605214

FORM 1

REGULATION 9

LODGED AT SUB-OFFICE
- 9 MAY 1988
Sydney

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952

APPLICATION FOR A STANDARD PATENT

We, RHONE-POULENC CHIMIE, of 25, quai Paul Doumer, 92408, Courbevoie, France, hereby apply for the grant of a Standard Patent for an invention entitled:-

PARTICLES HAVING MOLECULES OF AN AMPHIPHILE COMPOUND IN THEIR SURFACE AND METHOD OF PREPARATION--. POLYMER

which is described in the accompanying Complete Specification.

Details of basic application:-

Number: 87/06549

Country: France

Date: 11th May, 1987

APPLICATION ACCEPTED AND AMENDMENTS ALLOWED 16 - 8 - 90

Our address for service is: SHELSTON WATERS
55 Clarence Street
SYDNEY, N.S.W. 2000.

DATED this 9th day of May, 1988
RHONE-POULENC CHIMIE

by W Hlloyd

Fellow Institute of Patent Attorneys of Australia
of SHELSTON WATERS

To: The Commissioner of Patents
WODEN A.C.T. 2606

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PATENT OFFICE ONE HUNDRED DOLLARS - 9 MAY 1988 Sydney

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PATENT OFFICE TEN DOLLARS

AUSTRALIAN PATENT OFFICE LS

CONVENTION APPLICATION BY A COMPANY

FORM 8 - REGULATION 12 (2)

AUSTRALIA
PATENTS ACT 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT

In support of the Convention Application made by .....

(a) Here insert (in full) Name of Company. (a) RHONE-POULENC CHIMIE

(hereinafter referred to as "Applicant") for a patent for an invention entitled:

(b) Here insert Title of Invention. (b) "POLYMER PARTICLES IMPLANTED IN THEIR SURFACE MOLECULES WITH AN AMPHIPHILE COMPOUND AND METHOD OF PREPARATION"

(c) and (d) Here insert Full Name and Address of Company Official authorised to make declaration.

I, (c) Madeleine-France FABRE

of (d) RHONE-POULENC
25, quai Paul Doumer, 92408, Courbevoie, France

do solemnly and sincerely declare as follows:

1. I am authorised by Applicant to make this declaration on its behalf.

2. The basic Application(s) as defined by section 141 of the Act was ~~were~~ made

(e) Here insert Basic Country followed by date of Basic Application.

in (e) FRANCE on the 11th day of May, 19 87

(f) Here insert Full Name(s) of Applicant(s) in Basic Country.

by (f) RHONE-POULENC CHIMIE

in ..... on the ..... day of 19

by .....

in ..... on the ..... day of 19

by .....

in ..... on the ..... day of 19

by .....

(g) Here insert (in full) Name and Address of actual Inventor or Inventors.

3. (g) BERNARD CHAUVEL, of 24, chemin de la Commanderie - 95120 - ERMONT, FRANCE; JEAN-CLAUDE DANIEL, of 13, rue de Neuilly - 94120 - FONTENAY/SOUS/BOIS, FRANCE and CHRISTIAN PUSINERI, of 33, rue des Fleurs - Serezin du Rhone - 69360 - SAINT SYMPHORIEN D'OZON, FRANCE

~~is~~/are the actual Inventor(s) of the invention and the facts upon which Applicant is entitled to make the Application are as follows:

See reverse side of this form for guidance in completing this part.

Applicant is the Assignee of the said Inventors.

4. The basic Application(s) referred to in paragraph 2 of this Declaration was/~~were~~ the first Application(s) made in a Convention country in respect of the invention, the subject of the Application.

DECLARED at COURBEVOIE

this 5 day of July 19 88

(h) Personal Signature of Declarant (c) (no seal, witness or legalisation).

(h) Madeleine-France Fabre
Madeleine-France FABRE
(Signature of Declarant)

To THE COMMISSIONER OF PATENTS.

(12) PATENT ABRIDGMENT (11) Document No. AU-B-15832/88
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 603214

(54) Title
POLYMER PARTICLES HAVING MOLECULES OF AN AMPHIPHILE COMPOUND IN THEIR SURFACE

International Patent Classification(s)
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(57) Claim

1. A polymer particle comprising a macromolecular polymeric chain having a Tg glass transition temperature of greater than about 40°C and an amphiphile compound implanted in the surface of the particle, said amphiphile compound having an HLB of at least 10 and a molecular mass of at least 400, said amphiphile compound comprising a) a hydrophobic sequence enmeshed within the polymeric chain in the surface layer of the particle and b) a hydrophilic oligomer sequence terminating in at least one ionogenic or reactive group, whereby said polymer particle comprises about 10^{-5} to 10^{-1} moles of said amphiphile compound per 100 g of polymeric chain and has a diameter of from 0.01 to 50 microns.

