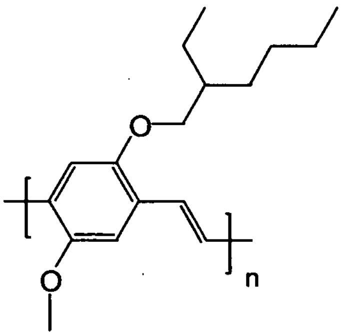
The FRET characteristics of the water dispersion solution of polymer nanoparticles 1 were evaluated by use of a spectrophotofluorometer (F-4500 manufactured by Hitachi, Ltd.) The results are shown in FIG. 5.

When the water dispersion solution of polymer nanoparticles 1 was irradiated with excitation light of 450 nm, the fluorescence from F8BT significantly decreased and the fluorescence emission of 680 nm from DiD was observed. From this, it was confirmed that FRET from F8BT to DiD efficiently occurs. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 2>

(Synthesis of polymer nanoparticles 2)

MEH-PPV (0.4 mg, average molecular weight of from 40000 to 70000 manufactured by Aldrich) represented by Formula 2 and DiD (0.04 mg) were dissolved in chloroform (0.8 g) to prepare a chloroform solution.



(2)

MEH-PPV

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (2% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for one hour to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 2 was obtained whose surface was protected by Tween 20 and having DiD dispersed in the MEH-PPV matrix.

The particle size of polymer nanoparticles 2 thus obtained was analyzed by the dynamic light scattering analyzer. As a result, the average particle size thereof was 102.3 nm.

(Evaluation of FRET characteristics)

The FRET characteristics of the water dispersion solution of polymer nanoparticles 2 were evaluated by use of the spectrophotofluorometer. The results are shown in FIG. 6.

When the water dispersion solution of polymer nanoparticles 2 was irradiated with excitation light of 500 nm, the fluorescence from MEH-PPV significantly decreased and the fluorescence emission of 680 nm from DiD was observed. From this, it was confirmed that FRET from MEH-PPV to DiD efficiently occurs. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 3>

(Synthesis of polymer nanoparticles 3)

A water dispersion solution of polymer nanoparticles 3 was obtained in the same manner as in Example 2 except that the use amount of DiD of Example 2 was changed from 0.04 mg to 0.004 mg. When the particle size of polymer nanoparticles 3 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size thereof was 59.3 nm. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 4>

(Synthesis of polymer nanoparticles 4)

A water dispersion solution of polymer nanoparticles 4 was obtained in the same manner as in Example 2 except that the use amount of DiD of Example 2 was changed from 0.04 mg to 0.0004 mg. When the particle size of polymer nanoparticles 4 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size thereof was 90.1 nm. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 5>

(Synthesis of polymer nanoparticles 5)

MEH-PPV (0.4 mg) and DiD (0.046 mg) were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (0.70% by weight, 2 g) dissolving DTAC (dodecyltrimethylammonium chloride manufactured by Alfa Aesar) to prepare a solution mixture. The solution mixture was stirred for 2 hours and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 60°C for 30 minutes to distil away chloroform. In this manner, a water dispersion solution of polymer nanoparticles 5 was obtained whose surface was protected by DTAC and having DiD dispersed in the MEH-PPV matrix.

When the particle size of polymer nanoparticles 5 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size thereof was 42.0 nm. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 6>

(Synthesis of polymer nanoparticles 6)

A water dispersion solution of polymer nanoparticles 6 was obtained in the same manner as in Example 5 except that the use amount of DiD of Example 5 was changed from 0.046 mg to 0.0046 mg. When the particle size of polymer nanoparticles 6 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size thereof was 51.7 nm. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 7>

(Synthesis of polymer nanoparticles 7)

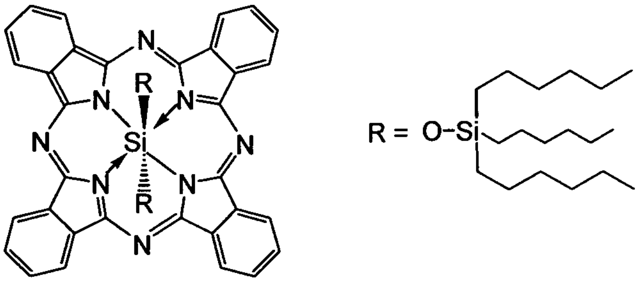
A water dispersion solution of polymer nanoparticles

7 was obtained in the same manner as in Example 5 except that the use amount of DiD of Example 5 was changed from 0.046 mg to 0.00046 mg. When the particle size of polymer nanoparticles 7 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size thereof was 19.7 nm. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 8>

(Synthesis of polymer nanoparticles 8)

F8BT (0.4 mg) represented by Formula 1 and SiPcTHSO (0.04 mg, manufactured by Aldrich) represented by Formula 18 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.



(18)

SiPcTHSO

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (0.70% by weight, 10 g) dissolving DTAC to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 60°C for 30 minutes to distil away chloroform. In this manner, a water dispersion solution of polymer nanoparticles 8 was obtained whose surface was protected by DTAC and having SiPcTHSO dispersed in the F8BT matrix. When the particle size of polymer nanoparticles 8 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size was 34.3 nm.

(Evaluation of FRET characteristics)

The FRET characteristics of the water dispersion solution of polymer nanoparticles 8 were evaluated by use of the spectrophotofluorometer. The results are shown in FIG. 7.

When the water dispersion solution of polymer nanoparticles 8 was irradiated with excitation light of 450 nm, the fluorescence from F8BT significantly decreased and the fluorescence emission from SiPcTHSO of 669.8 nm was observed. From this, it was confirmed that FRET from F8BT to SiPcTHSO efficiently occurs. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 9>

(Synthesis of polymer nanoparticles 9)

A water dispersion solution of polymer nanoparticles 9 was obtained in the same manner as in Example 8 except that the use amount of SiPcTHSO of Example 8 was changed from 0.04 mg to 0.02 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 10>

(Synthesis of polymer nanoparticles 10)

A water dispersion solution of polymer nanoparticles 10 was obtained in the same manner as in Example 8 except that the use amount of SiPcTHSO of Example 8 was changed from 0.04 mg to 0.01 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 11>

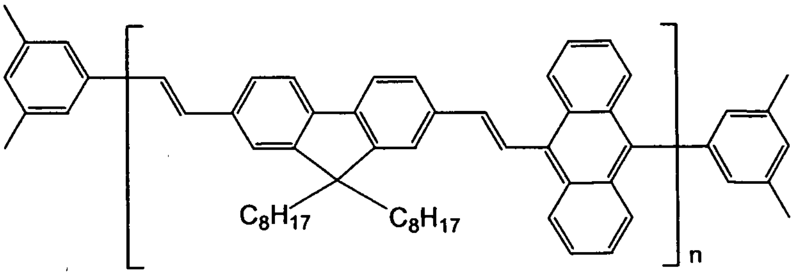
(Synthesis of polymer nanoparticles 11)

A water dispersion solution of polymer nanoparticles 11 was obtained in the same manner as in Example 8 except that the use amount of SiPcTHSO of Example 8 was changed from 0.04 mg to 0.004 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 12>

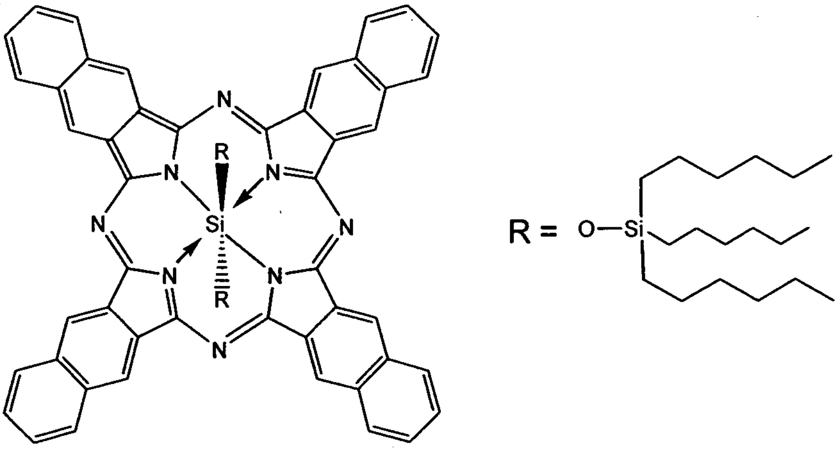
(Synthesis of polymer nanoparticles 12)

ADS106RE (0.4 mg, average molecular weight of from 50000 to 200000 manufactured by ADS) represented by Formula 10 and SiNPcTHSO (0.04 mg, manufactured by Aldrich) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.



(10)

ADS106RE



(21)

SiNPcTHSO

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (0.70% by weight, 10 g) dissolving DTAC (dodecyltrimethylammonium chloride manufactured by Alfa Aesar) to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 60°C for 30 minutes to distil away chloroform. In this manner, a water dispersion solution of polymer nanoparticles 12 was obtained whose surface was protected by DTAC and having SiNPcTHSO dispersed in the ADS106RE matrix. When the particle size of polymer nanoparticles 12 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size was 25.9 nm.

(Evaluation of FRET characteristics)

The FRET characteristics of the water dispersion solution of polymer nanoparticles 12 were evaluated by use of a spectrophotofluorometer (F-4500 manufactured by Hitachi, Ltd.). The results are shown in FIG. 8.

When the water dispersion solution of polymer nanoparticles 12 was irradiated with excitation light of 450 nm, the fluorescence from ADS106RE significantly decreased and the fluorescence emission from SiNPcTHSO of 777.0 nm was observed. From this, it was confirmed that FRET from ADS106RE to SiNPcTHSO efficiently occurs. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 13>

(Synthesis of polymer nanoparticles 13)

A water dispersion solution of polymer nanoparticles 13 was obtained in the same manner as in Example 12 except that the use amount of SiNPcTHSO of Example 12 was changed from 0.04 mg to 0.03 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 14>

(Synthesis of polymer nanoparticles 14)

A water dispersion solution of polymer nanoparticles 14 was obtained in the same manner as in Example 12 except that the use amount of SiNPcTHSO of Example 12 was changed from 0.04 mg to 0.02 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 15>

(Synthesis of polymer nanoparticles 15)

A water dispersion solution of polymer nanoparticles 15 was obtained in the same manner as in Example 12 except that the use amount of SiNPcTHSO of Example 12 was changed from 0.04 mg to 0.01 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 16>

(Synthesis of polymer nanoparticles 16)

A water dispersion solution of polymer nanoparticles 16 was obtained in the same manner as in Example 12 except that the use amount of SiNPcTHSO of Example 12 was changed from 0.04 mg to 0.004 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 17>

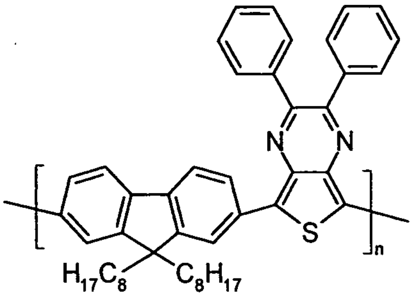
(Synthesis of polymer nanoparticles 17)

A water dispersion solution of polymer nanoparticles 17 was obtained in the same manner as in Example 12 except that the use amount of SiNPcTHSO of Example 12 was changed from 0.04 mg to 0.002 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 18>

(Synthesis of polymer nanoparticles 18)

CDPDOF (4 mg, average molecular weight of 18000 (in terms of polystyrene by GPC (Gel Permeation Chromatography))) synthesized by the present inventors) represented by Formula 13 and SiNPcTHSO (0.1 mg, manufactured by Aldrich) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.



(13)

CDPDOF

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for one hour to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 18 was obtained whose surface was protected by Tween 20 and having SiNPcTHSO dispersed in CDPDOF. When the particle size of polymer nanoparticles 18 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size was 78.6 nm.

(Evaluation of FRET characteristics)

The FRET characteristics of the water dispersion solution of polymer nanoparticles 18 were evaluated by use of a spectrophotofluorometer (FP-6600 manufactured by JASCO Corporation). The results are shown in FIG. 9.

When the water dispersion solution of polymer nanoparticles 18 was irradiated with excitation light of 600 nm, the fluorescence from CDPDOF significantly decreased and the fluorescence emission from SiNPcTHSO of 780 nm was observed. From this, it was confirmed that FRET from CDPDOF to SiNPcTHSO efficiently occurs. Furthermore, the results of FRET efficiency are shown in Table 1.

(In vivo fluorescence imaging of nude mouse)

The water dispersion solution of the polymer nanoparticles 18 was twofold concentrated by a sterilized ultrafiltration filter. After the concentrated solution was treated by a sterilized syringe filter (0.20 μm in diameter), the solution was diluted with PBS two fold and 0.2 ml of the solution was administered intravenously through the tail vein of a nude mouse (BALB/c-nu/nu, 9 weeks old, female). Fifteen minutes, 3 hours, 1, 2 and 3 days after the administration, a fluorescent image of the nude mouse was taken by a fluorescent imaging apparatus (IVIS200 manufactured by Xenogen Corporation). The results are shown in FIG. 31. Strong fluorescence was detected which was emitted from the body of the nude mouse. It was confirmed that in vivo fluorescent imaging can be performed.

<Example 19>

(Synthesis of polymer nanoparticles 19)

A water dispersion solution of polymer nanoparticles 19 was obtained in the same manner as in Example 18 except that the use amount of SiNPcTHSO of Example 18 was changed from 0.1 mg to 0.04 mg. Furthermore, the results of FRET efficiency are shown in Table 1. A TEM photograph of polymer nanoparticles 19 is shown in FIG. 10.

<Example 20>

(Synthesis of polymer nanoparticles 20)

A water dispersion solution of polymer nanoparticles 20 was obtained in the same manner as in Example 18 except that the use amount of SiNPcTHSO of Example 18 was changed from 0.1 mg to 0.17 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 21>

(Synthesis of polymer nanoparticles 21)

A water dispersion solution of polymer nanoparticles 21 was obtained in the same manner as in Example 18 except that the use amount of SiNPcHSO of Example 18 was changed from 0.1 mg to 0.3 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 22>

(Synthesis of polymer nanoparticles 22)

A water dispersion solution of polymer nanoparticles 22 was obtained in the same manner as in Example 19 except that the use amount of CDPDOF of Example 19 was changed from 4 mg to 0.4 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 23>

(Synthesis of polymer nanoparticles 23)

A water dispersion solution of polymer nanoparticles 23 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.01 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 24>

(Synthesis of polymer nanoparticles 24)

A water dispersion solution of polymer nanoparticles 24 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.013 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 25>

(Synthesis of polymer nanoparticles 25)

A water dispersion solution of polymer nanoparticles 25 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.017 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 26>

(Synthesis of polymer nanoparticles 26)

A water dispersion solution of polymer nanoparticles 26 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.02 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 27>

(Synthesis of polymer nanoparticles 27)

A water dispersion solution of polymer nanoparticles 27 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.03 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 28>

(Synthesis of polymer nanoparticles 28)

A water dispersion solution of polymer nanoparticles 28 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.054 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 29>

(Synthesis of polymer nanoparticles 29)

A water dispersion solution of polymer nanoparticles 29 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed from 0.04 mg to 0.08 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 30>

(Synthesis of polymer nanoparticles 30)

A water dispersion solution of polymer nanoparticles 30 was obtained in the same manner as in Example 22 except that the use amount of SiNPcHSO of Example 22 was changed

from 0.04 mg to 0.2 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 31>

(Synthesis of polymer nanoparticles 31)

CDPDOF (0.4 mg, synthesized by the present inventors) represented by Formula 13 and SiNPcTHSO (0.004 mg, manufactured by Aldrich) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (0.70% by weight, 10 g) dissolving DTAC (dodecyltrimethylammonium chloride manufactured by Alfa Aesar) to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 60°C for 30 minutes to distil away chloroform. In this manner, a water dispersion solution of polymer nanoparticles 31 was obtained whose surface was protected by DTAC and having SiNPcTHSO dispersed in CDPDOF. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 32>

(Synthesis of polymer nanoparticles 32)

A water dispersion solution of polymer nanoparticles 32 was obtained in the same manner as in Example 31 except that the use amount of SiNPcTHSO of Example 31 was changed

from 0.004 mg to 0.01 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 33>

(Synthesis of polymer nanoparticles 33)

A water dispersion solution of polymer nanoparticles 33 was obtained in the same manner as in Example 31 except that the use amount of SiNPcTHSO of Example 31 was changed from 0.004 mg to 0.03 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 34>

(Synthesis of polymer nanoparticles 34)

ADS106RE (0.4 mg) represented by Formula 10 and SiNPcTHSO (0.03 mg) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion..

