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(54) Title: FLUORINATED HYDROPHOBIN AND USES THEREOF

(57) Abstract: Modified protein comprising a molecule of hydrophobin and at least one fluorocarbon group bound covalently to an amino acid of hydrophobin, obtainable by reaction of a hydrophobin with a fluorinated compound of formula: $Y-R_{H}-R_{F}$, in which Y is a functional group capable of reacting with an amino acid residue of the hydrophobin forming a covalent bond between the fluorinated compound and said amino acid of the hydrophobin, R_{H} is an optional hydrocarbon group, non-fluorinated, aliphatic and/or aromatic, optionally including heteroatoms, and R_{F} is a fluorinated or perfluorinated hydrocarbon residue, linear, branched or cyclic, optionally including one or more heteroatoms.



FLUORINATED HYDROPHOBIN AND USES THEREOF

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The present invention relates to a new protein, called fluoro-hydrophobin hereinafter, obtained by modifying a hydrophobin with a fluorinated chain.

This new fluoroprotein combines the surface activity of fluorinated surfactants with the film-forming capacity of the hydrophobins, allowing them to be used in biological systems and in nanotechnology applications where high compatibility of non-fluorinated materials with highly fluorinated phases is required.

Today there is increased interest in highly fluorinated materials and phases in view of their remarkable properties, such as very low adhesion, friction and surface energy, heat resistance, high oleophobicity and higher hydrophobicity compared with hydrocarbon-based materials (such as oils).

In microfluidic systems, vehicles are currently used that consist of fluorinated oils, in which aqueous or organic microdroplets are dispersed. These systems constitute a route towards miniaturization of many chemical and biological systems (for example for the analysis of genetic materials, proteins and single cells) with benefits in terms of reduction of the amount of sample required, improved efficiency of reaction in small volumes and increased productivity. In these devices, stabilization of the droplets is critical, as the very low affinity between water and fluorinated oils gives rise to coalescence of the droplets.

The known fluorosurfactants are mainly synthetic molecules of low molecular weight and polymers that combine fluorophilic segments with hydrophilic and/or lipophilic segments. In the aforementioned microfluidic systems, the droplet stabilizers typically used include for example PFPE/PEG block copolymers, and the synthesis of numerous small molecules of surfactants for this purpose has been reported,

In formulations of artificial blood consisting of perfluorocarbon/water emulsions, in which the

fluorinated phase acts as a vehicle for oxygen, stabilization of the droplets constitutes a critical aspect that may hamper commercialization of suitable formulations. Many formulations use natural surfactants, such as egg yolk phospholipids, safflower oil and soya lecithin; more recently, some "French emulsions" have been proposed, containing compounds with two fluorocarbon-hydrocarbon blocks.

In flame-proofing or flame-retardant formulations, foams are currently used consisting of simple mixtures of protein lysates and fluorinated surfactants; in this case, these are mixtures in which the fluorinated surfactants constitute an additive of the protein lysates.

Another sector of interest for application of the fluoroproteins according to the invention is that of nanocomposite materials, containing fluoropolymers in which generally hydrophilic fillers are dispersed. Low compatibility between matrices based on fluoropolymers and the fillers has been reported for these nanocomposites, Therefore much effort has been expended for improving the compatibility (for example by terminal functionalization of the polymer, copolymerization of the latter with other polymers or fluorination of the fillers) to overcome the difficulty of obtaining intimate mixing between the fluorinated and non-fluorinated phases required for obtaining stable and durable nanocomposites of fluoropolymers.

In these nanocomposites, the dispersibility of the filler in the polymer may have a considerable influence on the properties of the material; for example, in fluoropolymer/graphene nanocomposites it has been reported that the conductivity behaviour is critically dependent on the dispersibility of the filler.

The aim of the invention is to provide a new surfactant, suitable for stabilization of foams and/or emulsions formed from a hydrophobic fluorinated phase and a hydrophilic phase.

Another aim of the invention is to provide, in general, a new compatibilizing agent between polymer matrix and dispersed phase, for use in the aforementioned applications.

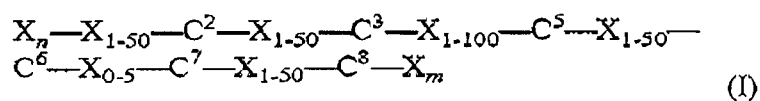
In view of these aims, the invention relates to a new modified protein, as defined in the claims given hereunder, which constitute an integral and supplementing part of the present description.

In particular, the new modified protein according to the invention comprises a molecule of hydrophobin and at least one fluorocarbon group bound covalently to an amino acid of the hydrophobin.

The natural hydrophobins are small proteins, typically having from 100 to 150 amino acids, which are present in filamentous fungi. Typically they have 8 cysteine units. The hydrophobins to which the invention refers can be isolated from natural sources, but also comprise hydrophobins obtained by recombinant techniques.