6. An aqueous dispersion of polymer particles, comprising from 1 to 50% by weight of the particles according to anyone of the claims 1 to 5.

.../2

8. A method of preparation of an aqueous dispersion according to claim 6 or 7 comprising the steps of:

a) selecting an amphiphile compound having an HLB of at least 10 and a molecular mass of least 400 and polymer particles each comprising a polymeric chain and having a diameter of 0.01 to 50 microns and a Tg glass transition temperature of greater than 40°C,

b) placing in contact about 10^{-4} to 1 mole of said amphiphile compound per 100 g of said polymer particles with a latex comprising from 1-50% by weight of said polymer particles, at a temperature between the glass transition zone of the polymer, until the polymeric chains and the hydrophobic sequences of the amphiphile compound become enmeshed and,

c) eliminating all the amphiphile compound not fixedly enmeshed.

COMMONWEALTH OF AUSTRALIA 603214

FORM 10

PATENTS ACT 1952

COMPLETE SPECIFICATION

FOR OFFICE USE:

Class Int.Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority:

Related Art:

This document contains the amendments made under Section 49 and is correct for printing.

Name of Applicant: RHONE-POULENC CHIMIE

Address of Applicant: 25, quai Paul Doumer, 92408, Courbevoie, France

Actual Inventor: Bernard Chauvel, Jean-Claude Daniel and Christian Pusineri

Address for Service: SHELSTON WATERS, 55 Clarence Street, Sydney

Complete Specification for the Invention entitled:

POLYMER PARTICLES HAVING MOLECULES OF AN AMPHIPHILE COMPOUND IN THEIR SURFACE AND METHOD OF PREPARATION--.

The following statement is a full description of this invention, including the best method of performing it known to me/us:-

POLYMER PARTICLES HAVING MOLECULES OF AN AMPHIPHILE COMPOUND ~~IMPLANTED~~ IN THEIR SURFACE AND METHOD OF PREPARATION.

This invention relates to polymer particles having, implanted in their surface, amphiphile molecules bearing ionogenic or reactive groups. The invention also relates to aqueous dispersions of these particles, a method for preparing the particles and their application in biology.

It is an object of the invention to provide polymer particles bearing ionogenic or reactive units at a sufficient distance from the surface of the particle so that the particle is capable of immobilizing, on its surface, encumbering molecules such as proteins without the activity of these encumbering molecules being modified.

In a first aspect, the invention relates to a polymer particle comprising a macromolecular polymeric chain having a Tg glass transition temperature of greater than about 40°C and an amphiphile compound implanted in the surface of the particle, said amphiphile compound having an HLB of at least 10 and a molecular mass of at least 400, said amphiphile compound comprising a) a hydrophobic sequence enmeshed within the polymeric chain in the surface layer of the particle and b) a hydrophilic oligomer sequence terminating in at least one ionogenic or reactive group, whereby said polymer particle comprises about 10^{-5} to 10^{-1} moles of said amphiphile compound

per 100 grams of polymeric chain and has a diameter of from 0.01 to 50 microns.

In a second aspect, the invention relates to a method of preparation of an aqueous dispersion of polymer particles according to the first aspect, said method comprising the steps of:

a) selecting an amphiphile compound having an HLB of at least 10 and a molecular mass of least 400 and polymer particles each comprising a polymeric chain and having a diameter of 0.01 to 50 microns and a Tg glass transition temperature of greater than 40°C,

b) placing in contact about 10^{-4} to 1 mole of said amphiphile compound per 100 g of said polymer particles with a latex comprising from 1-50% by weight of said polymer particles, at a temperature between the glass transition zone of the polymer, until the polymeric chains and the hydrophobic sequences of the amphiphile compound become enmeshed and,

c) eliminating all the amphiphile compound not fixedly enmeshed.

In a third aspect the invention relates to a method of preparation of polymer particles according to claim 1, said method comprising the steps of:

a) selecting an amphiphile compound having an HLB of at least 10 and a molecular mass of at least 400 and polymer particles each comprising a polymeric chain and having a diameter of 0.01 to 50 microns and a Tg glass transition temperature of greater than 40°C;

b) placing in contact about 10^{-4} to 1 mole of said amphiphile compound per 100 g of said polymer particles with a latex of said polymer particles, at a temperature between the glass transition zone of the polymer, until the polymeric chains and the hydrophobic sequences of the amphiphile compound become enmenshed,

c) eliminating all the amphiphile compound not fixedly enmeshed, and

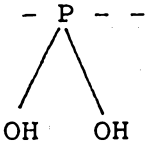
d) separating the particles obtained.