Next, the emulsion was heated at 40°C for one hour to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 34 was obtained whose surface was protected by Tween 20 and having SiNPcTHSO dispersed in ADS106RE.

When the particle size of polymer nanoparticles 34 thus obtained was analyzed by the dynamic light scattering analyzer, the average particle size was 73.1 nm. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 35>

(Synthesis of polymer nanoparticles 35)

A water dispersion solution of polymer nanoparticles 35 was obtained in the same manner as in Example 34 except that the use amount of SiNPcTHSO of Example 34 was changed from 0.03 mg to 0.01 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Example 36>

(Synthesis of polymer nanoparticles 36)

A water dispersion solution of polymer nanoparticles 36 was obtained in the same manner as in Example 34 except that the use amount of SiNPcTHSO of Example 34 was changed from 0.03 mg to 0.02 mg. Furthermore, the results of FRET efficiency are shown in Table 1.

<Comparative Example 1>

The nanoparticles obtained in Non-Patent Document 1 was used as Comparative Example 1. The polymer nanoparticles obtained in Examples 1 to 36 were compared with the nanoparticles of Comparative Example 1 for FRET efficiency. The results of FRET efficiency are collectively shown in Table 1.

From the results of Table 1, it was confirmed that the FRET efficiency of polymer nanoparticles 1 to 36 (excluding polymer nanoparticles 23 and 31 to 33) of the present invention are superior in FRET efficiency to those of Comparative Example 1 (Non-Patent Document 1).

Table 1

Sample	Fluorescent dye (mg)	Fluorescent polymer (mg)	Weight ratio of fluorescent dye/fluorescent polymer	Average particle size (nm) of polymer nanoparticles	FRET efficiency (%)
Example 1 Polymer nanoparticles1	8.70E-02	4.00E-01	2.18E-01	87.4	75.5
Example 2 Polymer nanoparticles2	4.00E-02	4.00E-01	1.00E-01	102.3	82.8
Example 3 Polymer nanoparticles3	4.00E-03	4.00E-01	1.00E-02	59.3	79.2
Example 4 Polymer nanoparticles4	4.00E-04	4.00E-01	1.00E-03	90.1	65.3
Example 5 Polymer nanoparticles5	4.60E-02	4.00E-01	1.15E-01	42.0	97.7
Example 6 Polymer nanoparticles6	4.60E-03	4.00E-01	1.15E-02	51.7	71.1
Example 7 Polymer nanoparticles7	4.60E-04	4.00E-01	1.15E-03	19.7	33.0
Example 8 Polymer nanoparticles8	4.00E-02	4.00E-01	0.1	34.3	100
Example 9 Polymer nanoparticles9	2.00E-02	4.00E-01	0.05	34.3	92
Example 10 Polymer nanoparticles10	1.00E-02	4.00E-01	0.025	34.3	85
Example 11 Polymer nanoparticles11	4.00E-03	4.00E-01	0.01	34.3	65
Example 12 Polymer nanoparticles12	0.04	4.00E-01	0.1	25.9	93
Example 13 Polymer nanoparticles13	0.03	4.00E-01	0.075	25.9	94
Example 14 Polymer nanoparticles14	0.02	4.00E-01	0.05	25.9	91
Example 15 Polymer nanoparticles15	0.01	4.00E-01	0.025	25.9	78
Example 16 Polymer nanoparticles16	0.004	4.00E-01	0.01	25.9	52
Example 17 Polymer nanoparticles17	0.002	4.00E-01	0.005	25.9	37

Table 1 (continued)

Sample	Fluorescent dye (mg)	Fluorescent polymer (mg)	Weight ratio of fluorescent dye/fluorescent polymer	Average particle size (nm) of polymer nanoparticles	FRET efficiency (%)
Example 18 Polymer nanoparticles18	0.1	4	0.025	78.6	55.9
Example 19 Polymer nanoparticles19	0.04	4	0.01	78.6	52.1
Example 20 Polymer nanoparticles20	0.17	4	0.04	78.6	64.5
Example 21 Polymer nanoparticles21	0.3	4	0.08	78.6	83.2
Example 22 Polymer nanoparticles22	0.04	0.4	0.1	100.1	55.6
Example 23 Polymer nanoparticles23	0.01	0.4	0.025	100.1	17.5
Example 24 Polymer nanoparticles24	0.013	0.4	0.033	100.1	49.2
Example 25 Polymer nanoparticles25	0.017	0.4	0.0416	100.1	44.9
Example 26 Polymer nanoparticles26	0.02	0.4	0.05	100.1	51.3
Example 27 Polymer nanoparticles27	0.03	0.4	0.075	100.1	52.4
Example 28 Polymer nanoparticles28	0.054	0.4	0.135	100.1	71.1
Example 29 Polymer nanoparticles29	0.08	0.4	0.2	100.1	80.6
Example 30 Polymer nanoparticles30	0.2	0.4	0.5	100.1	86.2
Example 31 Polymer nanoparticles31	0.004	0.4	0.01	ND	ND
Example 32 Polymer nanoparticles32	0.01	0.4	0.025	ND	ND
Example 33 Polymer nanoparticles33	0.03	0.4	0.075	ND	ND
Example 34 Polymer nanoparticles34	0.03	0.4	0.075	65.1	71.6
Example 35 Polymer nanoparticles35	0.01	0.4	0.025	65.1	21.0
Example 36 Polymer nanoparticles36	0.02	0.4	0.05	65.1	43.2
Comparative Example 1 Non-patent Document 1	9.58E-01	8.80E-01	1.09E+00	95.0	about 20

<Example 37>

(Comparison of brightness between polymer nanoparticles 37 and QD (quantum dot))

Polymer nanoparticles 37 of the present invention were compared with QD (quantum dot) for brightness. Polymer nanoparticles 37 used for evaluation were synthesized according to the following synthesis example. As the QD, QD565 (Invitrogen) was used.

(Synthesis example of polymer nanoparticles 37)

F8BT (4 mg) represented by Formula 1 and SiPcTHSO (0.04 mg, manufactured by Aldrich) represented by Formula 18 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for one hour to distil away chloroform. In this manner, a water dispersion solution of polymer nanoparticles 37 was obtained whose surface was protected by Tween 20 and having SiPcTHSO dispersed in the F8BT matrix.

(Comparison of brightness)

Polymer nanoparticles 37 and QD565 were dispersed in the same PVA (polyvinyl alcohol) film and observed by a fluorescent microscope. At that time, filters were used each of which can observe only the corresponding particles. In this manner, different fluorescent images were obtained in the same site. The results are shown in FIGS. 11 and 12, respectively.

Histograms showing brightness distribution of polymer nanoparticles 37 and QD565 are depicted in FIG. 13 for comparison. From the results above, it was confirmed that the brightness of polymer nanoparticles 37 is larger than that of QD565.

<Example 38>

(Comparison of brightness between polymer nanoparticles 38 and QD)

Polymer nanoparticles 38 were compared with QD for brightness. Polymer nanoparticles 38 used for evaluation were synthesized according to the following synthesis example. As the QD, the same QD565 (Invitrogen) as used in Example 37 was used.

(Synthesis example of polymer nanoparticles 38)

ADS106RE (4 mg) represented by Formula 10 and SiNPcTHSO (0.04 mg) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for one hour to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 38 was obtained whose surface was protected by Tween 20 and having SiNPcTHSO dispersed in ADS106RE. Furthermore, a TEM photograph of polymer nanoparticles 38 is shown in FIG. 14.

(Comparison of brightness)

Polymer nanoparticles 38 and QD565 were dispersed in the same PVA film and observed by a fluorescent microscope, in the same manner as in Example 37. At that time, filters were used each of which can observe only the corresponding particles. In this manner, different fluorescent images were obtained in the same site. The results are shown in FIGS. 15 and 16, respectively.

Histograms showing brightness distribution of polymer nanoparticles 38 and QD565 are shown in FIG. 17 for comparison. From the results above, it was confirmed that the brightness of polymer nanoparticles 38 is larger than that of QD565.

<Example 39>

(Dispersion stability evaluation for polymer nanoparticles)

Polymer nanoparticles 18, 39 and 40 of the present invention and polymer nanoparticles 41 obtained by the method of Non-Patent Document 3 were evaluated for dispersion stability. The polymer nanoparticles 39 to 41 used for evaluation were synthesized according to the following synthesis example. Note that polymer nanoparticles 18 were synthesized according to Example 18.

(Synthesis example of polymer nanoparticles 39)

ADS106RE (0.4 mg) represented by Formula 10 and SiNPcTHSO (0.04 mg) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (0.70% by weight, 10 g) dissolving DTAC to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 60°C for 30 minutes to distil away chloroform. In this manner, a water dispersion solution of polymer nanoparticles 39 was obtained whose surface was protected by DTAC and having SiNPcTHSO dispersed in the ADS106RE matrix.

(Synthesis example of polymer nanoparticles 40)

ADS106RE (0.4 mg) represented by Formula 10 and SiNPcTHSO (0.04 mg) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for one hour to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 40 was obtained whose surface was protected by Tween 20 and having SiNPcTHSO dispersed in ADS106RE.

(Synthesis example of polymer nanoparticles 41)

ADS106RE (0.08 mg) represented by Formula 10 and SiNPcTHSO (0.004 mg) represented by Formula 21 were dissolved in THF (2 g) to prepare a THF solution.

Subsequently, the aforementioned THF solution was added to ultrapure water (8 g) to prepare a solution mixture. The solution mixture was stirred and thereafter treated by the ultrasonic dispersion machine for 30 seconds.

Next, the solution was heated at 70°C for 30 minutes to distil away THF from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 41 was obtained having SiNPcTHSO dispersed in ADS106RE.

(Dispersion stability evaluation 1)

The particle size of polymer nanoparticles 39, 40 and 41 obtained above was analyzed by the dynamic light scattering analyzer. After the particle size was measured, a 1.5 mol/L aqueous sodium chloride solution (0.2 g) was added to each of the water dispersion solutions (1.8 g) of the polymer nanoparticles. After the solution mixtures were allowed to standstill overnight, the particle size was again analyzed by the dynamic light scattering analyzer. The results are shown in FIG. 18.

From FIG. 18, remarkable coagulation was not observed in polymer nanoparticles 39 or 40 of the present invention even after the dispersion solutions were prepared and allowed to standstill overnight. In particular, polymer nanoparticles 40 were confirmed to have excellent dispersion stability. On the other hand, in polymer nanoparticles 41 of Comparative Example, a large coagulation was produced after the standstill overnight since the surface of the nanoparticles is not protected by a surfactant. From this, it was confirmed that polymer nanoparticles 41 of Comparative Example are inferior in dispersion stability compared to polymer nanoparticles 39 and 40 of the present invention.

(Dispersion stability evaluation 2)

The particle size of polymer nanoparticles 18, 40 and 41 obtained above was analyzed by the dynamic light scattering analyzer. After the particle size was measured, a phosphate buffer solution (PBS, 0.2 g) and fetal bovine serum (FBS, 0.2 g) were added to each of the water dispersion solutions (1.8 g) of the polymer nanoparticles. After the solution mixtures were allowed to standstill overnight, the particle size was again analyzed by the dynamic light scattering analyzer. The results are shown in FIGS. 19 to 21.

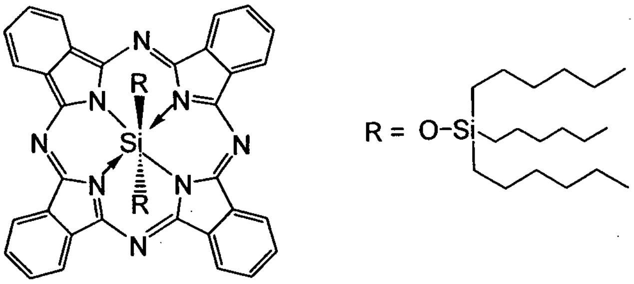
From Figures 19 to 21, remarkable coagulation was not observed in polymer nanoparticles 18 and 40 of the present invention placed in the phosphate buffer solution or the fetal bovine serum even after the dispersion solutions were prepared and allowed to standstill overnight. It was thus confirmed that polymer nanoparticles are excellent in dispersion stability. On the other hand, in polymer nanoparticles 41 of Comparative Example, a large coagulation was produced in the phosphate buffer solution after the standstill overnight since the surface of the nanoparticles was not protected by a surfactant. From this, polymer nanoparticles 41 of Comparative Example are confirmed to be inferior in dispersion stability compared to polymer nanoparticles 18 and 40 of the present invention.

Next, the second invention will be described by way of Examples.

<Example 40>

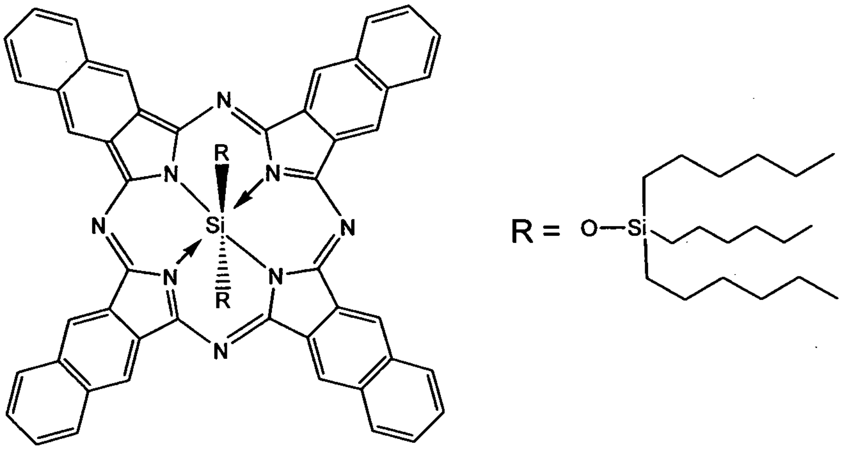
(Synthesis of polymer nanoparticles 42)

PS (4 mg, molecular weight: 22,000 manufactured by SCIENTIFIC POLYMER PRODUCTS), SiPcTHSO (0.756 mg, manufactured by Aldrich) represented by Formula 18, and SiNPcTHSO (0.042 mg, manufactured by Aldrich) represented by Formula 21 were dissolved in chloroform (0.8 g) to prepare a chloroform solution.



