

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 May 2006 (11.05.2006)

PCT

(10) International Publication Number
WO 2006/049497 A1

- (51) International Patent Classification⁷: **B01J 13/04**
- (21) International Application Number:
PCT/NL2005/000777
- (22) International Filing Date:
3 November 2005 (03.11.2005)
- (25) Filing Language: Dutch
- (26) Publication Language: English
- (30) Priority Data:
1027428 5 November 2004 (05.11.2004) NL
- (71) Applicant (for all designated States except US): **ENCAP-SON V.O.F.** [NL/NL]; Groenestraat 217-Q, NL-6531 HH Nijmegen (NL).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **VRIEZEMA, Dennis, Manuel** [NL/NL]; Groenestraat 217-Q, NL-6531 HH Nijmegen (NL). **ROWAN, Alan, Edward** [NL/NL]; Dominicanessenstraat 3, NL-6584 GL Molenhoek (NL). **NOLTE, Roeland, Johannes, Maria** [NL/NL]; Sophiaweg 73a, NL-6523 NG Nijmegen (NL).
- (74) Agents: **DOHMEN, Johannes, Maria, Gerardus et al.**; Algemeen Octrooi- en Merkenbureau, P.O. Box 645, NL-5600 AP Eindhoven (NL).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PERMEABLE CAPSULES, METHOD FOR THE PREPARATION THEREOF AS WELL USE THEREOF

(57) Abstract: The present invention relates to a permeable capsule formed through stabilisation of a vesicle, comprising a membrane formed from amphiphilic copolymers comprising polymerisable groups, wherein the polymerisable groups are thiophene groups. The present invention furthermore relates to a method for the preparation of permeable capsules, comprising the formation of a vesicle from an amphiphilic copolymer comprising polymerisable groups and the stabilisation of the vesicles thus formed through polymerisation of polymerisable groups so as to obtain a capsule comprising a membrane that encloses a cavity, wherein thiophene groups are used as the polymerisable groups.



WO 2006/049497 A1

PERMEABLE CAPSULES, METHOD FOR THE PREPARATION AND USE THEREOF

5 The present invention relates to a permeable capsule formed through stabilisation of a vesicle, comprising a membrane formed from amphiphilic copolymers comprising polymerisable groups.

Such a capsule is known from WO 01/32146, said capsule being composed of amphiphilic copolymers comprising acrylate groups, for example, stabilised through polymerisation of the acrylate groups.

10 In certain solvents, such as ethanol, the permeability of the capsules according to WO 01/32146 can be induced through swelling of the polymer parts. When these capsules are placed in another solvent, viz. water, the permeability of the capsule drastically decreases. It is a drawback of the capsules according to WO 01/32146 that the permeability strongly depends on the solvent and that it is especially low in water, which is a common solvent.

15 International application WO 2005/038968 (not pre-published) relates to the use of hollow vesicles in suspension for generating current, wherein a polypeptide is captured within the hollow vesicle, possibly for use as a battery.

20 From the publication by Vriezema et al., Angew. Chem. Int. Ed. 2003, 42, 7, a conductive polymeric vesicle and a method for the preparation thereof are known.

25 The permeability of the capsules is important for certain applications, such as the controlled delivery of pharmaceuticals. Such medicines can be introduced into the central cavity of the capsules during the preparation thereof, after which the capsules are taken to the desired delivery location, where controlled diffusion of the pharmaceuticals takes place.

It is an object of the present invention to provide a permeable capsule that exhibits a sufficient and controllable degree of permeability in water and organic solvents.

30 Another object of the present invention is to provide a permeable capsule and a method for the preparation thereof wherein the permeability is already obtained during the preparation of the capsules.

In addition, it is an object of the present invention to provide a permeable capsule suitable for containing and releasing a large number of different

materials.

In addition to that it is an object of the present invention to provide a permeable capsule that is at least partially electrically conductive.