Polymers suitable for use in the present invention include monopolymers and copolymers containing units derived from:

- vinylaromatic monomers such as styrene and vinyltoluene;
- unsaturated α-β acid alkylesters such as methyl and ethyl acrylates and methoacrylates;
- the esters of unsaturated carboxylic acid such as vinyl acetate;
- vinyl chloride and vinylidene chloride;
- dienes such as butadiene; and
- those compounds having nitrile functions such as acrylonitril.

Preferably, the amphiphile compounds have an HLB of from 10 to less than 20. Amphiphile compounds suitable for use in the present invention comprise a hydrophilic oligomer sequence preferably selected from the group comprising a polyoxyalkylated sequence containing from 5 to 100, and preferably 5 to 50 oxyalkylated units of

C_2-C_3. The hydrophilic sequence may also comprise a polycarboxyethylated, polyamidoethylated, or polycyanoethylated sequence containing from 4 to 50 carboxyethylated, amidoethylated or cyanoethylated units respectively, or a combination of these units. The hydrophilic oligomer sequence terminates in an ionogenic or reactive group.

Ionogenic or reactive groups suitable for inclusion in the hychrophilic oligomer sequence include -OH, $-SO_3H$, -CHO, $-OCH_2Cl$, $-NH_2$, NR_2NR_3 (where R is an alkyl radical of C_1 - C_2), $-CONH_2$, $-NH-NH_2$, $-NH-NH-CO-NH_2$, -SH, and  O.

Specific examples of the amphiphile compounds suitable for use in the present invention include

a) fatty alcohols or fatty polyoxyethylated acids and/or polyoxypropylated acids having from 5 to 50 oxyalkylene units wherein the hydrophobic sequence comprises approximately 8 to 20 carbon atoms (such as the CEMULSOL DB marketed by the Societe Francaise d'Organo Synthese or the SOPROPHORS LA marketed by Rhone-Poulenc);

b) the esters of these fatty alcohols having (i) semicarbozide functions (such as the esters of these alcohols and of phenylcarbamic meta-semicarbazide par-methyl), (ii) acid functions (such as the monoesters of these alcohols and of azealic acid) and (iii) thiol functions (such as the esters of the alcohols with

thiogylcolic acid);

c) the amides of these fatty acids (such as the ETHOMIDS marketed by Armour Industrial Chemical Co.);

d) the alkylphenols, polyoxyalkylenes and/or polyoxypropylenes having from 5 to 50 oxyalkylene units and of which the, or the several alkyl radicals contain from 8 to 12 carbon atoms, and their esters with phosphoric acid (such as the SOPROPHOS BC and OP marketed by Rhone-Poulenc, the SOPROPHORS NFP marketed by Geronazzo and the GAFACS RE marketed by General Aniline and Film Corp.); and

e) the polyoxyethylated fatty amines, the hydrophobic sequence of which contain from 8 to 22 carbon atoms (such as the SOPROMINES marketed by Rhone-Poulenc, the ETHOMEENS marketed by Armour Industrial Co).

The particles according to the invention may correspond to a predetermined distribution or else may be calibrated.

In addition, the particles may be magnetised by the incorporation of finely divided metal or metal compound particles. Preferably, the magnetisable polymer particles contain from 0.05 to 50% by weight preferably from 0.5 to 35% and more preferably from 0.7 to 20% of a metal or metal compound particle capable of exhibiting a magnetic charge. These metal or metal compound particles are typically less than 1 µm in diameter, and preferably between 0.002 - 0.05 µm. Typically, the metal and metal oxide particles are selected from the group comprising:

. metals or their alloys such as, iron, silicon-iron, nickel, cobalt or their alloys with molybdenum, chromium, copper, vanadium, maganese, aluminium, titanium;

. iron oxide: Fe_3O_4 or $q\text{-}Fe_2O_3$ pure or combined or mixed with other oxides such as cobalt, manganese, zinc, barium, rare-earth oxides; and

. chromium dioxide.

Preferably approximately 10^{-3} to 10^{-1} mole of amphiphile compound per 100 g of polymer, is placed in contact with one another.

The latexes suitable for use in the present invention comprise approximately 1 to 50%, and preferably 5 to 30% and more particularly 10 to 15% by weight of polymer particles. The polymer particles have a diameter which is preferably from 0.01 to 15 microns and usually 0.05 to 3 microns. These polymer particles can have a particular distribution or be calibrated.

When the magnetisable particles are required, the laxexes of magnetisable particles can be obtained using the method described in the European Patent No. 38.730 and American Patent No. 4.157.323.