The hydrophobins are known for their emulsifying, thickening and surfactant properties and have been used for increasing the hydrophilicity of hydrophobic phases and for improving the water resistance of hydrophilic substrates and thus also for preparing oil-in-water or water-in-oil emulsions, for example in the pharmaceutical and cosmetic sectors,

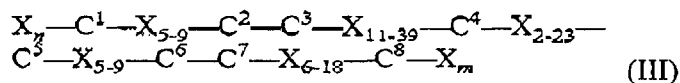
In the context of the present invention, it is generally possible to use hydrophobins, as defined in US 7 981 313, whose description (from column 3, line 22 to column 4, line 45) is to be considered to be incorporated in the present specification by reference. In particular, the scope of the invention is to be understood as including hydrophobins having general formula (I):



in which X can be one of the twenty naturally occurring amino acids, where the groups X can be identical or different in each case; in formula (I), C represents cysteine, alanine, serine, glycine, methionine or threonine, provided that at least four of the residues identified with C are cysteine, where the subscripts n and m are, each independently, integers between 0 and 500, preferably between 15 and 300.

In the aforementioned formula, in particular, the amino acids designated with C¹ to C^s are preferably cysteine; however, the scope of the invention includes the use of hydrophobins having the aforementioned formula (I), in which at least four or preferably at least five of the groups C¹ to C^s are cysteine.

A more restricted group of hydrophobins usable in the context of the invention can be defined by formula (III):



in which the groups X and C are as defined above and the subscripts n and m are integers, preferably between 0 and 200; also in this case, a hydrophobin is preferred in which at least six of the residues indicated with C are cysteine.

Genetically modified hydrophobins, i.e. variants in which the amino acid sequence has been modified by recombinant DNA techniques, are also usable in the context of the invention.

In particular, the use of modified hydrophobins, such as NCys-HFBI and HFBI-CysC, as described by G. Szilvay et al., Febs. Letters 581 (2007) 2721, constitutes a preferred embodiment. It is possible to use natural (wild-type) hydrophobins of class I or class II, in particular natural or modified hydrophobins derived from *Trichoderma reesei*.

As already mentioned, the choice of hydrophobin is not particularly critical and it is possible to use all the hydrophobins having at least one amino acid capable of reacting with a fluorocarbon compound, as defined below, to form a covalent bond.

In particular, the modified hydrophobins according to the invention are obtainable by reacting a hydrophobin with a compound of formula **Y-RH-RF**, in which:

- **Y** is a functional group capable of reacting with an amino acid residue of the protein to form a covalent bond between the fluorinated molecule and the protein. The amino acids

involved in this reaction are preferably selected from cysteine, lysine, aspartic acid and glutamic acid. The functional group Y can include, as non-limiting examples;

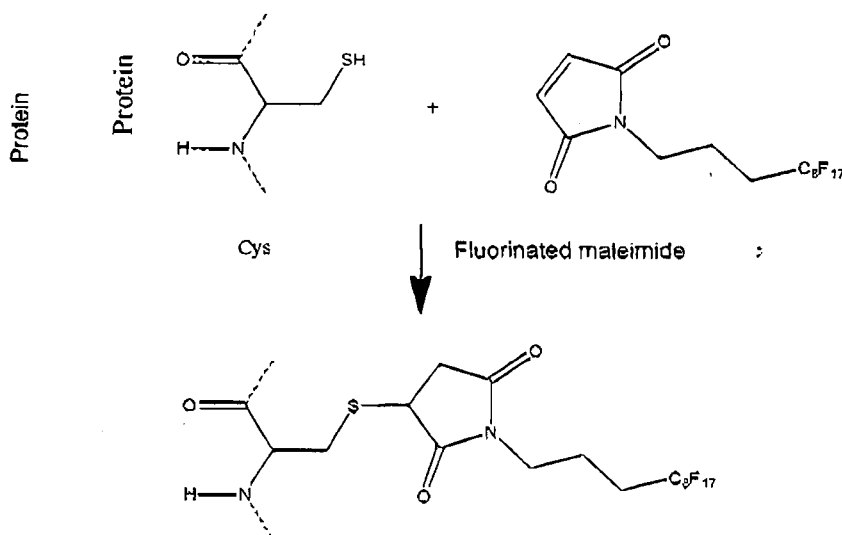
- i) maleimide, iodoacetamide and sulphydryl group, for reaction with cysteine residues; the maleimide group being particularly preferred;
 - ii) a carboxylic acid or a reactive ester, for reaction with lysine residues; and
 - iii) an amino group for reaction with aspartic acid and glutamic acid residues;
- the group R_H is an optional group, which can be a non-fluorinated hydrocarbon residue, of aliphatic and/or aromatic nature, optionally containing heteroatoms (such as oxygen, sulphur and nitrogen); however, in the preferred embodiment, the group R_H is a $-(CH_2)_n-$ alkyl chain, where n is typically between 2 and 8, preferably 3 or 4;
 - R_F is a fluorinated hydrocarbon residue, which can be linear, branched or cyclic and saturated or unsaturated and which optionally can contain one or more heteroatoms (for example oxygen, nitrogen or other halogens); preferably it is a perfluorinated or highly fluorinated residue, where the term highly fluorinated means that at least 30% of the hydrogen atoms in the corresponding hydrocarbon residue have been replaced with fluorine atoms; in a preferred embodiment, the group R_F is a perfluoroalkyl chain, preferably having from 6 to 20 carbon atoms; other examples comprise partially fluorinated alkyl residues and perfluoropolyethers of varying chain length, preferably comprising from 3 to 30 repeating groups $-(CF_2-O)-$ or $-(CF_2-CF_2-O)-$.