(18)

SiPcTHSO



(21)

SiNPcTHSO

Subsequently, the aforementioned chloroform solution was added to an aqueous solution (1.5% by weight, 10 g) dissolving Tween 20 to prepare a solution mixture. After the solution mixture was stirred and treated by the ultrasonic dispersion machine for 30 seconds to prepare an O/W type emulsion.

Next, the emulsion was heated at 40°C for two hours to distil away chloroform from the dispersoid. In this manner, a water dispersion solution of polymer nanoparticles 42 was obtained whose surface was protected by Tween 20 and having SiPcTHSO and SiNPcTHSO dispersed in PS. Furthermore, a TEM photograph of polymer nanoparticles 42 is shown in FIG. 22. The particle size of polymer nanoparticles 42 thus obtained was observed by a transmission electron microscope. As a result, the average particle size thereof was 66.9 nm.

(Evaluation of FRET characteristics)

The FRET characteristics of the water dispersion solution of polymer nanoparticles 42 were evaluated by use of the spectrophotofluorometer. The results are shown in FIG. 23.

When the water dispersion solution of polymer nanoparticles 42 was irradiated with excitation light of 600 nm, the fluorescence from SiPcTHSO significantly decreased and the fluorescence emission of 780 nm from SiNPcTHSO was observed. From this, it was confirmed that FRET from SiPcTHSO to SiNPcTHSO efficiently occurs. Furthermore, the results of FRET efficiency are shown in Table 2.

(Comparison of brightness)

PVA films having polymer nanoparticles 42 and QD800 separately dispersed therein were formed on different slide glasses. They were separately observed by a fluorescent microscope. At that time, the same excitation filter, dichroic mirror and fluorescence filter were used for observation and individual fluorescent images were separately obtained. The results are shown in Figures 24 and 25. The absorbance index, fluorescence quantum yield and fluorescence intensity per particle are shown in Table 3.

Furthermore, histograms showing brightness distributions of polymer nanoparticles 42 and QD800 are prepared and compared in FIG. 26. From the results above, it was confirmed that the brightness of polymer nanoparticles 42 is larger than that of QD800.

Table 3

Sample	Abs		Absorbance index (ε)		Fluorescence quantum yield (φ)	Fluorescence intensity (ε×φ)	
	610 nm	668 nm	610 nm	668 nm		610 nm	668 nm
Polymer nanoparticles 42	0.07	0.79	1.29×10^{8}	1.56×10^{9}	0.08	1.00×10^{7}	1.21×10^{8}
QD800	0.10	0.05	9.80×10^{5}	5.12×10^{5}	0.13	1.27×10^{5}	6.66×10^{4}
SiPcTHSO	-	-	-	-	0.26	-	-
SiNPcTHSO	-	-	-	-	0.07	-	-

(Dispersion stability evaluation)

Polymer nanoparticles 43 consisting of PS alone were prepared in the same manner as in Example 40 except that SiPcTHSO and SiNPcTHSO were not contained. The particle size thereof was analyzed by the dynamic light scattering analyzer. After the particle size was measured, a phosphate buffer (PBS, 0.1 g) or fetal bovine serum (FBS, 0.1 g) was added to the water dispersion solution (0.9 g) of the polymer nanoparticles 43. After the solution mixture was allowed to standstill at room temperature overnight, then the particle size thereof was again analyzed by the dynamic light scattering analyzer. The results are shown in FIG. 27.

From FIG. 27, remarkable coagulation was not observed in polymer nanoparticles 43 either in the phosphate buffer or the fetal bovine serum and even after the dispersion solution was prepared and allowed to standstill overnight. It was confirmed that the nanoparticles have excellent dispersion stability.

(In vivo fluorescence imaging of nude mouse)

The water dispersion solution of the polymer nanoparticles 42 was diluted with PBS four fold. After the solution was treated by a sterilized ultrafiltration filter (0.22 μm in diameter), 0.2 ml of the solution was administered intravenously through the tail vein of a nude mouse (BALB/c-nu/nu, 9 weeks old, female). Fifteen minutes, 3 hours, 1, 2 and 3 days after the administration, a fluorescent image of the nude mouse was taken by a fluorescent imaging apparatus (IVIS200 manufactured by Xenogen Corporation). The results are shown in FIG. 28. Strong fluorescence was detected which was emitted from the body of the nude mouse. It was confirmed that in vivo fluorescent imaging can be performed.

<Example 41>

(Synthesis of polymer nanoparticles 44)

A water dispersion solution of polymer nanoparticles 44 was obtained in the same manner as in Example 40 except that the use amount of SiNPcTHSO of Example 40 was changed

from 0.042 mg to 0.0084 mg. Furthermore, the results of FRET efficiency are shown in Table 2.

<Example 42>

(Synthesis of polymer nanoparticles 45)

A water dispersion solution of polymer nanoparticles 45 was obtained in the same manner as in Example 40 except that the use amount of SiNPcTHSO of Example 40 was changed from 0.042 mg to 0.021 mg. Furthermore, the results of FRET efficiency are shown in Table 2.

<Example 43>

(Synthesis of polymer nanoparticles 46)

A water dispersion solution of polymer nanoparticles 46 was obtained in the same manner as in Example 40 except that the use amount of SiNPcTHSO of Example 40 was changed from 0.042 mg to 0.084 mg. Furthermore, the results of FRET efficiency are shown in Table 2.

<Example 44>

(Synthesis of polymer nanoparticles 47)

A water dispersion solution of polymer nanoparticles 47 was obtained in the same manner as in Example 40 except that the use amount of SiNPcTHSO of Example 40 was changed from 0.042 mg to 0.14 mg. Furthermore, the results of FRET efficiency are shown in Table 2.

Table 2

Example	SiNPcTHSO (mg)	SiPcTHSO (mg)	Molar ratio of SiNPcTHSO/ SiPcTHSO	Average particle size of polymer nanoparticles (nm)	FRET efficiency (%)
Example 40 Polymer nanoparticles 42	0.042	0.756	0.1	66.9	96.4
Example 41 Polymer nanoparticles 44	0.0084	0.756	0.02	66.9	70.4
Example 42 Polymer nanoparticles 45	0.0021	0.756	0.05	66.9	91.6
Example 43 Polymer nanoparticles 46	0.084	0.756	0.2	66.9	98.2
Example 44 Polymer nanoparticles 47	0.14	0.756	0.33	66.9	99.1

<Example 45>

(Synthesis example of polymer nanoparticles 48)

Polymer nanoparticles 48 were synthesized according to the method of U.S. Patent No. 5,763,189.

To a Latex solution (0.6 mL, 4% w/v: Interfacial Dynamics Corp. Inc.) under stirring, DMF (1.33 mL) was added dropwise for 5 minutes. The mixture was further stirred for 30 minutes to allow Latex to swell. Subsequently, DMF (0.07 mL) having dyes (SiPcTHSO: 0.668 mg, SiNPcTHSO: 0.2 mg dissolved in the total volume (2 mL) of the solution) was added dropwise for 5 minutes and further stirred for 30 minutes. Finally, dialysis was performed in a refrigerator (about 7°C) for 15 hours to remove DMF. In this manner, polymer nanoparticles 48 were obtained.

(Comparison of FRET efficiency of polymer nanoparticles)

Polymer nanoparticles containing only SiPcTHSO alone, SiNPcTHSO alone, and SiPcTHSO and SiNPcTHSO were prepared according to the method of U.S. Patent No. 5,763,189 and fluorescence was measured. Normalized spectra of the fluorescence are shown in FIG. 29. Polymer nanoparticles 48 containing SiPcTHSO and SiNPcTHSO, the fluorescence of SiPcTHSO was quenched and fluorescence of SiNPcTHSO was observed.

Using polymer nanoparticles 42 of the present invention and polymer nanoparticles 48 obtained by the manufacturing method of U.S. Patent No. 5,763,189, FRET efficiency was compared. The FRET efficiency of polymer nanoparticles 48 was 32.3%. Thus, it was confirmed that polymer nanoparticles 42 of the present invention (FRET efficiency: 96.4%) is superior.

(Comparison of fluorescence intensity of polymer nanoparticles)

Nanoparticles were prepared such that the polymer nanoparticles of the present invention and polymer nanoparticles of U.S. Patent No. 5,763,189 were contained in substantially the same concentration (0.0089% w/v). They were respectively designated as polymer nanoparticles 49 and polymer nanoparticles 50, which were compared for fluorescence intensity. The results are shown in FIG. 30. The fluorescence intensity of polymer nanoparticles 49 at 781 nm is 2243, whereas that of polymer nanoparticles 50 is 19. From this, it was confirmed that the fluorescence intensity of polymer nanoparticles 49 is stronger by 100 times or more.

Furthermore, when the concentration of the dye based on the polymer was calculated from absorbance, polymer nanoparticles 49 contained 2.7 wt% of SiPcTHSO and 0.3 wt% of SiNPcTHSO; whereas polymer nanoparticles 50 contained 0.054 wt% of SiPcTHSO and 0.013 wt% of SiNPcTHSO.

Moreover, the content of the dye per particle was obtained. As a result, it was found that polymer nanoparticle 49 contained 3800 molecules of SiPcTHSO and 400 molecules of SiNPcTHSO; whereas, polymer nanoparticle 50 contained 150 molecules of SiPcTHSO and 30 molecules of SiNPcTHSO. From this, it was found that it is difficult to efficiently incorporate a donor dye and an acceptor dye into a polymer particle by the method of U.S. Patent No. 5,763,189. Since SiPcTHSO and SiNPcTHSO are less soluble in DMF, it is conceivably difficult to increase the concentration of them beyond the aforementioned concentrations. The results mentioned above are summarized in Table 4.

Table 4

Example	Fluorescence intensity (Em 781 nm)	Dye concentration/polymer (wt%)	Number of dye molecules per particle (number)
Polymer nanoparticles 49 (Invention)	2243	SiPcTHSO: 2.7 SiNPcTHSO: 0.3	SiPcTHSO: 3800 SiNPcTHSO: 400
Polymer nanoparticles 50 (U.S. P. No. 5,763,189)	19	SiPcTHSO: 0.054 SiNPcTHSO: 0.013	SiPcTHSO: 150 SiNPcTHSO: 30

As described in the foregoing, according to preferred embodiments of the present invention, because of a structure in which a fluorescent dye is dispersed in the matrix of a fluorescent polymer, it is possible to provide polymer nanoparticles having excellent FRET efficiency compared to the prior art.

Furthermore, according to another preferred embodiment of the present invention, because the polymer nanoparticles have a structure in which two types of fluorescent dyes are dispersed in the matrix of a polymer, it is possible to provide polymer nanoparticles having excellent FRET efficiency compared to the prior art.

The polymer nanoparticles according to preferred embodiments of the present invention can emit fluorescence excellent in penetration through a living body via FRET caused by a combination of a fluorescent dye emitting fluorescence within a near-infrared wavelength region of 600 nm or more and 1000 nm or less and a fluorescent polymer.

Furthermore, in the polymer nanoparticles according to preferred embodiments of the present invention, since the surface of the particles is protected by a surfactant, excellent dispersibility is shown even in an aqueous solution containing a salt such as physiological saline.

Furthermore, the polymer nanoparticles according to preferred embodiments of the present invention can serve as an extremely excellent contrast agent for optical molecular imaging by use of excellent FRET efficiency between a fluorescent polymer and a fluorescent dye of each of the nanoparticles.

Furthermore, the polymer nanoparticles according to another preferred embodiment of the present invention can serve as an extremely excellent contrast agent for optical molecular imaging by use of excellent FRET efficiency between two types of fluorescent dyes within each of the nanoparticles.

The present invention is directed to providing polymer nanoparticles excellent in FRET efficiency and dispersibility, and a method of manufacturing the same. Furthermore, the polymer nanoparticles of the present invention can emit fluorescence excellent in penetration through a living body via FRET caused by a combination of a fluorescent dye and a fluorescent polymer emitting fluorescence within a near-infrared wavelength region of 600 nm or more and 1000 nm or less or by a combination of two types of fluorescent dyes emitting fluorescence within a near-infrared wavelength region of 600 nm or more and 1000 nm or less, and used as a contrast agent for optical molecular imaging.

While the present invention has been described with reference to exemplary embodiments, it is to be understood that the invention is not limited to the disclosed exemplary embodiments. The scope of the following claims is to be accorded the broadest interpretation so as to encompass all such modifications and equivalent structures and functions.

This application claims the benefit of Japanese Patent Application No. 2008-050395, filed February 29, 2008, Japanese Patent Application No. 2008-120603, filed May 2, 2008 and Japanese Patent Application No. 2008-230140, filed September 8, 2008, which are hereby incorporated by reference herein in their entirety.

CLAIMS

1. Polymer nanoparticles each having a fluorescent dye dispersed in the matrix of a fluorescent polymer, wherein the surface of the nanoparticles is protected by a surfactant and fluorescence is emitted via fluorescence resonance energy transfer (FRET) between the fluorescent polymer and the fluorescent dye.

2. The polymer nanoparticles according to claim 1, wherein the fluorescent polymer is a conjugated polymer.

3. Polymer nanoparticles each having two types of fluorescent dyes dispersed in the matrix of a polymer, wherein the surface of the nanoparticles is protected by a surfactant and fluorescence is emitted via FRET between the two types of fluorescent dyes.

4. The polymer nanoparticles according to any one of claims 1 to 3, wherein the fluorescence emitted via FRET has a near-infrared wavelength of 600 nm or more and 1000 nm or less.

5. The polymer nanoparticles according to any one of claims 1 to 4, wherein the surfactant is a nonionic surfactant.

6. The polymer nanoparticles according to any one of claims 1 to 5, wherein an average particle size of the nanoparticles is 10 nm or more and 200 nm or less.

7. A contrast agent for optical molecular imaging, containing the polymer nanoparticles according to any one of claims 1 to 6.