The placing in contact of the amphiphile compound and the latex is done by introducing the amphiphile compound possibly in an aqueous solution into the aqueous phase of the said latex, for example at the ordinary temperature, then raising the temperature to a temperature between the

vitreous transition zone of the polymer which constitutes the latex. In this temperature zone the macromolecular chains acquire a certain mobility which allows them to mix with the hydrophobic part of the amphiphile compound and to fix this compound to the surface of the latex particles. This operation last approximately from 15 minutes to 4 hours and usually from 1 to 3 hours.

For a polystyrene latex, for example, this enmeshing operation is carried out at a temperature of 70 to 80°C.

This temperature zone can be lowered if necessary by the use of plasticizers such as the dialkyl phthalates, aliphatic or atomic hydrocarbons containing at least 5 carbon atoms.

Another way of reducing this temperature zone consists of a process of placing in contact, in the presence of a solvent, the amphiphile compound and inflating the polymer particles. The solvent facilitates the penetration of the amphiphile compound by it's hydrophobic part in the surface layer of the polymerparticle where it enmeshes with the polymer chains. The solvent can then be removed, for example by evaporation under a vacuum.

Solvents suitable for use in the methods according to the invention include the aromatic derivatives (toluene, ethylbenzene, xylenes, ...) the chlorinated aromatic derivatives (mono, di and trichlorobenzene ...) aliphatic and cyclic hydrocarbons (heptane, decane, cyclohexane,

decalin, ...), ether dialkyls, alcohols (pentanol, cyclohexanol, ...) esters (methyl propionate, ...) etc.

The amphiphile compounds not implanted during the placing in contact as well as all emulsifying constituents which were previously present in the latex are then eliminated by aqueous washing, for example by ultrafiltration.

The polymer particles having the amphiphile compound implanted on the surface obtained in this way can be separated from the aqueous medium by the usual methods of sedimentation by centrifugation.

This invention also relates to aqueous dispersions or latexes of these polymer particles having, implanted on their surface molecules of amphiphile compounds, the rate of dry extraction from these dispersions is from 1 to 50%, preferably 5 to 30% and more precisely 10 to 15% by weight.

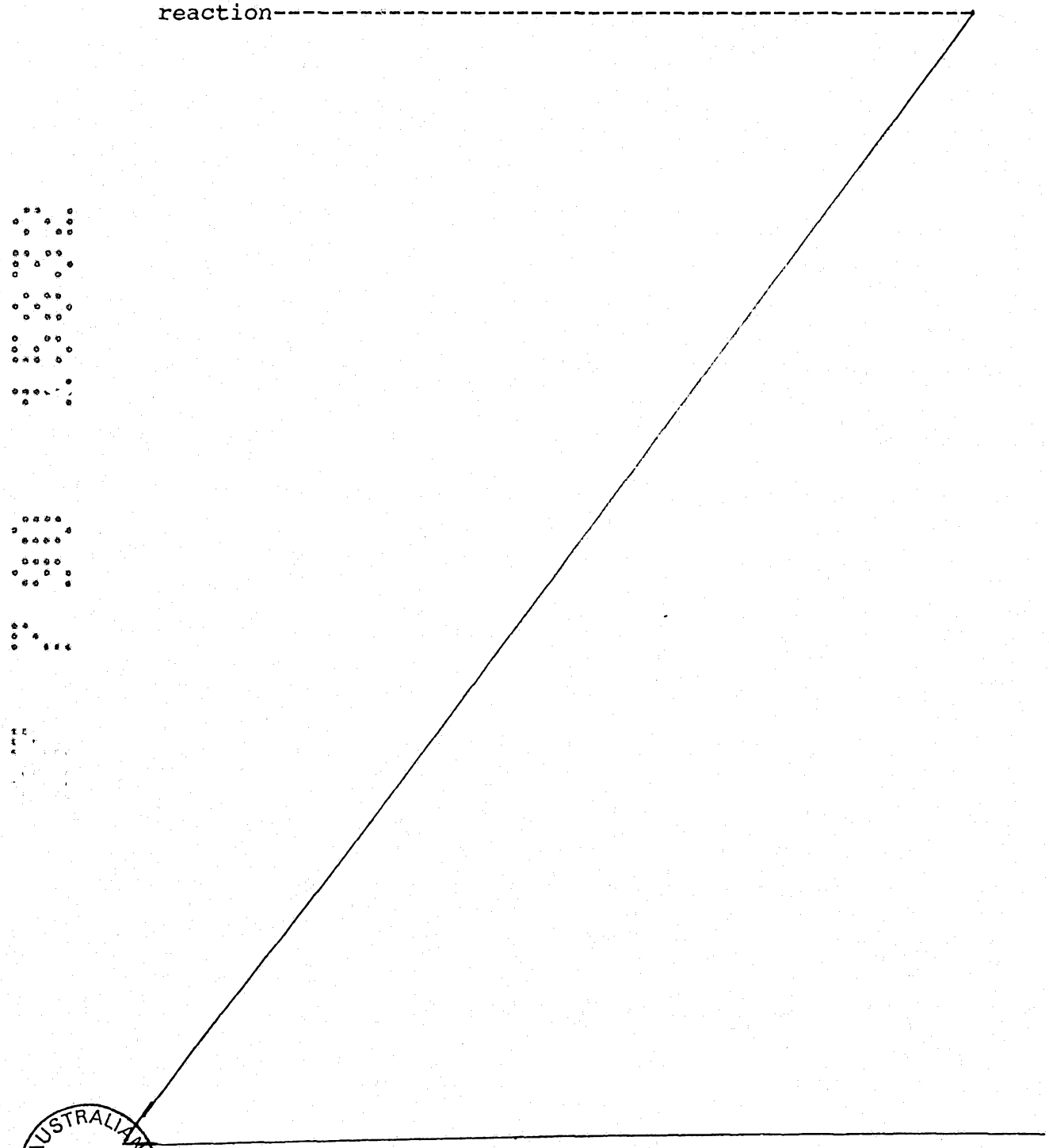
These latexes can be prepared using the known methods, by direct dispersion in water of the previously mentioned particles.