One or more of the above objects are achieved by means of a capsule according to the preamble, which is characterized in that the polymerisable groups are thiophene groups.

The advantage of the use of thiophene groups as polymerisable groups is that the present inventors have found that permeability is already introduced into the capsule wall upon preparation of the present capsules through polymerisation of the thiophene groups in the membrane of the vesicle. Said permeability is retained when the capsules are introduced into aqueous mediums.

The present inventors assume that thiophene groups of one copolymer molecule are linked to thiophene groups of another adjacent copolymer molecule upon stabilisation of the vesicles through polymerisation of the thiophene groups, which leads to crosslinking of the membrane of the vesicle. Each copolymer may comprise a number of thiophene groups (up to a few dozen). During polymerisation, an oligomer or even a polymer of thiophene groups is formed in and on the membrane of the vesicle. The oligomer or polymer of thiophene thus formed is potentially electrically conductive and exhibits a high degree of stability. The capsule that is formed consists of a central cavity surrounded by a membrane. The wall of the capsule (also called membrane) contains pores, which render the capsule permeable. Said permeability can be utilized by certain materials contained in the capsule, which diffuse from the capsule into the environment outside the capsule (as is the case with the controlled delivery of pharmaceuticals) or by materials that diffuse into the capsule from outside (as is the case when the capsule is used as a nanoreactor).

The present capsules thus provide a shell for encapsulated materials, whilst communication through the membrane remains possible.

In another embodiment of the present capsule, one or more of the thiophene groups have a substituent. The thiophene groups are linked to the present copolymer molecules via the 3-position or the 4-position of the thiophene ring. The 2-position and the 5-position of the thiophene ring must be non-substituted, since the polymerisation of the thiophene groups takes place via said positions. The 3-position or the 4-position that is still available can be substituted, if desired. The

advantage of this is that it is possible to influence the solubility of the copolymer molecule.

The substituent on the thiophene group is preferably selected from the group consisting of alkyl groups and alkoxy groups, both linear and branched, without being limited to these groups. Examples of suitable groups are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, methoxy, ethoxy, propoxy, butoxy, pentoxy, hexoxy, heptoxy, octoxy, nonoxy and decoxy. These alkyl or alkoxy substituents help to influence the solubility without interfering with the polymerisation of the thiophene groups.

Preferably, the amphiphilic copolymer is built up of hydrophobic blocks, hydrophilic blocks and combinations thereof. Depending on the intended use, the type of blocks can be selected therefrom. When a lipophilic material is to be contained in the cavity of the capsules, it may be elected to use hydrophilic blocks, with the capsules being formed in a hydrophobic solvent. It is possible to use copolymers consisting of two blocks, for example one hydrophobic block and one hydrophilic block. It is also possible to use copolymers consisting of more than two blocks, for example two hydrophobic blocks with a hydrophilic block therebetween, or two hydrophilic blocks with a hydrophobic block therebetween. The terms hydrophilic and hydrophobic as used herein indicate the difference in hydrophobicity, the hydrophilic block having a lower hydrophobicity than the hydrophobic block.

In a preferred embodiment, the blocks in the block copolymer molecule are isolated from each other by a spacer, thereby facilitating the synthesis of the block copolymers and introducing additional flexibility. The spacer as meant herein may have any desired structure, such as an alkyl group, an aryl group, a peptide or combinations thereof, as long as the spacer can be coupled between two blocks in the copolymer molecule.

A spacer is used in particular for isolating a hydrophobic block from a hydrophilic block for the purpose of facilitating the synthesis thereof and creating a spacing between the two blocks exhibiting different degrees of hydrophobicity. Interference between the two blocks might occur in particular during the synthesis of a copolymer comprising two blocks exhibiting different degrees of hydrophobicity, which interference can be prevented by using a spacer.