FIG. 1

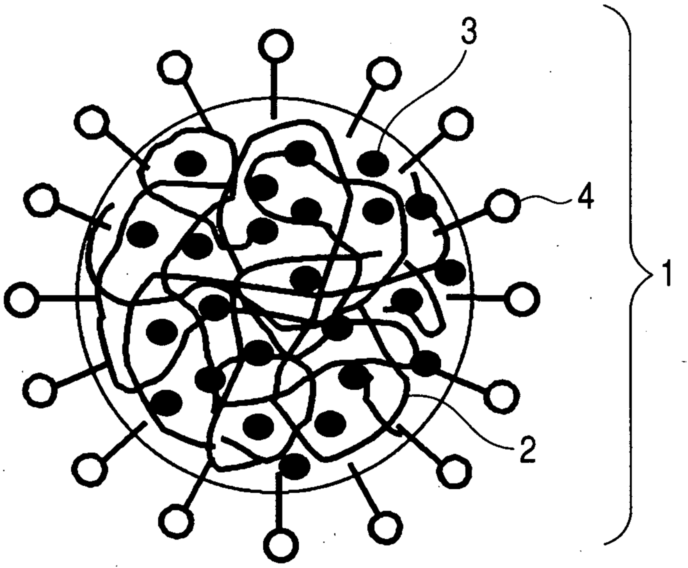


FIG. 2

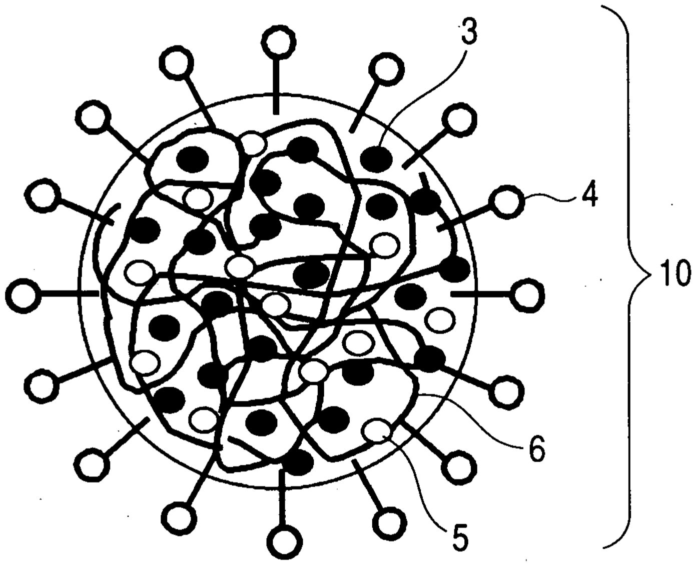


FIG. 3

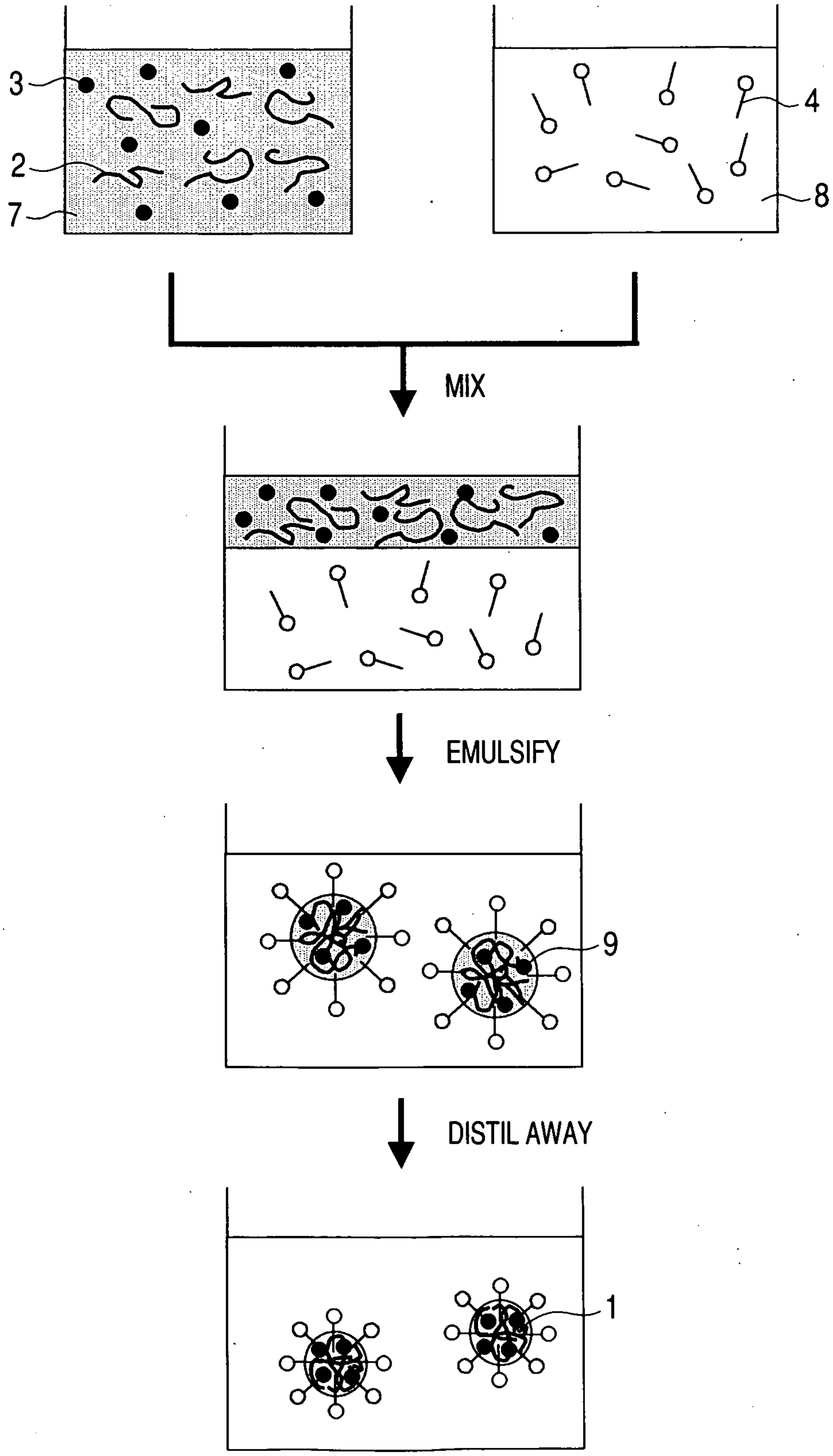


FIG. 4

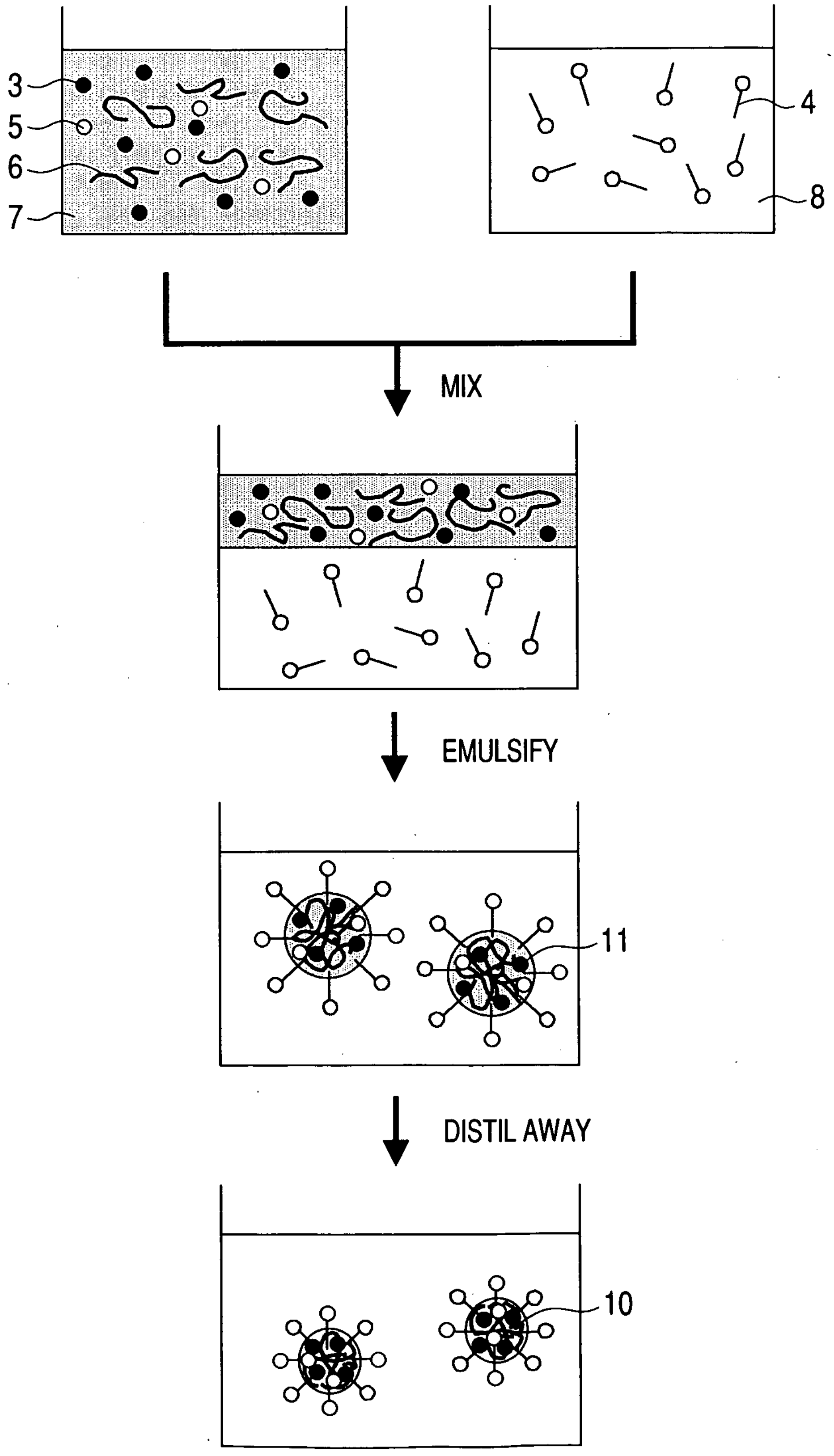


FIG. 5

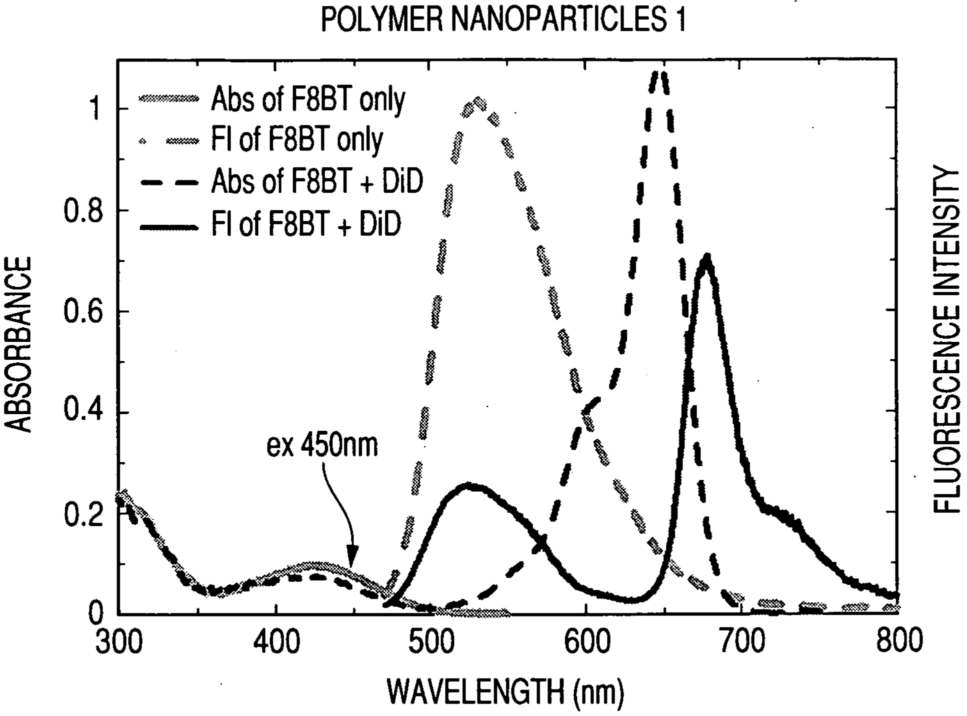

POLYMER NANOPARTICLES 1
Abs of F8BT only
Fl of F8BT only
Abs of F8BT + DiD
Fl of F8BT + DiD
ex 450nm
ABSORBANCE
FLUORESCENCE INTENSITY
WAVELENGTH (nm)
0
0.2
0.4
0.6
0.8
1
300
400
500
600
700
800

FIG. 6

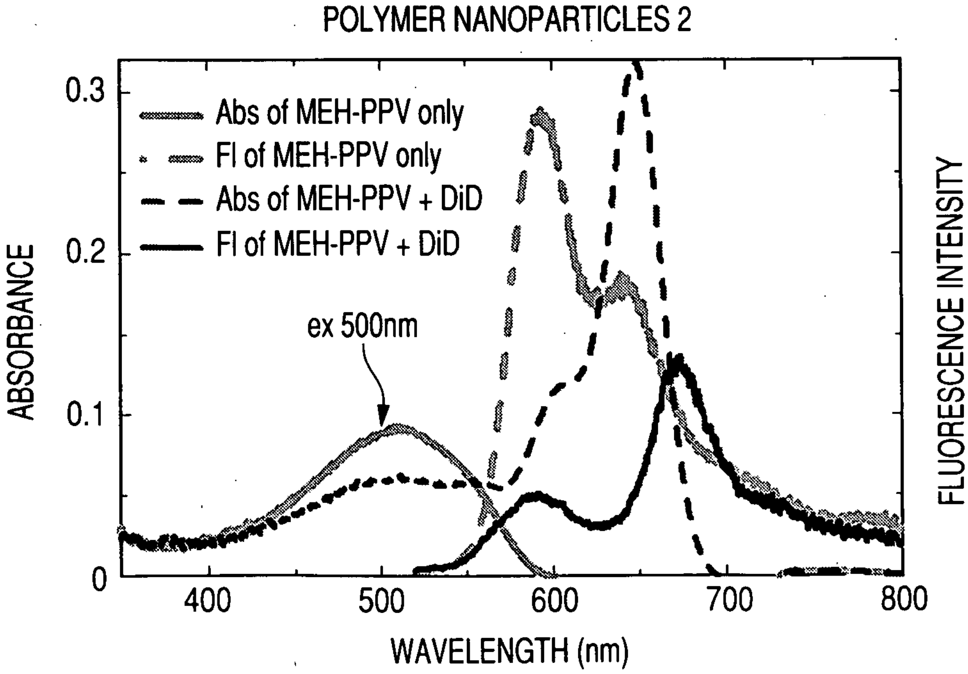

POLYMER NANOPARTICLES 2
Abs of MEH-PPV only
Fl of MEH-PPV only
Abs of MEH-PPV + DiD
Fl of MEH-PPV + DiD
ex 500nm
ABSORBANCE
FLUORESCENCE INTENSITY
WAVELENGTH (nm)
0
0.1
0.2
0.3
400
500
600
700
800