A preferred method for obtaining these latexes consists of eliminating the third stage (separation of the particles from the contact medium after eliminating all amphiphile compounds not implanted) of the process for the preparation of the polymer particles described above.

The particles or the particle latexes to which this invention relates are particularly useful in biology, for fixing biological molecules (antibodies, antigens, ...) by

covalency.

The fixing of biological molecules by covalency on the polymer particles can be done by coupling reaction, a reaction----------



which involves the terminal groupings of the implanted amphiphile molecule and the functional groupings of the biological molecule to be fixed.

This coupling reaction can be done using the well known methods, for example :

- . by using coupling agents (such as glutaraldehyde, hydrosoluble carbodiimide)
- . by activating the polymer functions (for example by diazotizing, by the action of cyanogen bromide, of hydrazine ...) then reacting with the molecule to be fixed.
- . etc.

The products can therefore be used for carrying out diagnostic tests such as agglutination, radioimmunology, enzymatic.

The following examples are given only as indications and cannot be considered as limiting the area or concept of the invention.

EXAMPLE 1

- Implantation of CEMULSOL DB 25/18, (abbreviation CA-OH : amphiphile compound with alcoholfunction) as amphiphile agent on the particles of a calibrated polystyrene latex.

Average formula of the amphiphile compound :

$C_{18}H_{37}(O-CH_2-CH_2)_{50}OH$

A solution at 2 % by weight of the amphiphile agent is prepared in water.

50 g of this solution, which corresponds to 0.535 mole of amphiphile agent is mixed with 10 g of particles of a calibrated polystyrene latex of an average diameter of 0.9 microns and with 33 % by weight of dry extract.

The mixture is agitated for 2 hours at 70°C,

After cooling, the latex obtained is washed by ultrafiltration using 2.5 l of purified water for 10 g of latex particles, to eliminate the molecules of amphiphile compound not implanted as well as the emulsifiers present in the original latex.

The latex remains stable after ultrafiltration which is an evident indication of the implantation of the amphiphile compound.

The latex obtained is titrated by nuclear magnetic resonnance.

It is noted that the amount in weight of amphiphile agent implanted in 100 g of the final particles is 6.4 (theory = 9.1).

The results are shown on table I

EXAMPLE 2

The process described in example 1 is repeated under the same conditions, replacing the 10 g of CA-OH by respectively 5 and 20 g of CA-OH. The results of the titration by NMR are shown on table I.

EXAMPLE 3

- Implantation of CEMULSOL DB 25/8 as amphiphile agent on the particles of a magnetisable polystyrene latex.

The process described in example 2 is repeated replacing the calibrated polystyrene latex used by 10 g of particles of a polystyrene latex having a dry extract of 33 %, composed of particles with a diameter from 0.5 to 0.9 micron, with an average diameter of 0.7 micron and containing 41.5 % by weight of γFe_2O_3 in relation to the weight of polymer. The mixture is treated as in example 1.

The titration results by conductimetry are shown on table I.

EXAMPLE 4

Implantation of CEMULSOL DB 25/18 as amphiphile agent on the particles of a polychloride vinyl latex.

The process described in example 1 is repeated, replacing the calibrated polystyrene latex used, by 10 g of particles of a vinyl polychloride calibrated latex with a dry extract of 37 % by weight and composed of calibrated particles with an average diameter of 0.7 micron.

The mixture is agitated for 2 hours at 80°C. After cooling, the latex obtained is ultrafiltered.

The titration results by conductimetry are shown on table I.