Polymerisation of the thiophene groups will result in part of the

outer surface (membrane) or the entire outer surface of the capsules becoming potentially electrically conductive. Said conductance is caused by the oligomer or polymer of thiophene that has been formed. This (partially) conductive membrane makes it possible to use the capsules for applications in which electrical conductivity is of relevance, for example in a sensor, as will be explained hereinafter.

The amphiphilic copolymers may be molecules of the so-called rod-coil type, the coil-coil type or the rod-rod type. A "rod" indicates a more rigid copolymer block, whilst a "coil" indicates a more flexible copolymer block. Thus it is possible to use block copolymers built up of one rigid part and one more flexible part, of two more flexible parts or of two rigid parts, depending on the required properties of the final capsule. Furthermore it is possible to use block copolymer molecules consisting of more than two types of blocks, for example a copolymer consisting of two "rods" with a "coil" therebetween, or two "coils" with a "rod" therebetween. Thus it is possible to control the properties of the membrane, such as strength and flexibility, as desired.

A rod-coil type polymer is preferred, because it gives the final capsule sufficient strength and also an adequate flexibility.

Preferably, polystyrene-6-poly(isocyano-alanine)2-thiophene-3-yl-ethyl)amide) (PS-PIAT) is used as the amphiphilic block copolymer molecule. The structural formula and the schematic representation of the polymer are shown in figure 1. The PS-PIAT has a rigid hydrophilic polyisocyanide head group and a flexible hydrophobic polystyrene tail, which makes it an amphiphilic rod-coil type block copolymer. PS-PIAT is capable of forming very stable and well-defined aggregates in water and organic solvents.

The present invention also relates to a method for the preparation of permeable capsules, comprising the formation of a vesicle from an amphiphilic copolymer comprising polymerisable groups and the stabilisation of the vesicles thus formed through polymerisation of polymerisable groups so as to obtain a capsule comprising a membrane that encloses a cavity, characterized in that thiophene groups are used as the polymerisable groups.

In a preferred embodiment, the polymerisable groups are polymerised by using metal ions, which has shown to enable an excellent control of the rate and the degree of polymerisation. Such control makes it possible to influence the degree of permeability.

Chemical oxidative polymerisation, using metal ions, comprises the oxidative linear coupling of thiophene groups on the 2-position and the 5-position of the thiophene ring. The present inventors assume that the mechanism of oxidative coupling takes place via radical cations of a metal or organometal complex. The present inventors by no means intend to be bound by such a theory, however.

The metal ions are preferably selected from the group consisting of Fe(III) ions, Ru(II) ions and combinations thereof, which types of ions are suitable catalysts for the polymerisation of thiophene groups.

In an especially preferred embodiment, bis(2,2'-bipyridine) ruthenium(II) bispyrazolyl (BRP) is used as the polymerisation catalyst, which catalyst enables an adequate control of the polymerisation reaction and which can thus be used for influencing the degree of permeability.

Another method for the polymerisation the thiophene groups is oxidative anodic electrochemical polymerisation. The advantage of such a polymerisation method is that no catalyst is needed, since a potential difference is used for controlling the polymerisation reaction. The polymerisation reaction can be carried out in a suitable electrolyte solution with a constant potential and a constant current, or by changing the potential cyclically.

In a preferred embodiment of the present method, an indium tin oxide (ITO) is used during the electrochemical polymerisation process. The use of such an ITO electrode ensures an adequate control of the degree of polymerisation, so that permeable capsules having the desired properties are obtained.

Polymerisation by means of metal cations is preferable to electrochemical polymerisation, because the three-dimensional shape of the vesicle is retained better during the polymerisation of the thiophene groups for forming the capsule.

The polymerisation reaction is preferably carried out at a temperature between 0 and 90 °C, which temperature is determined in dependence on the reactants, the reaction time and the solvent. When temperatures higher than 90 °C are used, the vesicles that are formed will not be stable enough, so that no capsules can be obtained. When temperatures lower than 0 °C are used, aggregation will occur to such an extent that no individual vesicles but larger aggregates or coagulated vesicles are obtained, which is undesirable. Such coagulated vesicles do not exhibit satisfactory permeability properties, for example

because they exhibit holes or because the membranes of the individual vesicles have fused together, so that diffusion through the membrane is not possible.