FIG. 7

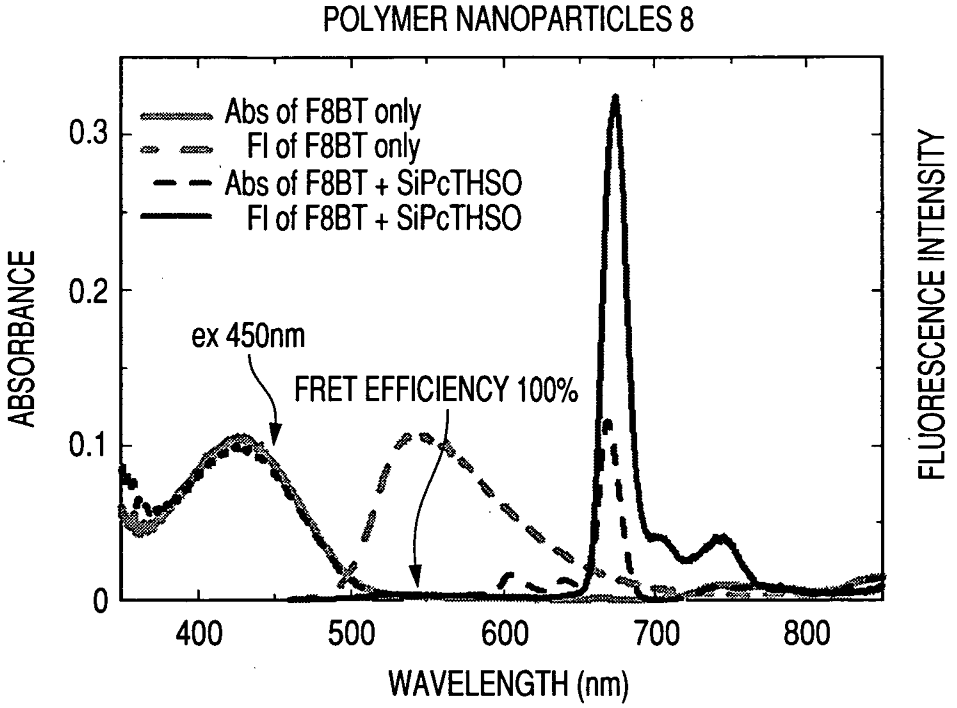

POLYMER NANOPARTICLES 8
Abs of F8BT only
Fl of F8BT only
Abs of F8BT + SiPcTHSO
Fl of F8BT + SiPcTHSO
ex 450nm
FRET EFFICIENCY 100%
ABSORBANCE
FLUORESCENCE INTENSITY
0
0.1
0.2
0.3
400
500
600
700
800
WAVELENGTH (nm)

FIG. 8

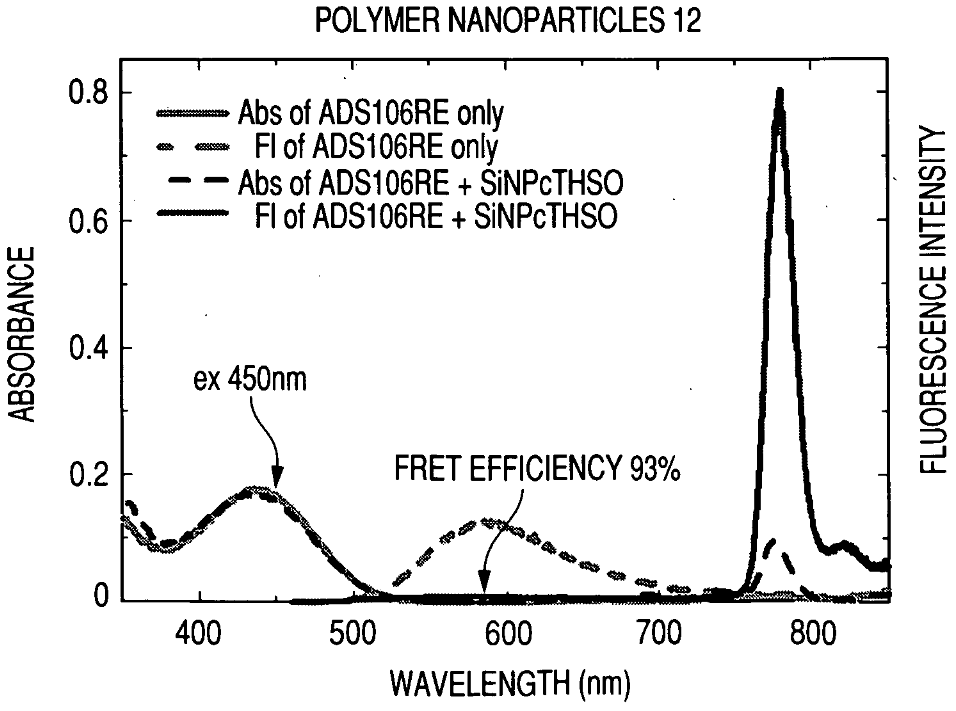

POLYMER NANOPARTICLES 12
Abs of ADS106RE only
Fl of ADS106RE only
Abs of ADS106RE + SiNPcTHSO
Fl of ADS106RE + SiNPcTHSO
ex 450nm
FRET EFFICIENCY 93%
ABSORBANCE
FLUORESCENCE INTENSITY
0
0.2
0.4
0.6
0.8
400
500
600
700
800
WAVELENGTH (nm)

FIG. 9

POLYMER NANOPARTICLES 18

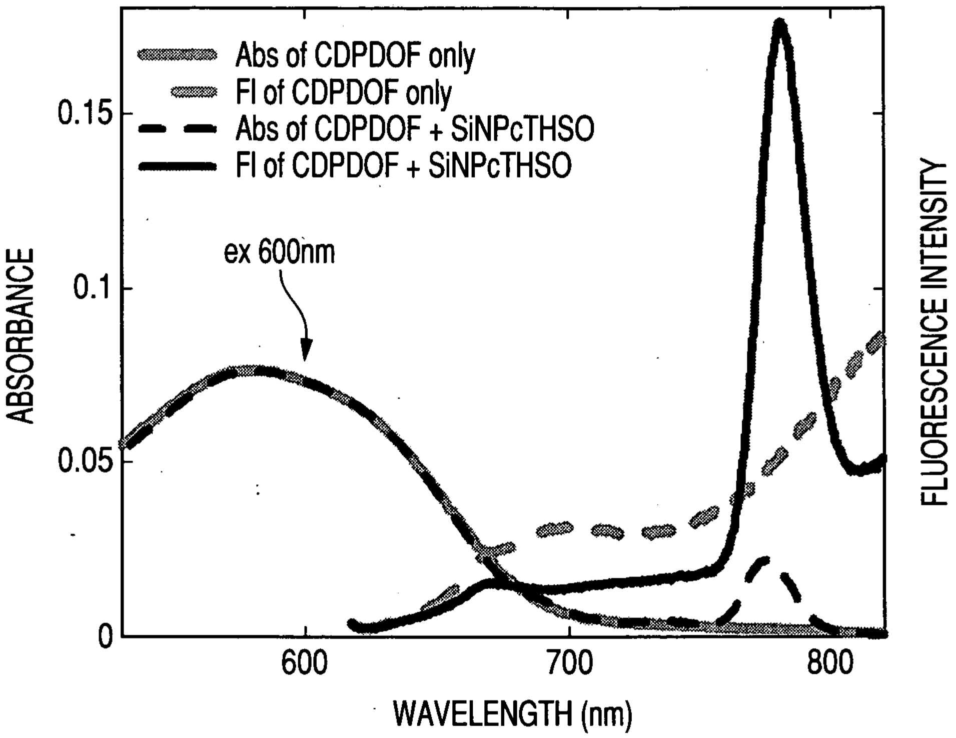
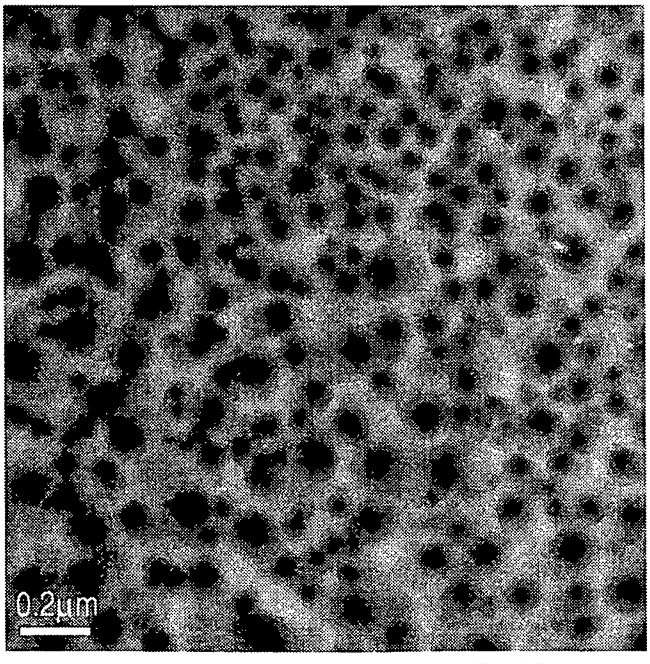


FIG. 10

TEM PHOTOGRAPH OF POLYMER NANOPARTICLES 19



(SCALE BAR: 200nm)

FIG. 11

FLUORESCENT IMAGE OF POLYMER NANOPARTICLES 37

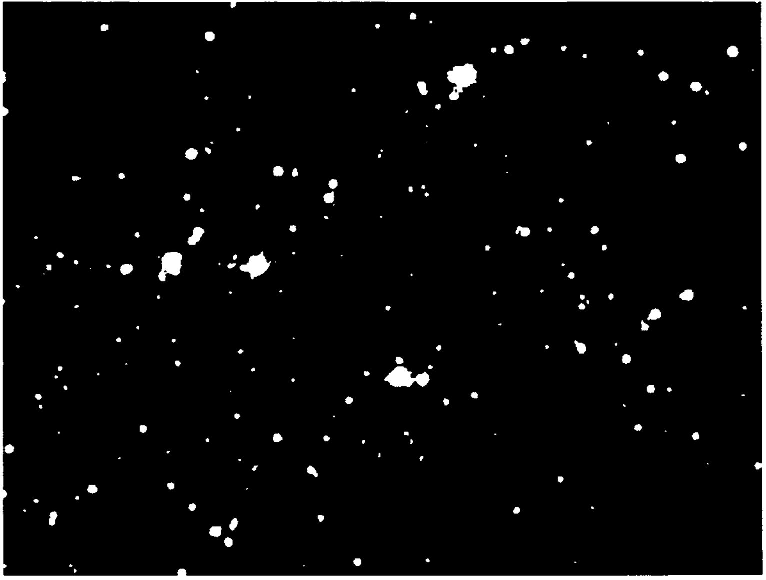


FIG. 12

FLUORESCENT IMAGE OF QD565

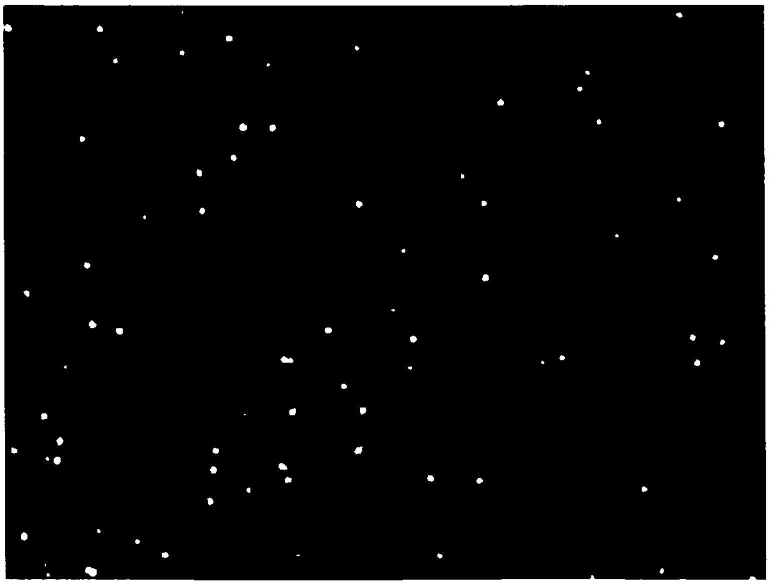
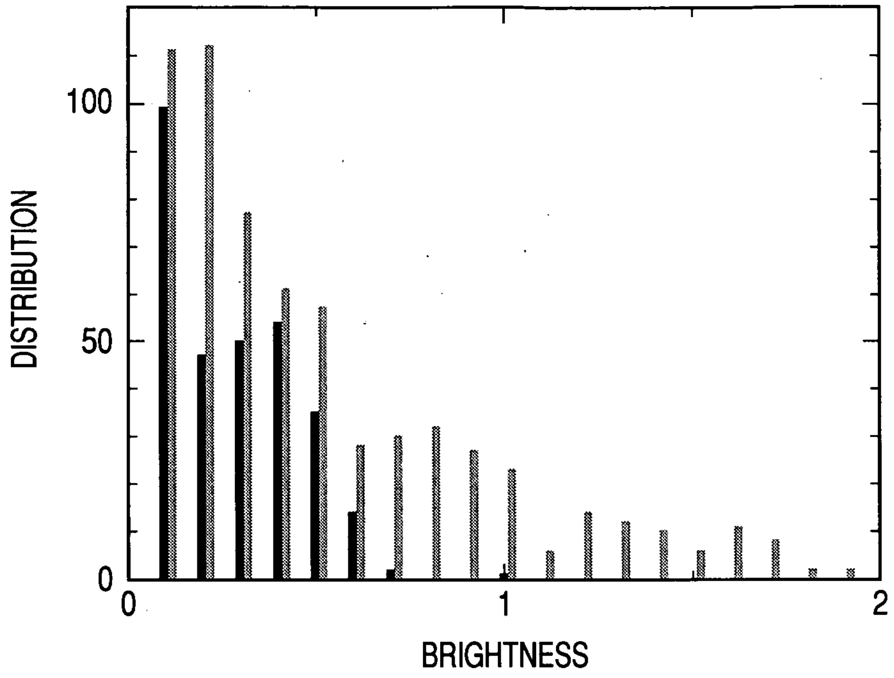


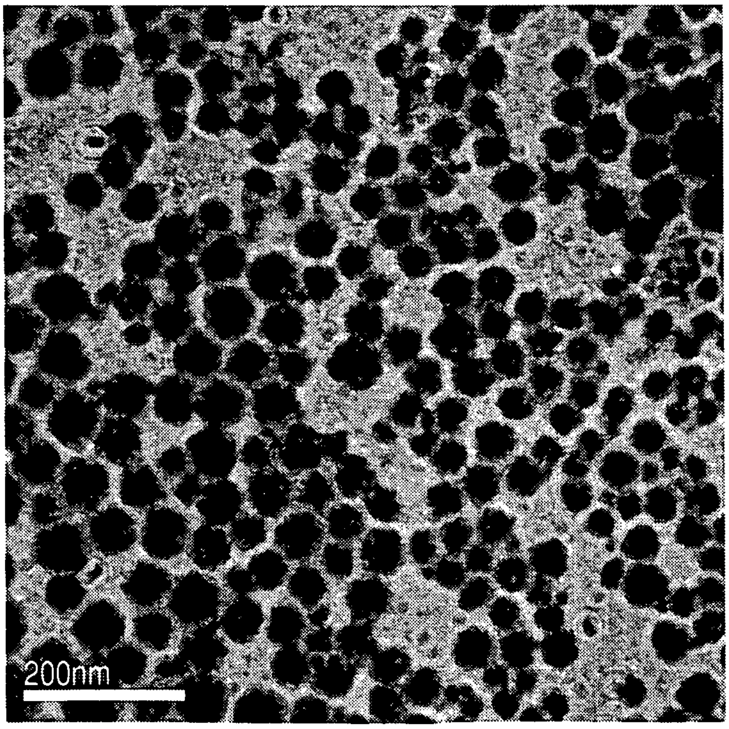
FIG. 13



BRIGHTNESS HISTOGRAM (GREY REPRESENTS POLYMER NANOPARTICLES 37; BLACK REPRESENTS QD565)