In general, the method of preparation is carried out by reacting the hydrophobin (having an accessible Cys residue or other accessible residue, such as Lys, Asp or Glu) with a molar excess of the fluorinated compound in a mixture of organic solvent and water at room temperature. The product is then purified by HPLC and lyophilized.

The following example illustrates the preparation of a fluorinated hydrophobin according to a preferred embodiment, in which the hydrophobin is NCys-HFBI and the fluorinated compound is a fluorinated maleimide.

Preparative_example_1

The reaction is carried out according to the following reaction scheme:



5 mg of reduced NCys-HFBI is dissolved in a mixture of 2 ml of THF and 3,5 ml of phosphate buffer (100 mM, pH 7.2). Then a solution of 5.8 mg of 3-(perfluorooctyl)-propyl-1-maleimide in 1.5 ml of THF is added. The test tube containing the mixture is put under a nitrogen atmosphere, wrapped in aluminium foil and shaken gently at room temperature for 5 hours.

At this point, 8 mg of polystyrene particles functionalized with N-(2-mercaptoethyl)-aminomethyl] (1.33 mmol/g N) is added, for sequestering the unreacted fluorinated maleimide, and the mixture is shaken for 30 minutes at room temperature. The polystyrene particles are then separated from the supernatant by centrifugation and washed twice with 2 ml of THF at 50% in water, which is added to the mother liquor after further centrifugation. The solution is then lyophilized and the solid is dissolved again in 2 ml of 50% ethanol for purification by reversed-phase HPLC on a Resource RPC column (GE Healthcare), using a water/acetonitrile gradient (both containing 0.1% trifluoroacetic acid).

The fluorinated protein is then recovered by lyophilization, typically obtaining a yield of 65-70%.

Similarly, following the procedure described above, fluorinated hydrophobins were prepared according to the following examples:

- preparative example 2: protein reacted: HFBI-CysC, fluorinated compound: 3-(perfluorooctyl)-propyl-1-maleimide.

- preparative example 3: protein reacted: NCys-HFBI, fluorinated compound: N-[(3-perfluorooctyl)propyl]-iodoacetamide.

Preparative example 4

5 mg of HFBI is dissolved in a mixture of 2 ml of THF and 3.5 ml of borate buffer (25 mM, pH 8). A solution of 16.2 mg of N-succinimidyl-3-perfluorohexyl-propionate in 1.5 ml of THF is then added. The test tube containing the mixture is shaken gently at room temperature for 3 hours, then 25 μ l of 50% hydroxylamine is added. The solution is then lyophilized and the solid is dissolved again in 2 ml of 50% ethanol for purification by reversed-phase HPLC on a Resource RPC column (GE Healthcare), using a water/acetonitrile gradient (both containing 0.1% trifluoroacetic acid).

The fluorinated protein is then recovered by lyophilization, typically obtaining a yield of 55-60%.

The properties of the fluorinated protein prepared according to example 1, designated F-HFBI hereinafter, were determined, in comparison with the non-fluorinated native hydrophobin HFBI, in the practical examples given below.

Example A - Reduction of interfacial energy

The interfacial energy between milli-Q water and the perfluoropolyether Galden SV90 was monitored over time by the sessile drop technique on a CAM200 instrument (KSV Ltd.). A 10 μ l drop of Galden SV90 was introduced into an aqueous solution containing F-HFBI, or the non-fluorinated native protein HFBI, at a concentration of 0.01 mM and the interfacial energy

was found at intervals of 30 seconds from the shape of the drop of Galden.

As shown in the graph in Fig. 1, the fluorinated protein is quicker and more effective in reducing the interfacial tension between the two liquids (which is about 46-5 mN/m in the absence of any surfactant). For F-HFBI, the largest part of the reduction in tension occurs within the first minutes of contact between the immiscible liquids, with a significant portion within a few seconds.