Suitable solvents for the polymerisation reaction include water, dichloromethane, chloroform, tetrahydrofuran, diethyl ether and acetonitrile. These solvents can easily be removed from the reaction mixture through evaporation, if required. The selection of the solvent depends on the desired properties of the final capsules, such as the degree of permeability, and also on the materials to be contained in the capsules and the solubility thereof in the solvents. Solvents that are especially preferred for polymerisation are acetonitrile and chloroform or a mixture thereof.

The present permeable capsules may contain a material selected from the group consisting of a catalytically active material, a diagnostic agent, a reagent, a colorant, a pharmaceutically active agent, a preservation agent, a flavouring substance, an antibacterial agent, an aromatic substance, a photosensitive substance and combinations thereof.

The catalytically active material is preferably selected from the group consisting of a protein, an enzyme, and inorganic complex, an organic compound and combinations thereof.

Although a few enzymes exist that are capable of withstanding severe conditions, such as high/low temperatures or extreme pH values, most enzymes are only active in a narrow range of temperature and pH and require a buffered solution, moderate temperatures and protection against harmful substances. In nature, these requirements are met through compartmentalisation. Membranes surround a cell, and the enzyme-containing cell plasma is thus isolated from the environment. One function of the capsules according to the present invention is to protect enzymes and other materials against external influences.

Three examples of suitable enzymes, to which examples the present invention is not limited, that can be contained in the cavity or in the membrane of the present vesicles are *Candida antarctica* lipase B (cal B), horseradish peroxidase (HRP) and glucose oxidase (GO_x).

Cal B is a 33 kDA enzyme, which hydrolyses esters in aqueous solutions and which carries out amidation reactions, esterification reactions and transesterification reactions in anhydrous organic solutions. The enzymes can be introduced into the present capsule by means of an injection method, for example,

which will be explained on the basis of the sample enzyme Cal B.

The method comprises the injection of a solution of copolymer molecules into an aqueous solution of Cal B, after which vesicles will be formed in which Cal B will be contained. Excess Cal B will be removed by ultrafiltration, for example. After the addition of a substrate (for example DiFMU octanoate, 6,8-difluor-4-methyl-umbelliperyl octanoate), the substrate appears to be capable of passing the membrane of the vesicle, whereupon it is hydrolysed in the internal cavity of the vesicle by Cal B.

The membrane of the vesicle can subsequently be polymerised with Ru(II) ions. The Cal B present in the cavity of the capsules thus formed still exhibits hydrolytic activity, albeit at a lower rate (about three times lower) than the activity in the non-stabilised vesicles. The present capsules thus exhibit permeability to the substrate.

The degree of polymerisation of the membrane controls the degree of permeability of the capsule. The permeability of the capsule, measured by determining the activity of encapsulated Cal B (figure 2a), decreases as the duration of the polymerisation reaction increases.

The concentration of the metal cation in an oxidative chemical oxidation reaction plays an important part as well. As the concentration of metal cations increases, the permeability decreases, because of a higher degree of polymerisation. The concentration of metal cations must be precisely determined, therefore, according to the desired degree of permeability. This effect is shown in Figure 2b.

The amount of voltage that is applied during the electrochemical polymerisation also has an influence on the polymerisation process; the higher the amount of voltage, the higher the degree of polymerisation and the lower the degree of permeability.

The pores of the present capsule must be such that the encapsulated enzymes will not leak out, whilst diffusion of compounds from outside the membrane can take place to a sufficient degree, which compounds can be converted by the enzyme after having passed the membrane.

Horseradish peroxidase (HRP) is a 44 kDa enzyme that is found in plants. It catalyses the oxidation of compounds, using hydrogen peroxide. The present inventors have produced capsules in which HRP is contained in the central

cavity of the capsule.