FIG. 14

TEM PHOTOGRAPH OF POLYMER NANOPARTICLES 38



(SCALE BAR: 200nm)

FIG. 15

FLUORESCENT IMAGE OF POLYMER NANOPARTICLES 38

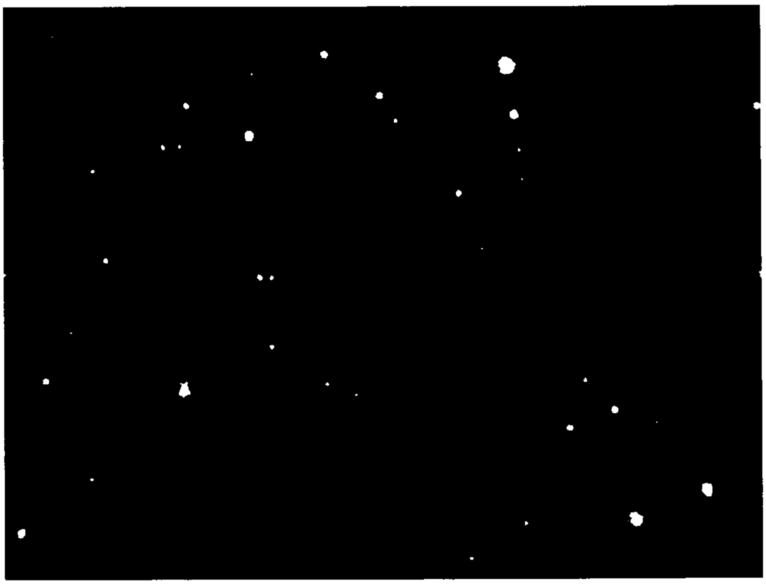


FIG. 16

FLUORESCENT IMAGE OF QD565

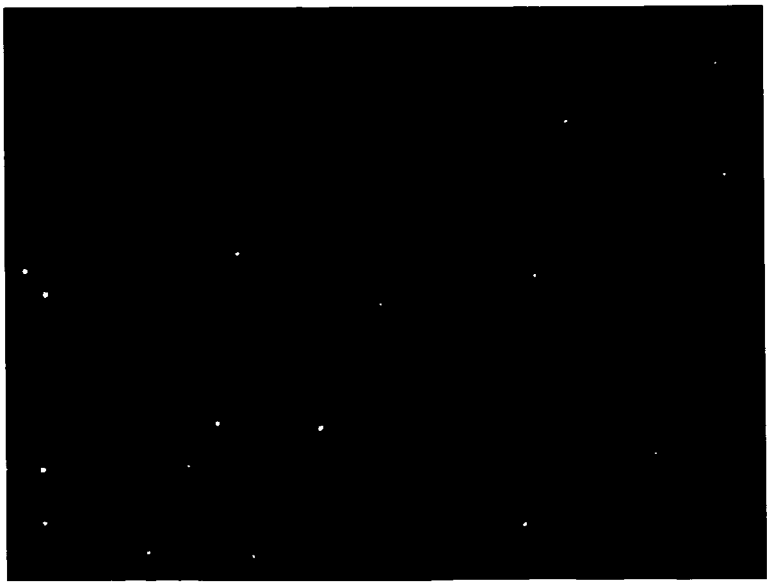
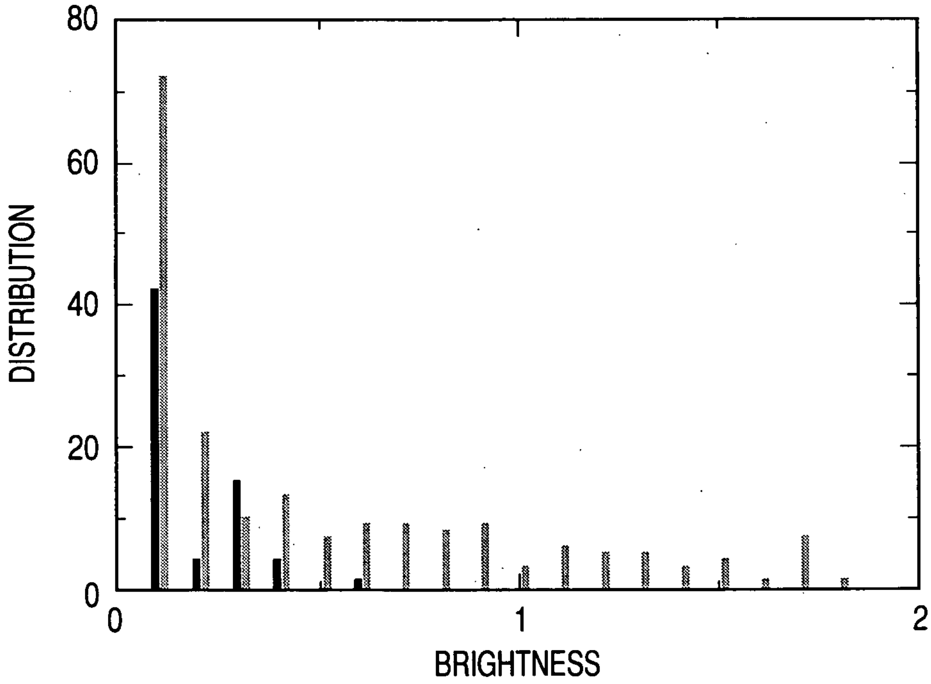


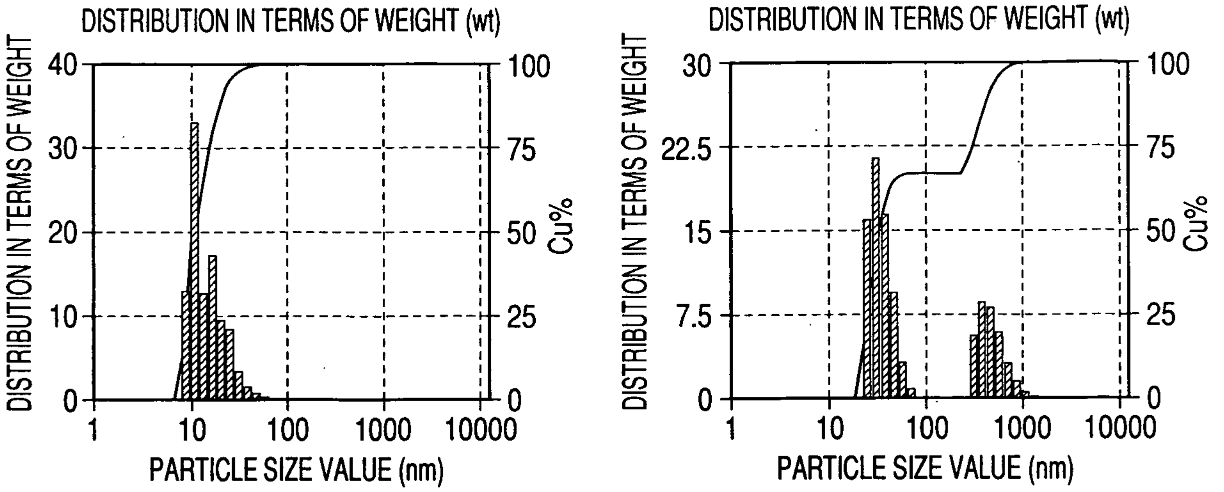
FIG. 17



BRIGHTNESS HISTOGRAM (GREY REPRESENTS POLYMER NANOPARTICLES 38; BLACK REPRESENTS QD565)