EXAMPLE 5

Implantation of CEMULSOL DB 25/18 as amphiphile agent on the particles of a methyl copolymer styrene/methacrylate copolymer 40/60 by weight.

The process described in example 1 is repeated but the calibrated polystyrene latex used is replaced with 10 g of particles of a calibrated methyl styrene/methacrylate latex 40/ 60 having a rate of dry extract of 30 % by weight and composed of calibrated particles of an average diameter of 0.8 micron.

The mixture is kept agitated for 2 hours at 90°C.

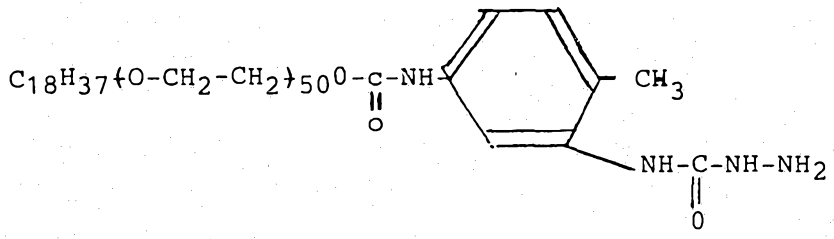
After cooling, the latex obtained is ultrafiltered.

The titration results obtained by NMR are shown on table I.

EXAMPLE 6

Implantation of the ester CEMULSOL DB 25/18 and phenyl-carbamic meta-semicarbazide para-methyle acid (abreviated to CASC : amphiphile compound with a semi-carbazide function) as the amphiphile agent, on the particles of a calibrated polystyrene latex.

Average formula of the amphiphile compound :



Preparation of the amphiphile compound

The compound is prepared in two stages :

- a first stage consists in functionalising the 25/18 CEMULSOL DB with toluene diisocyanate in the presence of dioxanne as solvent at a temperature of 90°C.
- a second stage consists of functionalising the 25/18 CEMULSOL DB obtained in this way by adding hydrazine hydrate in the presence of dioxanne, at the normal temperature.

The required amphiphile compound is precipitated cold in ethylic ether.

Implantation.

The process described in example 1 is repeated, substituting the 25/18 CEMULSOL DB by 50 g of an aqueous solution at 2 % of its derivative, prepared as above, which corresponds to 0.494 mole of amphiphile compound.

The titration results obtained by NMR are shown on table II.

EXAMPLE 7

Implantation of the 25/18 CEMULSOL ester and thioglycolic acid (abreviated to CA-SH : amphiphile compound with a thiol function) as amphiphile agent, on the particles of a polystyrene calibrated latex.

Average formula of the amphiphile compound

$$C_{18}H_{37}(O-CH_2-CH_2)_{50}-\underset{\underset{O}{\|}}{C}-CH_2-SH$$

This compound can be prepared following the process described in the american patent No. 2.461.920

The operation described in example 1 is repeated, replacing the 25/18 CEMULSOL DB by 50 g of an aqueous solution at 2 % of its thioglycolic ester with a formula as above, this corresponds to 0.255 mmole of amphiphile compound.

The results of titration by conductimetry are shown on table II.

EXAMPLE 8

Implantation of the 25/18 DB ester and phenyl-carbamic meta-semicarbazide para-emthyl acid (abreviation CASC), on the particles of a magnetisable polystyrene latex.

The process described in example 1 is repeated, replacing the calibrated polystyrene latex by 10 g of polystyrene latex having a dry extract of 33 % composed of particles with a diameter from 0.5 to 9 micron with an average diameter of 0.7 micron and containing 41.5 % by weight of γ Fe_2O_3 in relation to the weight of polymer.

The mixture is treated as in example 1.

The reults of titration by conductimetry are shown on table II.

EXAMPLE 9

Implantation of the monoester of 25/18 CEMULSOL DB and azelaic acid (abreviation CA-COOH : amphiphile compound with an acid funtion), as amphiphile compound, on the particles of a calibrated polystyrene latex.

Average formula of the amphiphile compound :

$$C_{18}H_{37}(O-CH_2-CH_2)_{50}O-\underset{\underset{O}{\|}}{C}-(CH_2)_7-COOH$$

This can be prepared by esterification of the 25/18 CEMULSOL DB with azelaic acid in pyridine in the presence of dicyclohexyl-carbodiimide as agent of condensation.

The process described in example 1 is repeated, replacing the 25/18 EMULSOL DB by 50 g of a 2 % solution of its ester with the above formula, which corresponds to 0.495 mmole of amphiphile compound.

The results of titration obtained by NMR are shown on table II.

EXAMPLE 10

Implantation of the 25/18 CEMULSOL DB ester and phenyl-carbamic meta-semicarbazide para-methyl acid (CASC), on a calibrated polystyrene latex in the presence of an inflating solvent.