Glucose oxidase (GO_x) is a 160 kDa enzyme that catalyses the oxidation of β -D-glucose whilst forming hydrogen peroxide and two electrons. GO_x can therefore be used in sensors for determining the amount of glucose in a particular medium by measuring the amount of H_2O_2 that has been formed or the amount of electrons that have been formed, which electrons may be led to a measuring electrode via the conductive surface of the capsules.

Each of the enzymes Cal B, HRP and GO_x , as well as other enzymes, can be introduced into the capsules according to the present invention either individually or in combination with each other for the purpose of carrying out one or more specific reactions and forming sensors.

The reagent is preferably selected from the group consisting of an amino acid, a peptide, a protein, a nucleotide, a monomer and other chemical compounds and combinations thereof. Such capsules containing these reagents are used for reactions in which the retarded delivery of the reagent is of importance, for example in the case of controlled polymerisation reactions and the like.

The diagnostic agent is preferably selected from the group consisting of an antibody, an antibody fragment, a gene, a colorant, a fluorescent substance, a radioactive substance, an antigen, a nucleic acid, magnetic particles and combinations thereof.

The pharmaceutically active agent may be a medicine, a prophylactic or the like, but also hormones and other types of medicines. The type of medicine is not specifically limited, it can be determined by a person skilled in this field of the art. Capsules containing pharmaceuticals can be used for the retarded delivery of medicines or for transporting acid-sensitive medicines through the stomach after oral administration thereof.

Capsules containing aromatic or flavouring substances and/or colorants may be used as additives in foodstuffs or cosmetics.

Furthermore, a photosensitive substance may be introduced into the capsule, so that said substance is screened from ambient light by the capsule wall, thus enabling the storage and processing thereof, so that the photosensitive substance will not be released until it is to be reacted.

The present invention also relates to the use of the present permeable capsules as a nanoreactor.

The permeable capsules may furthermore be used as components for paints and coatings. Such capsules can be mixed through water-based and/or oil-based paints or coatings. After application of the paint or coating to the surface to be treated, the capsule can protect the material that is present in the cavity and deliver it in a time-controlled manner, whilst even externally added components in the paint or the coating can diffuse into the capsule, depending on the intended effect.

The present permeable capsules may also be used for lubricating purposes, with the capsules functioning as microscopically small ball bearings that reduce the resistance between moving parts.

Moreover, the permeable capsules can be used as a component for inks, wherein the component that is retained within the capsule can diffuse from the capsule or be protected after the ink has been applied to a support, or wherein other components of the ink can diffuse into the capsule, depending on the intended effect.

Furthermore, the capsules according to the present invention can be used as components for a dielectric. The value for the dielectric constant can be varied by changing the pore size of the membranes of the capsules.

The present capsules can also be used as an antistatic material. The conductive membrane allows the capsules to discharge static charge and thus protect their contents.

Yet another application of the present invention is the use thereof as an antirefractive material. The index of refraction can be altered by changing the diameter of the capsules.

Another application of the present capsules is the use thereof as an antireflective material, wherein the capsules, once they have dried on the surface, scatter incident light thereon, as a result of which the surface does not reflect said light.

According to yet another application of the present capsules, the capsules, when filled with antimicrobial components, can be incorporated in packaging materials, for example packaging materials that are used for perishable products, such as foodstuffs and beverages, so that the contents of said packaging materials will remain fresh longer.

According to yet another application of the present capsules, the

capsules, when filled with certain types of enzymes, can be used in washing powder, as a result of which the enzymes in the washing powder will be washed away less easily than in the situation in which said enzymes would not be present in the capsules, and consequently the enzymes will remain active for a longer period of time during the washing treatment.

According to another possibility, an energy source is developed by enclosing the enzyme glucose oxidase in the capsules, wherein glucose may be used as a fuel, which can be converted into gluconolacton and two electrons by the enzyme. The released electrons can be transported through the conductive membranes of the capsule to a cathode, where they deliver energy.