FIG. 18

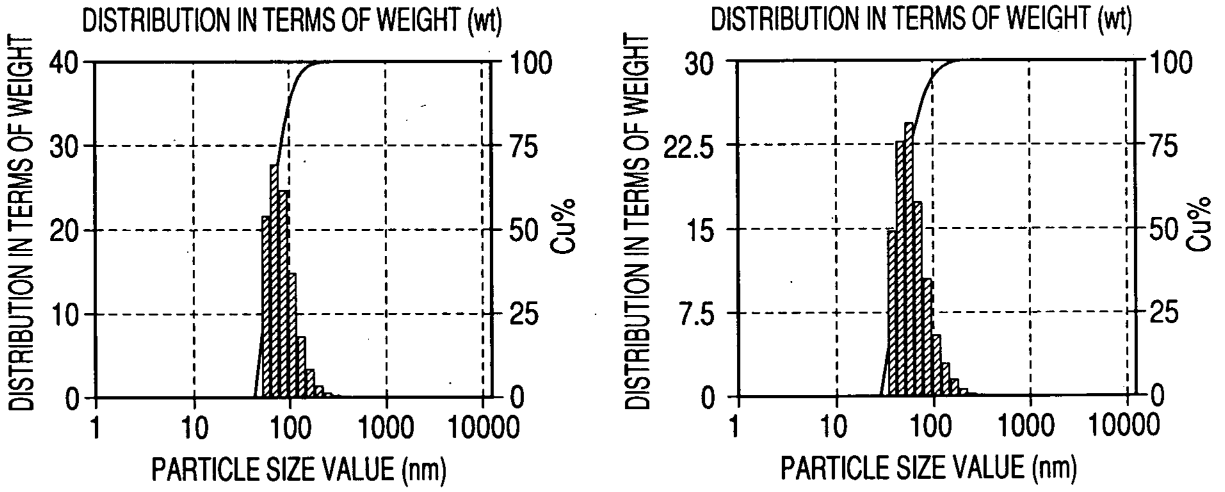
POLYMER NANOPARTICLES 39



BEFORE ADDITION OF AQUEOUS SODIUM CHLORIDE SOLUTION

AFTER STANDSTILL OVERNIGHT

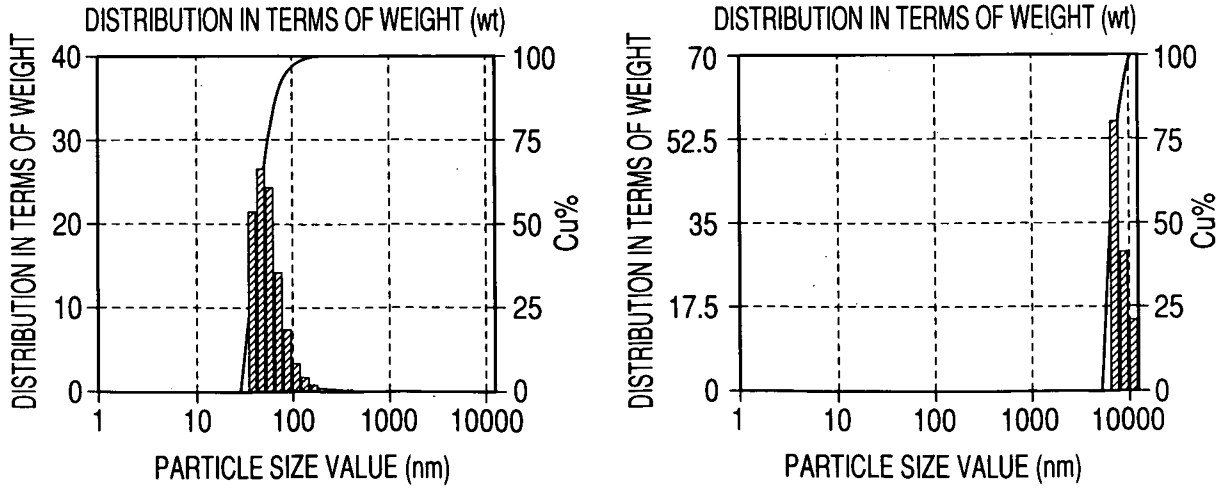
POLYMER NANOPARTICLES 40



BEFORE ADDITION OF AQUEOUS SODIUM CHLORIDE SOLUTION

AFTER STANDSTILL OVERNIGHT

POLYMER NANOPARTICLES 41

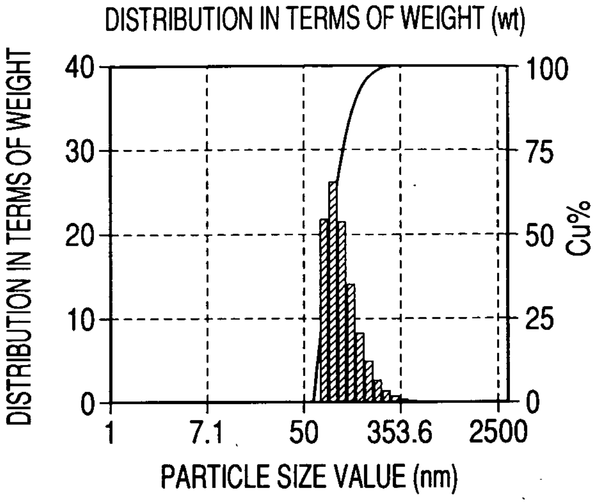


BEFORE ADDITION OF AQUEOUS SODIUM CHLORIDE SOLUTION

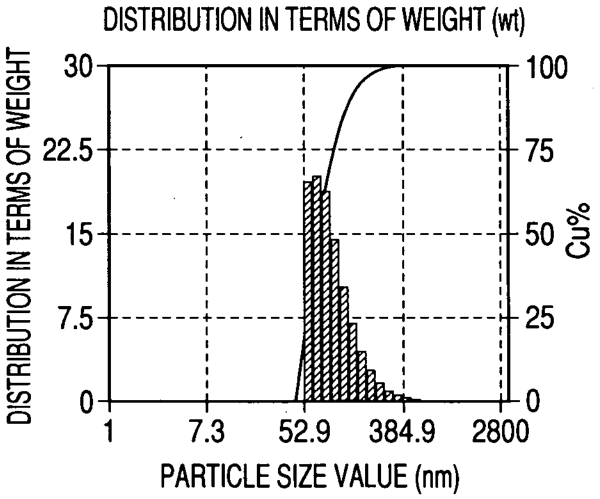
AFTER STANDSTILL OVERNIGHT

FIG. 19

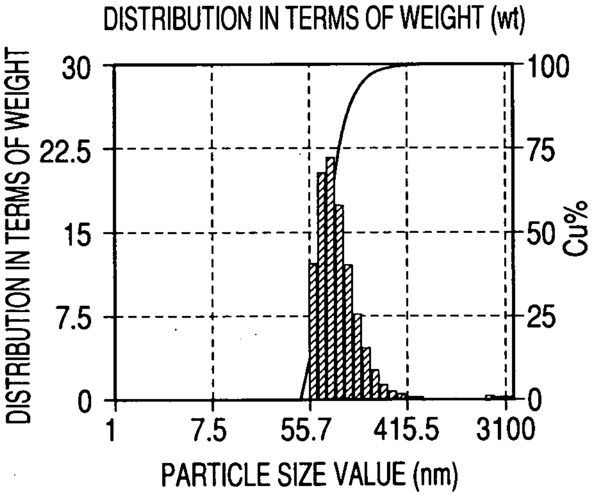
POLYMER NANOPARTICLES 18



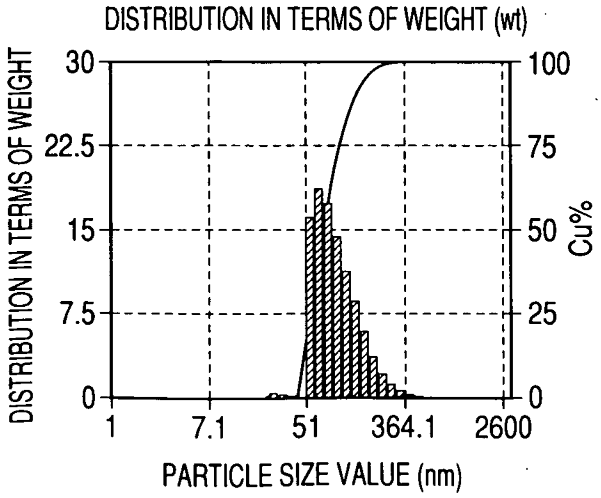
ORIGINAL



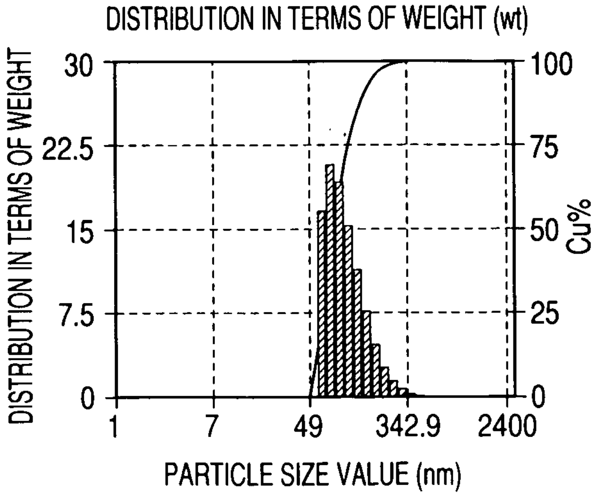
IMMEDIATELY AFTER PBS ADDITION



ONE DAY AFTER PBS ADDITION



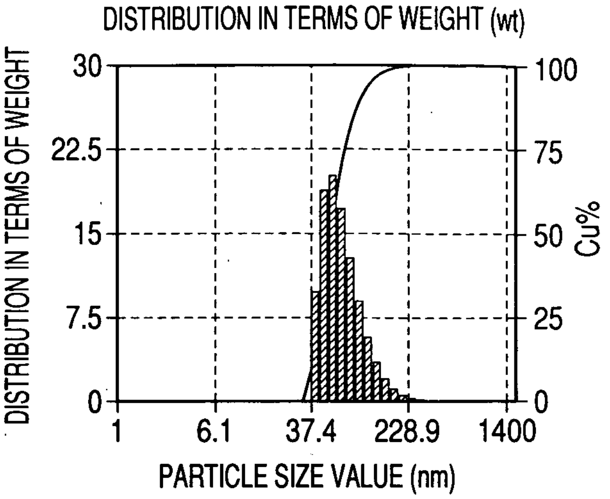
IMMEDIATELY AFTER FBS ADDITION



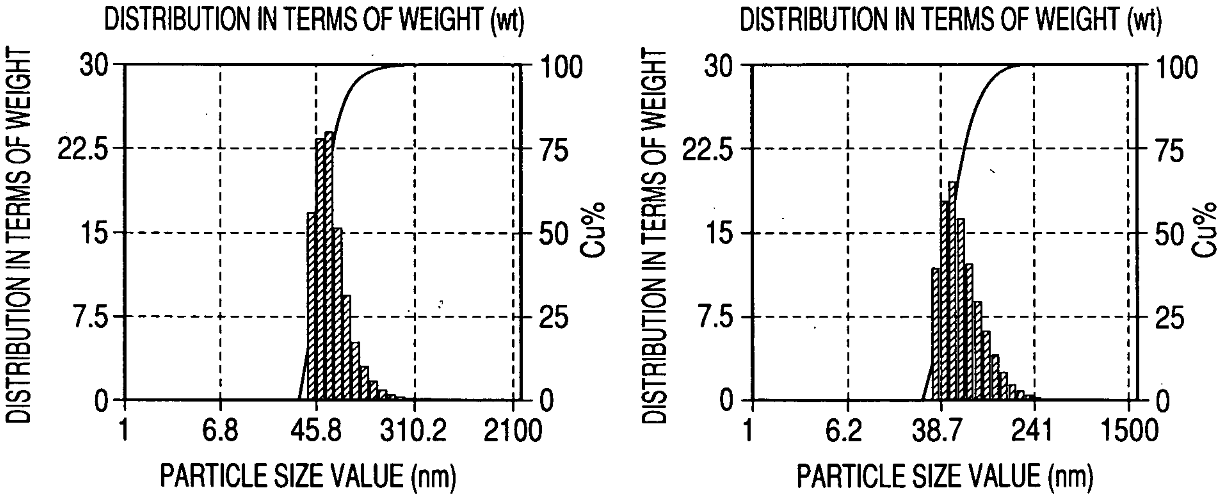
ONE DAY AFTER FBS ADDITION

FIG. 20

POLYMER NANOPARTICLES 40

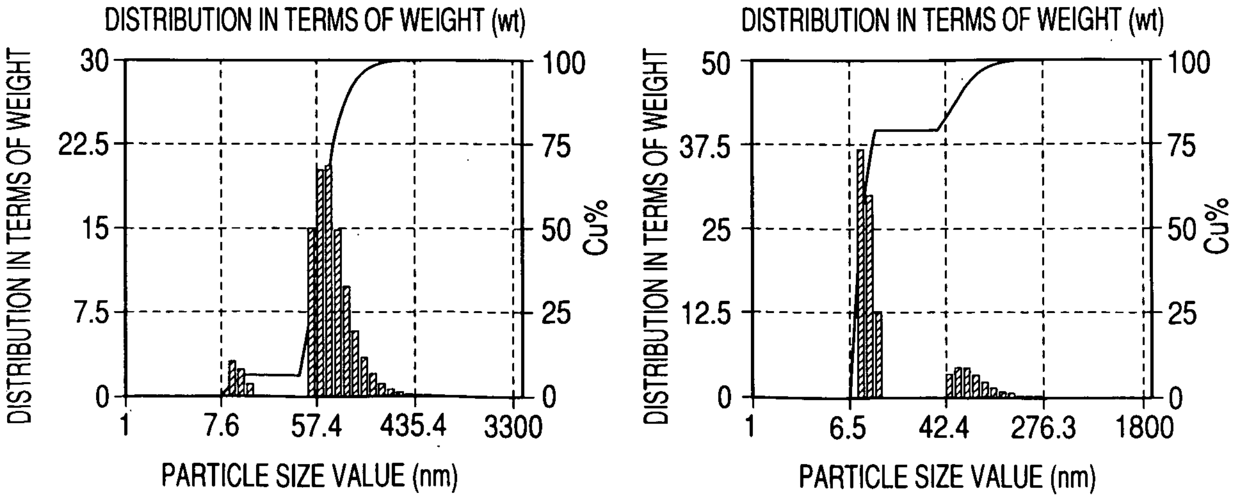


ORIGINAL



IMMEDIATELY AFTER PBS ADDITION

ONE DAY AFTER PBS ADDITION

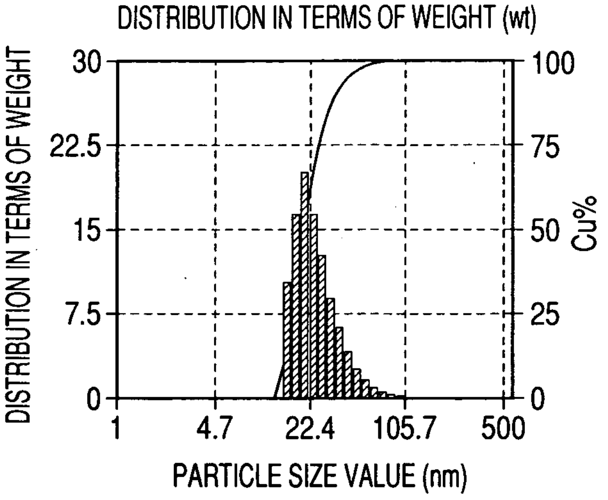


IMMEDIATELY AFTER FBS ADDITION

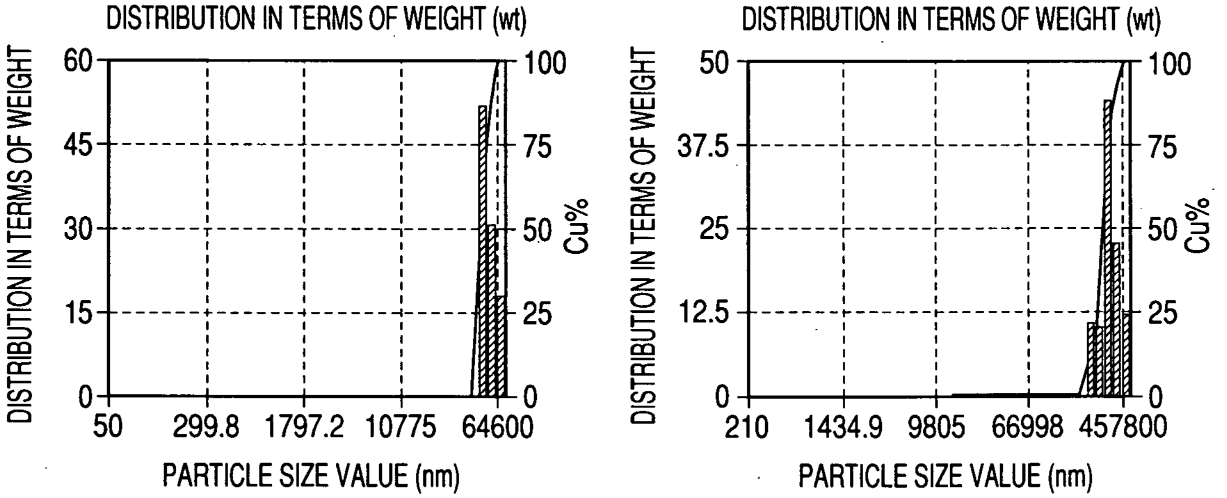
ONE DAY AFTER FBS ADDITION

FIG. 21

POLYMER NANOPARTICLES 41

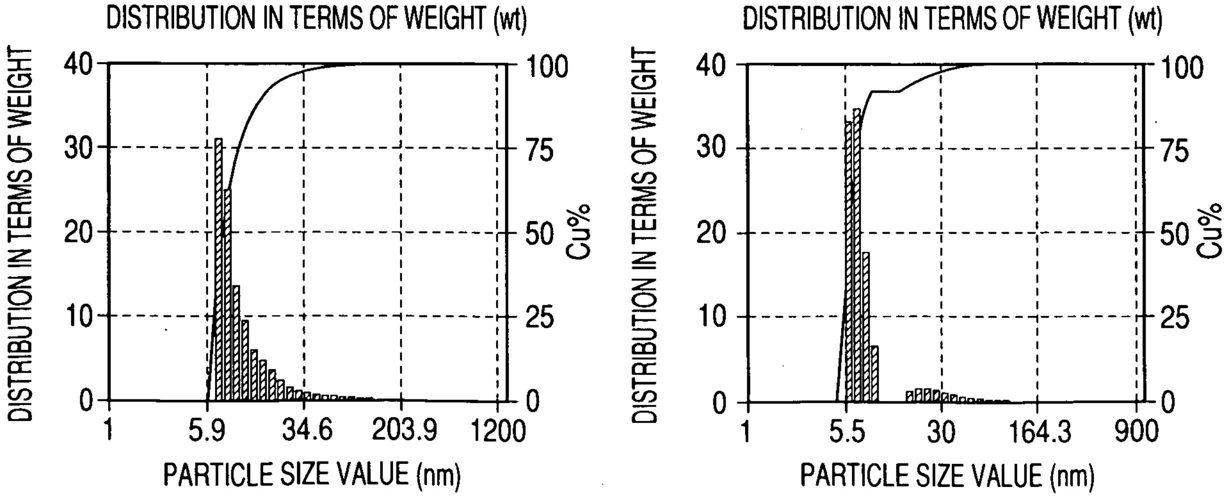


ORIGINAL



IMMEDIATELY AFTER PBS ADDITION

ONE DAY AFTER PBS ADDITION

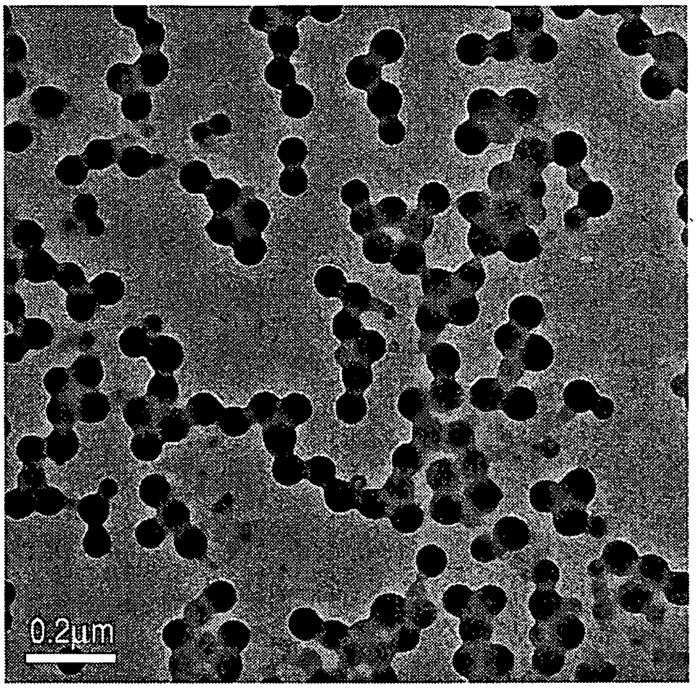


IMMEDIATELY AFTER FBS ADDITION

ONE DAY AFTER FBS ADDITION

FIG. 22

TEM PHOTOGRAPH OF POLYMER NANOPARTICLES 42



(SCALE BAR: 200nm)