25 g of a 2 % solution by weight of the amphiphile compound is prepared in toluene.

10 g of a 0.1 % solution by weight of ammonium laurate is prepared in water, this is added to 10 g of particles of a polystyrene latex with a rate of dry extract of 33 % by weight and composed of calibrated particles with a diameter of 0.8 micron. The toluenic solution of amphiphile agent is introduced into the latex. The mixture is agitated continuously for 2 hours at 45°C.

The toluene is scoured out by stripping at 70°C under a pressure of 160 mm Hg in a rotating evaporator.

The latex is then washed by ultrafiltration.

The results of titration obtained by NMR are shown on table II.

EXAMPLE 11

The latex particles obtained in example 1 are sedimented using a BECKMAN L 50 centrifuge equipped with a rotor 30 (device marketed by BECKMANN) spinning at 10.000 t/mn for 15 mn, then oven dried under vacuuum at 40°C and kept under nitrogen.

The characteristics of the particles are maintained.

The abreviations relating to the initial polymer are shown in tables I and II and their meaning is as follows :

PS : polystyrene

PS mag : magnetic polystyrene

PVC : polyvinyl chloride
P(S/MAM) : methyl styrene/methacrylate copolymer
DE : dry extract

TABLE I

EXAMPLE	1	2	3	4	5	6
LATEX - Polymer	PS	PS	PS	PS mag	PVC	P(S/MAM
LATEX - Ø µm	0.9	0.9	0.9	0.5-0.9	0.7	0.8
LATEX - DE %	33	33	33	33	37	30
AMPHIPHILIC POLYMER - nature	CA-OH	CA-OH	CA-OH	CA-OH	CA-OH	CA-OH
AMPHIPHILIC POLYMER - g/100g of polymer	10	5	20	5	10	10
AMPHIPHILIC POLYMER - moles/100 g of polymer	5.35	2.675	11.70	2.675	5.35	5.35
temparature °C	70	70	70	70	80	90
duration h	2	2	2	2	2	2
% implanted - theory	9.1	4.8	16.7	4.8	9.1	9.1
% implanted - titrated	6.4	3.1	12.7	2.9	5.8	7.1

TABLE II

EXAMPLE	7	8	9	10	11	12
LATEX - Polymer	PS	PS	PS mag	PS	PS	PS
LATEX - 0 um	0.9	0.9	0.5-0.9	0.9	0.8	0.9
LATEX - DE %	33	33	33	33	33	33
AMPHIPHILE - nature	CASC	CA-SH	CASC	CA-COOH	CASC	CA-OH
AMPHIPHILE - g/100 g of polymer	10	10	10	10	5	10
AMPHIPHILE - moles/100 g of polymer	4.95	2.55	4.95	4.95	2.47	5.35
temperature °C	70	70	70	70	45	70
duration h	2	2	2	2	2	2
% implanted - theory	9.1	9.1	9.1	9.1	4.8	9.1
% implanted - titrated	5.3	4.8	3.1	6.2	4.3	6.4

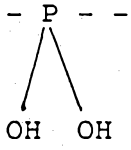
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. A polymer particle comprising a macromolecular polymeric chain having a Tg glass transition temperature of greater than about 40°C and an amphiphile compound implanted in the surface of the particle, said amphiphile compound having an HLB of at least 10 and a molecular mass of at least 400, said amphiphile compound comprising a) a hydrophobic sequence enmeshed within the polymeric chain in the surface layer of the particle and b) a hydrophilic oligomer sequence terminating in at least one ionogenic or reactive group, whereby said polymer particle comprises about 10^{-5} to 10^{-1} moles of said amphiphile compound per 100 g of polymeric chain and has a diameter of from 0.01 to 50 microns.

2. A particle according to claim 1 wherein the amphiphile compound has an HLB of less than 20, the polymer particle comprises about 10^{-4} to 10^{-2} moles of amphiphile compound per 100 g of polymeric chain, the polymeric chain has a Tg greater than approximately 50°C and the diameter of the particle is from 0.01 to 15 microns.

3. A particle according to claim 1 or 2 wherein the polymeric chain comprises a homopolymer or a copolymer containing units derived from vinylaromatic monomers, unsaturated α-β acid alkylesters, esters of unsaturated carboxylic acid, vinyl chloride, vinylidene chloride, dienes, or compounds having nitrile functions.