The present invention will now be explained in more detail by means of the following examples.

Example 1: formation of vesicles of PS-PIAT

A solution of 1 mg PS-PIAT in 1 ml THF was injected into 5 ml of water at a temperature of 25 °C in order to obtain vesicles of PS-PIAT in a solvent mixture of THF/water (1:5 v/v).

Example 2: chemical polymerisation from vesicle to capsule

Chemical oxidative polymerisation of the thiophene groups in vesicles of PS-PIAT was carried out, using BRP Ru(II)-ions bis(2,2'-bipyridine) ruthenium(II) bispyrazolyl. It is a strong base with a pKa of the acid (BRDH+) of >13. Vesicles of PS-PIAT were prepared through injection of a THF solution of PS-PIAT into an aqueous solution of BRP at 70°C. This resulted in the formation of vesicles of PS-PIAT, the thiophene groups of which were polymerised, using chemical oxidative polymerisation with Ru(II) ions (BRP) so as to form permeable capsules. It was found that the capsules were substantially spherical in shape.

Example 3: electrochemical polymerisation from vesicle into capsule

Electrochemical polymerisation of the thiophene groups into vesicles of PS-PIAT was carried out by drying a drop of a dispersion of the aggregate in water/THF (5:1 v/v) both on a platinum electrode and on an indium tin oxide platelet, which functioned as the work electrode.

The ITO plate was submersed in an acetonitrile solution, which contained an electrolyte, together with a reference electrode and a counter electrode. A constant potential of 1,6 V was applied to the ITO platelet for 60 seconds so as to obtain permeable capsules.

Comparative Example 1: capsules of copolymers with acrylate groups

Vesicles were formed, using copolymers comprising acrylate groups, which vesicles were stabilised so as to form capsules. The obtained capsules were tested against the present capsules according to Examples 2 en 3.

Example 4: evaluating the permeability of capsules

The capsules, to be prepared in accordance with the procedure described in Examples 2 en 3 and Comparative Example 1, were produced anew, but this time the enzyme Cal B was present during the formation of the vesicles. The hydrolytic activity of Cal B, using DiFMU octanoate (6,8-difluor-4-methylumbelliferyl octanoate) as a substrate, was evaluated for Cal B in water (control) and Cal B contained in the three types of capsules (according to Example 2, Example 3 en Comparative Example 1). The Cal B exhibits hydrolytic activity both in aqueous solution and in capsules according to Examples 2 en 3. The activity of Cal B in the capsules according to Examples 2 en 3 is three times lower than the activity of free Cal B in solution. The capsules obtained in accordance with Comparative Example 1 do not exhibit hydrolytic activity in water on account of the low permeability of said capsules in water.

The fact that Cal B, contained in the present capsules according to Examples 2 en 3, exhibits hydrolytic activity in water demonstrates that the substrate (DiFMU octanoate) and the hydrolysis products are capable of passing the capsule wall, which demonstrates the permeability of the present capsules in water as well as the fact that the present capsules according to Examples 2 en 3 are better than the capsules according to Comparative Example 1. Moreover, this example demonstrates the use of the present capsules as nanoreactors.