FIG. 23

POLYMER NANOPARTICLES 42

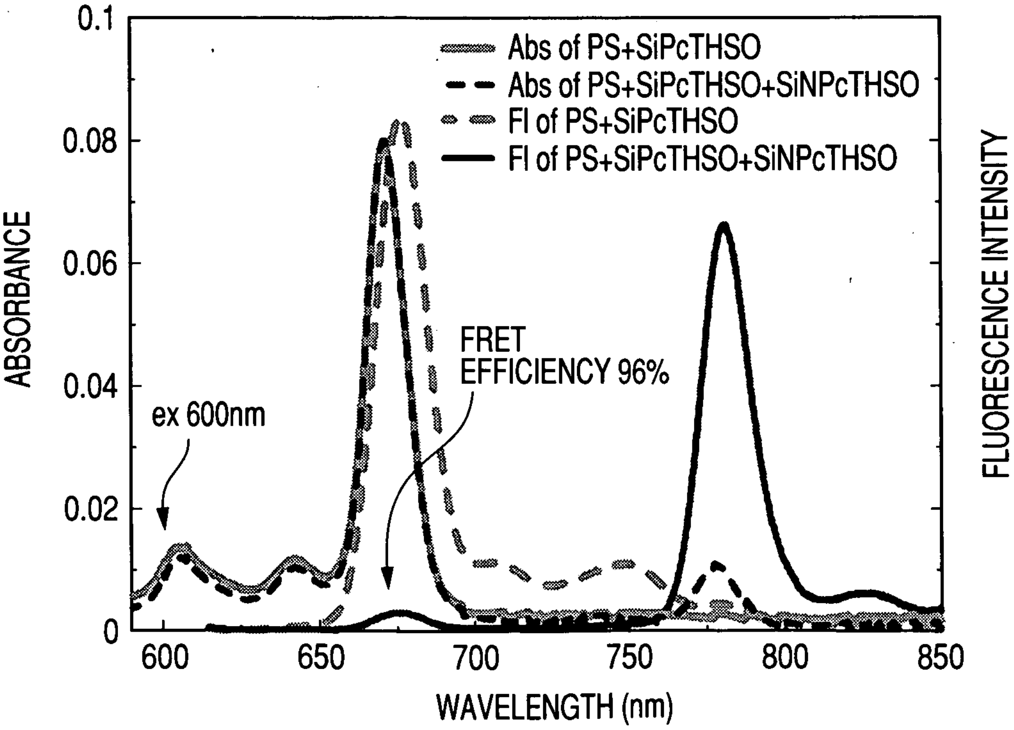


FIG. 24

FLUORESCENT IMAGE OF POLYMER NANOPARTICLES 42

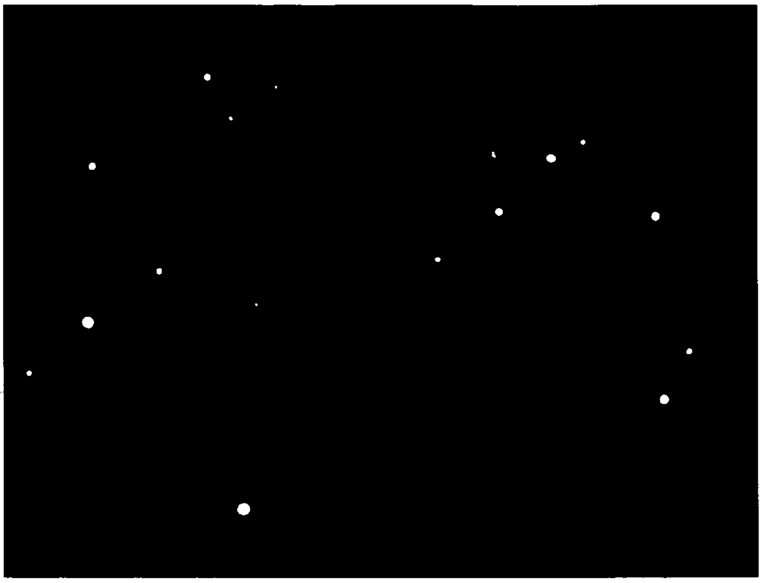


FIG. 25

FLUORESCENT IMAGE OF QD800

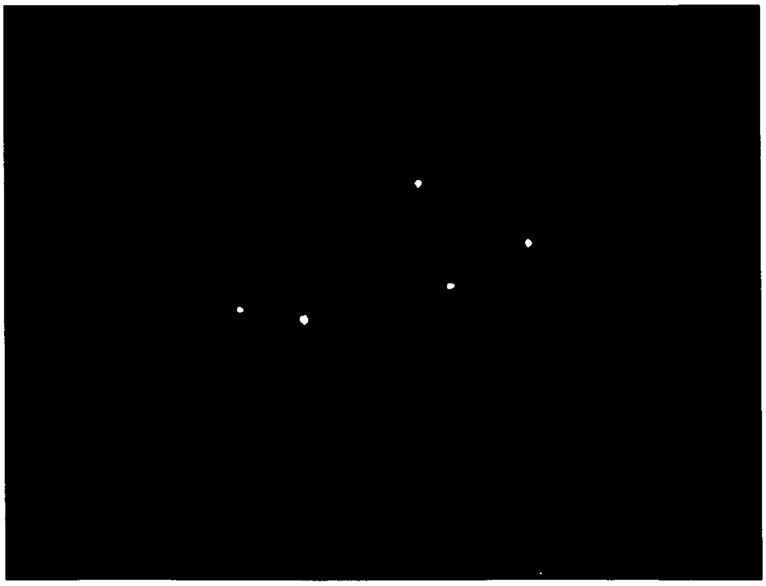
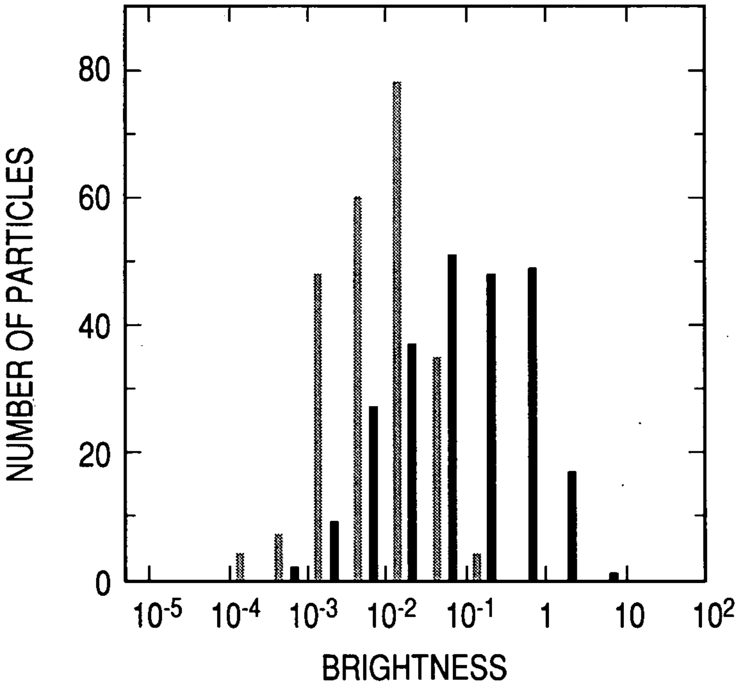


FIG. 26



BRIGHTNESS HISTOGRAM (BLACK REPRESENTS POLYMER NANOPARTICLES 42; GRAY REPRESENTS QD800)

FIG. 27

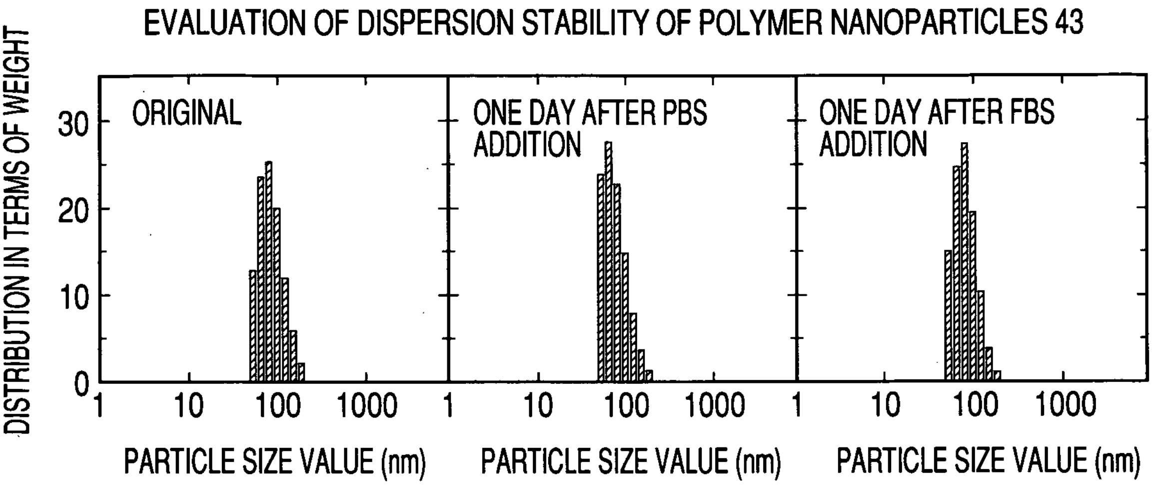
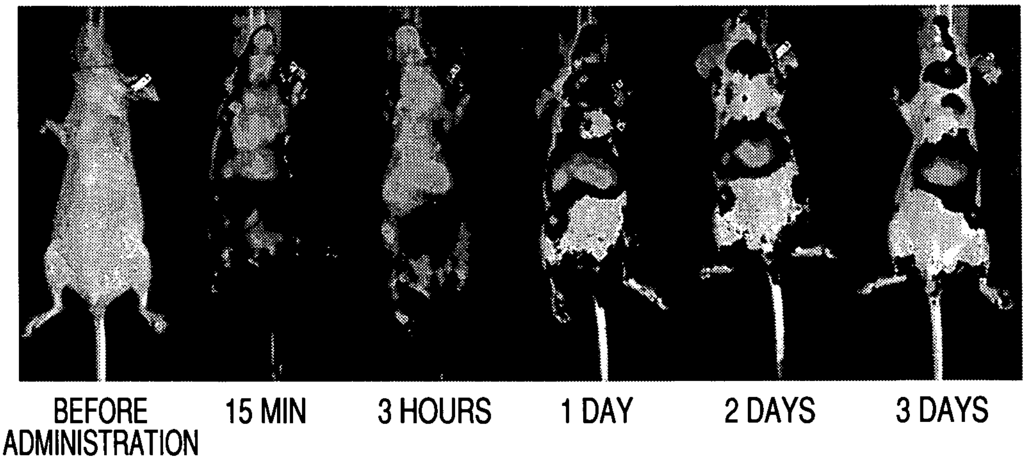


FIG. 28

FLUORESCENT IMAGE OF NUDE MOUSE ADMINISTERED WITH POLYMER NANOPARTICLES 42



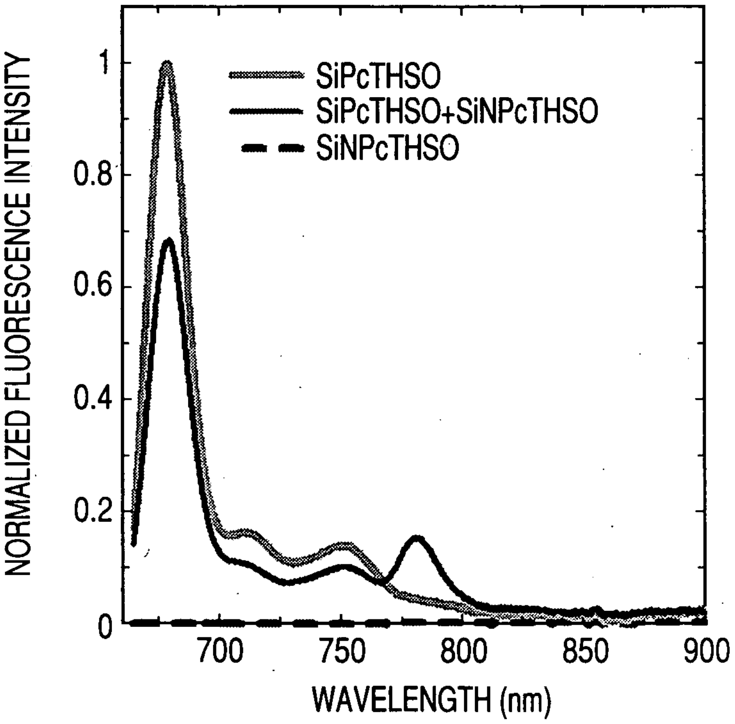
IMAGING RESULTS OF MOUSE
(SUBTRACTING Bkg FILTER FROM FLUORESCENCE FILTER)

A-B* 1.7
A: FLUORESCENCE FILTER (Ex: 675/Em780)
B: Bkg FILTER (Ex: 640/Em780)

Max 2.2e+07, Min 7e+07

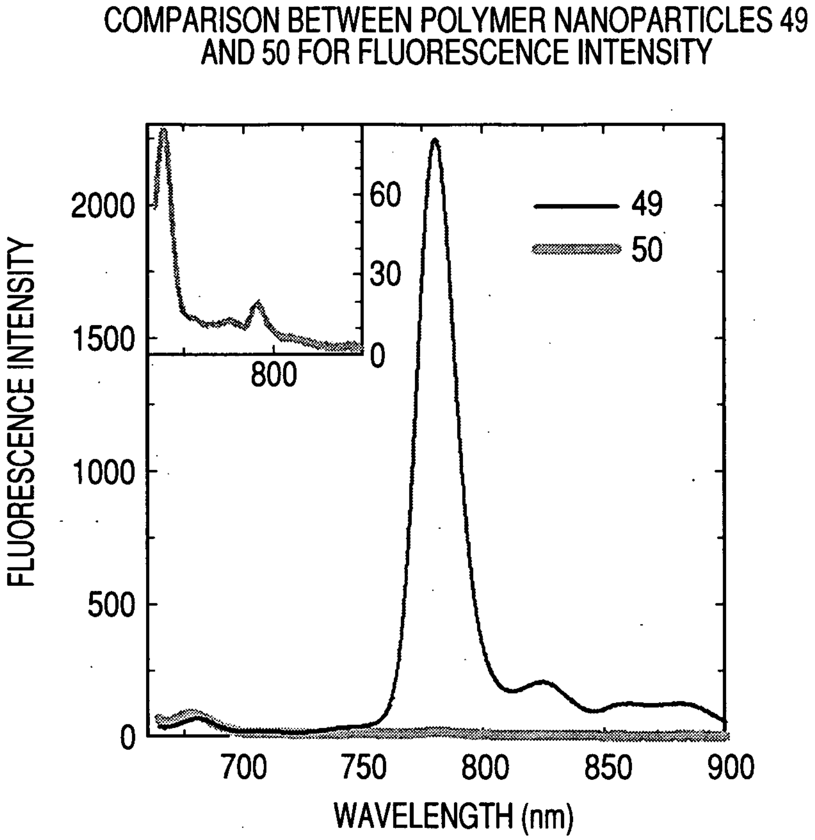
FIG. 29

NORMALIZED FLUORESCENCE SPECTRA OF POLYMER NANOPARTICLES SYNTHESIZED ACCORDING TO U.S.PATENT No. 5,763,189 (Ex650nm)



SiPcTHSO: POLYMER NANOPARTICLES CONTAINING SiPcTHSO
SiNPcTHSO: POLYMER NANOPARTICLES CONTAINING SiNPcTHSO
SiPcTHSO+SiNPcTHSO: POLYMER NANOPARTICLES 48 CONTAINING SiPcTHSO AND SiNPcTHSO

FIG. 30



49: POLYMER NANOPARTICLES 49
50: POLYMER NANOPARTICLES 50

FIG. 31

FLUORESCENT IMAGE OF NUDE MOUSE ADMINISTERED WITH POLYMER NANOPARTICLES 18