4. A particle according to anyone of the preceding

claims wherein the amphiphile compound comprises a hydrophilic oligomer sequence selected from the group comprising a polyoxyalkylated hydrophilic oligomer sequence comprising from 5 to 100 oxyalkylated units of C_2-C_3; or a polycarboxyethylated sequence, a polyamidoethylated sequence, or a polycyanoethylated sequence comprising from 4 to 50 carboxyethylated, amidoethylated, or cyanoethylated units respectively or a combination of these units; said hydrophilic oligomer sequence terminating in a group selected from -OH, $-SO_3H$, -COOH, -CHO, $-OCH_2Cl$, $-NH_2$, $-NR_2$. NR_3 wherein R is an alkyl radical of C_1-C_2, $-CONH_2$, $-NH-NH_2$, $-NH-NH-CO-NH_2$, -SH, or 

5. A particle according to anyone of the preceding claims being magnetisable.

6. An aqueous dispersion of polymer particles, comprising from 1 to 50% by weight of the particles according to anyone of the claims 1 to 5.

7. A dispersion according to claim 6, comprising from 5 to 30% by weight of particles according to anyone of the claims 1 to 5.

8. A method of preparation of an aqueous dispersion according to claim 6 or 7 comprising the steps of:

a) selecting an amphiphile compound having an HLB of at least 10 and a molecular mass of least 400 and polymer particles each comprising a polymeric chain and having a

diameter of 0.01 to 50 microns and a Tg glass transition temperature of greater than 40°C,

b) placing in contact about 10^{-4} to 1 mole of said amphiphile compound per 100 g of said polymer particles with a latex comprising from 1-50% by weight of said polymer particles, at a temperature between the glass transition zone of the polymer, until the polymeric chains and the hydrophobic sequences of the amphiphile compound become enmeshed and,

c) eliminating all the amphiphile compound not fixedly enmeshed.

9. A method of preparation of polymer particles according to claim 1, said method comprising the steps of:

a) selecting an amphiphile compound having an HLB of at least 10 and a molecular mass of at least 400 and polymer particles each comprising a polymeric chain and having a diameter of 0.01 to 50 microns and a Tg glass transition temperature of greater than 40°C;

b) placing in contact about 10^{-4} to 1 mole of said amphiphile compound per 100 g of said polymer particles with a latex of said polymer particles, at a temperature between the glass transition zone of the polymer, until the polymeric chains and the hydrophobic sequences of the amphiphile compound become enmenshed,

c) eliminating all the amphiphile compound not fixedly enmeshed, and

d) separating the particles obtained.

10. A method according to claim 8 to 9 wherein the

selected amphiphile compound has a HLB of less than 20.

11. A method according to anyone of the claims 8 to 10, wherein the amphiphile compound comprises a hydrophilic oligomer sequence selected from the group comprising a polyoxyalkylated sequence containing 5 to 100 oxyalkylated units of C_2-C_3; or a polycarboxyethylated sequence, a polyamidoethylated sequence, or a polycyanoethylated sequence comprising from 4 to 50 carboxyethylated, amidoethylated, or cyanoethylated units respectively or a combination of these units; said hydrophilic oligomer sequence terminating in group selected from -OH, $-SO_3H$, -COOH, -CHO, $-OCH_2Cl$, $-NH_2$, $-NR_2$. NR_3 wherein R is an alkyl radical of C_1-C_2, $-CONH_2$, $-NH-NH_2$, $-NH-NH-CO-NH_2$, -SH, or $-P(OH)(OH)=O$.

12. A method according to anyone of the claims 9 to 11 wherein the latex of polymer particles comprises from 1 to 50-% by weight of said polymer particles.

13. A method according to anyone of claims 8 to 12, wherein the latex of particles comprises from 5 to 30% by weight of polymer particles.

14. A method according to anyone of the claims 8 to 13, wherein said polymer particles have a diameter from 0.01 to 15 microns.

15. A method according to anyone of the claims 8 to 14, wherein the polymer particles comprise a homopolymer or a copolymer containing units derived from vinylaromatic

monomer, alkylesters of unsaturated α-β acids, esters of unsaturated carboxylic acids, vinyl chlorides, vinylidene chlorides, dienes, or compounds having nitrile functions.

16. A method according to anyone of the claim 8 to 15, wherein the polymer particles of the latex are magnetisable.

17. A method according to anyone of the claims 8 to 16, wherein the amphiphile compound is introduced into the aqueous phase of the latex at ambient temperature and the temperature is subsequently elevated to a level within the glass transition zone of the polymer.

18. Use of the particles according to anyone of the claims 1 to 5 in biology.

19. Use of the aqueous dispersions according to claim 6 or 7 in biology.

20. A polymer particle substantially as herein described with reference to anyone of the examples.

21. A method of preparation of an aqueous dispersion substantially as herein described with reference to anyone of the examples.

22. A method of preparation of polymer particles substantially as herein described with reference to anyone of the examples.

Dated this 27th day of JULY, 1990
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