CLAIMS

1. A permeable capsule formed through stabilisation of a vesicle, comprising a membrane formed from amphiphilic copolymers comprising polymerisable groups, characterized in that the polymerisable groups are thiophene groups.
2. A capsule according to claim 1, characterized in that one or more of the thiophene groups have a substituent.
3. A capsule according to claim 2, characterized in that the substituent is selected from alkyl groups and alkoxy groups.
4. A capsule according to any one or more of the preceding claims, characterized in that the copolymers are block copolymers built up of hydrophobic blocks, hydrophilic blocks and combinations thereof.
5. A capsule according to claim 4, characterized in that two or more blocks in the block copolymers are isolated from each other by a spacer.
6. A capsule according to claim 5, characterized in that the block copolymer is built up of, successively, a hydrophilic block, a spacer and a hydrophobic block.
7. A capsule according to any one or more of the preceding claims, characterized in that the outer surface of the capsule is electrically conductive.
8. A capsule according to any one or more of the preceding claims, characterized in that the amphiphilic copolymers are of the rod-coil type, the coil-coil type or the rod-rod type.
9. A capsule according to claim 8, characterized in that the amphiphilic copolymers are of the rod-coil type.
10. A capsule according to any one or more of the preceding claims, characterized in that the amphiphilic copolymer is polystyrene-6-poly(isocyanoalanine)2-thiophene-3-yl-ethyl)amide) (PS-PIAT).
11. A capsule according to any one or more of the preceding claims, characterized in that the capsule contains a material selected from the group consisting of a catalytically active material, a diagnostic agent, a reagent, a colorant, a pharmaceutically active agent, a preservation agent, a flavouring substance, an antibacterial agent, an aromatic substance, a photosensitive substance and combinations thereof.
12. A capsule according to claim 11, characterized in that the

catalytically active material is selected from the group consisting of a protein, an enzyme, and inorganic complex, an organic compound and combinations thereof.

13. A capsule according to claim 12, characterized in that the enzyme is selected from the group consisting of *Candida antarctica* lipase B (cal B),
5 horseradish peroxidase (HRP), glucose oxidase (GO_x) and combinations thereof.

14. A capsule according to claim 11, characterized in that the reagent is selected from the group consisting of an amino acid, a peptide, a protein, a nucleotide, a monomer and other chemical compounds and combinations thereof.

15. A capsule according to claim 11, characterized in that the diagnostic
10 agent is selected from the group consisting of an antibody, an antibody fragment, a gas, a colorant, a fluorescent substance, a radioactive substance, an antigen, a nucleic acid, magnetic particles and combinations thereof.

16. A method for the preparation of permeable capsules, comprising the formation of a vesicle from an amphiphilic copolymer comprising polymerisable
15 groups and the stabilisation of the vesicles thus formed through polymerisation of polymerisable groups so as to obtain a capsule comprising a membrane that encloses a cavity, characterized in that thiophene groups are used as the polymerisable groups.

17. A method according to claim 16, characterized in that one or more
20 of the thiophene groups have a substituent.

18. A method according to claim 17, characterized in that the substituent is selected from alkyl groups and alkoxy groups.

19. A method according to any one of the claims 16-18, characterized in that the polymerisable groups are polymerised by using metal cations.

20. A method according to claim 19, characterized in that the metal
25 cations are selected from the group consisting of Fe(III) ions, Ru(II) ions and combinations thereof.

21. A method according to claim 20, characterized in that bis(2,2'-bipyridine) ruthenium(II) bispyrazolyl (BRP) is used as the Ru(II) ions.

22. A method according to any one of the claims 16-18, characterized in that the polymerisable groups are electrochemically polymerised.

23. A method according to claim 22, characterized in that an indium tin oxide or a platinum electrode is used during the electrochemical polymerisation process.

24. A method according to any one of the claims 16-23, characterized in that the polymerisation reaction is carried out at a temperature between 0 and 90 °C.

25. A method according to any one of the claims 16-24, characterized in that the polymerisation reaction is carried out in a solvent selected from the group consisting of water, dichloromethane, chloroform, tetrahydrofuran, diethyl ether, acetonitrile and combinations thereof.

26. A method according to any one of the claims 16-25, characterized in that the copolymers are block copolymers built up of hydrophobic blocks, hydrophilic blocks and combinations thereof.

27. A method according to claim 26, characterized in that two or more blocks in the block copolymers are isolated from each other by a spacer.

28. A method according to claim 27, characterized in that the block copolymer is built up of, successively, a hydrophilic block, a spacer and a hydrophobic block.

29. A method according to any one of the claims 16-28, characterized in that the amphiphilic copolymers are of the rod-coil type, the coil-coil type or the rod-rod type.

30. A method according to claim 29, characterized in that copolymers of the rod-coil type are used as the amphiphilic copolymers.

31. A method according to claim 30, characterized in that polystyrene-6-poly(isocyano-alanine)2-thiophene-3-yl-ethyl)amide) (PS-PIAT) is used as the amphiphilic copolymer.

32. A method according to any one of the claims 16-31, characterized in that one or more materials selected from the group consisting of a catalytically active material, a diagnostic agent, a reagent, a colorant, a pharmaceutically active agent, a preservation agent, a flavouring substance, an antibacterial agent, an aromatic substance, a photosensitive substance and combinations thereof are present during the formation of the vesicle, which materials are enclosed during the stabilisation of the vesicle.

33. A method according to claim 32, characterized in that one or more materials are enclosed in the cavity of the capsule.

34. A method according to any one of the claims 32-33, characterized in that the catalytically active material is selected from the group consisting of a protein, an enzyme, and inorganic complex, an organic compound and combinations

thereof.

35. A method according to claim 34, characterized in that the enzyme is selected from the group consisting of *Candida antarctica* lipase B (cal B), horseradish peroxidase (HRP), glucose oxidase (GO_x) and combinations thereof.

5 36. A method according to claim 32, characterized in that the reagent is selected from the group consisting of an amino acid, a peptide, a protein, a nucleotide, a monomer and other chemical compounds and combinations thereof.

37. A method according to claim 32, characterized in that the diagnostic agent is selected from the group consisting of an antibody, an antibody fragment, a gas, a colorant, a fluorescent substance, a radioactive substance, an antigen, a nucleic acid, magnetic particles and combinations thereof.

38. A pharmaceutical intended for retarded delivery, comprising a permeable capsule according to any one or more of the claims 1-15 containing a pharmaceutically active agent.

15 39. A nanoreactor for carrying out one or more reactions, comprising a permeable capsule according to any one or more of the claims 1-15, which contains one or more catalytically active agents or reagents.

40. A paint comprising a permeable capsule according to any one or more of the claims 1-15.

20 41. A coating comprising a permeable capsule according to any one or more of the claims 1-15.

42. A lubricant comprising a permeable capsule according to any one or more of the claims 1-15.

25 43. An ink comprising a permeable capsule according to any one or more of the claims 1-15.

44. A diagnostic substance comprising a permeable capsule according to any one or more of the claims 1-15.

45. A sensor comprising a permeable capsule according to any one or more of the claims 1-15.

30 46. A dielectric material comprising a permeable capsule according to any one or more of the claims 1-15.

47. A foodstuff comprising a permeable capsule according to any one or more of the claims 1-15.

48. A cosmetic substance comprising a permeable capsule according to

any one or more of the claims 1-15.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL2005/000777

A. CLASSIFICATION OF SUBJECT MATTER
B01J13/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
B01J A01N A23K A61K B41M A23L

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 2005/038968 A (JANSSEN, CATHARINA, PHILIPPINA) 28 April 2005 (2005-04-28) the whole document	1, 4-12, 14, 16, 22, 24-34, 36, 38-48
A	WO 01/32146 A (BIOCURE, INC; MEIER, WOLFGANG; HIRT, THOMAS; NARDIN, CORINNE) 10 May 2001 (2001-05-10) cited in the application the whole document	1

☐

Further documents are listed in the continuation of box C.

☒

Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

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

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

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

* & * document member of the same patent family

Date of the actual completion of the international search

24 January 2006

Date of mailing of the international search report

31/01/2006

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Willsher, C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL2005/000777

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005038968 A	28-04-2005	NONE	
WO 0132146 A	10-05-2001	AU 3267301 A	14-05-2001
		CA 2388344 A1	10-05-2001
		EP 1225873 A2	31-07-2002
		JP 2003518015 T	03-06-2003
		US 6916488 B1	12-07-2005