A minimum surface tension of about 10 mN/m is reached after 30 minutes. Although this value of interfacial tension is not found to remain constant at the measured time, it was not possible to perform the measurements for longer times owing to the considerable deformation of the drop and the formation of wrinkles on its surface, making fitting of the shape unreliable.

Example B - Preparation of emulsions

Emulsions of fluorinated oil in water were prepared from a) perfluorooctyl bromide (PFOB) and b) Galden SV90 in solutions of milli-Q water containing various amounts of F-HFBI. The volume fraction of the aqueous phase was maintained at 5% and the total volume of the emulsions was 1 ml. The emulsions were formed by ultrasonication with an ultrasonic probe (Soniprep 150 MSE, 24 kHz, amplitude 26 μm , 150 W) using, for 30 minutes, a cycle comprising 20 seconds of sonication and 30 seconds of rest, in a water bath. The amplitude of the sonication was fixed at 40% of the maximum attainable by the instrument. The emulsions were investigated immediately by optical microscopy and the skimming was monitored visually.

The protein concentrations tested are equal to 0,01 mg/ml, 0.1 mg/ml and 1 mg/ml for all the tests. Fluorinated microdroplets were obtained with dimensions ranging from 0.5 μm to 6 μm . Average dimensions under 1 μm were observed for PFOB at the highest protein concentrations.

When Galden SV90 was used as the oily phase, sedimentation of the microdroplets occurred after one day, whereas the process proved slower for PFOB, requiring 7 days for complete sedimentation. It is known that homogeneous dispersion can then be obtained again in both cases by simple manual stirring, even at least 1 month after preparation and without showing visible signs of macroscopic phase separation.

Example C - Coating of solid substrates

The coating capabilities of F-HFBI were tested for four substrates with different affinity: (i) soda-lime glass, (ii) silanized glass (treated with trimethylchlorosilane), (iii) fluorinated slides for microarrays (obtained from Fluorous Technologies, Inc.), (iv) graphite plates,

The following method of coating was used in all cases.

Three samples of solid substrate (0.5x1.5 cm) were put in a glass Petri dish and immersed in 3 ml of 0.01 mM solution of F-HFBI in milli-Q water, The Petri dish was sealed and shaken gently overnight on a rotary shaker. The samples were then removed from the solution and washed in a new Petri dish on a rotary shaker, sequentially with 3 ml of milli-Q water for 10 minutes, 3 ml of acetonitrile at 20% in water for 5 minutes and finally again with 3 ml of milli-Q water for 5 minutes, and then dried under a nitrogen stream. The presence of the protein on the coated surfaces was verified by measuring the contact angle of water (see the following table) and AFM microscopy.

	Soda-lime glass	Silanized glass	Fluorous glass	Graphite
Original substrate	17°	104°	103°	83°
Substrate coated with F-HFBI	40°	87°	70°	82°

It should be noted that although the value of the contact angle in the case of coating on graphite is very similar to that found for the original substrate, the presence of a homogeneous protein layer was verified by AFM imaging. The images showed compact and homogeneous protein layers for all the substrates, except for the soda-lime glass, on which the protein formed an incomplete covering.

The coating capabilities of F-HFBI (and for comparison, of HFBI) were also investigated by Quartz Crystal Microbalance (QCM) experiments on gold sensor chips previously coated with (i) 1H,1H,2H,2H-perfluorodecano-1-thiol (fluorophilic), (ii) hexane-1-thiol (lipophilic), (iii) 11-mercaptoundecan-1-ol (hydrophilic),

During the experiments, the sensor chips were exposed to 0.01 mM solutions of protein dissolved in 20% acetonitrile in a 50 mM phosphate buffer (pH 7.2) and then washed with the same acetonitrile/buffer mixture until the amount of protein adsorbed on the substrates had stabilized. The resultant values of adsorbed mass are given in the following table.

Protein	Substrate	Mass adsorbed (ng/cm ²)
HFBI	fluorophilic	321.2
F-HFBI	fluorophilic	516.0
HFBI	lipophilic	367.1
F-HFBI	lipophilic	818.3
HFBI	hydrophilic	207.7
F-HFBI	hydrophilic	317.3

The results show greater capacity for adsorption of the fluorinated protein, particularly for fluorophilic and lipophilic substrates. The variation in mass adsorbed cannot be attributed to the simple difference of molecular weights of the proteins (7532 Da for HFBI, 9232 Da for F-HFBI) and is more probably linked to the occurrence of so-called "fluorous-fluorous" interactions between the perfluorinated alkyl chains of various F-HFBI proteins, which are

therefore more likely to form adsorbed multilayers (for comparison, a monolayer of HFBI is estimated to correspond approximately to 200 ng/cm²).

Example D - Exfoliation of graphite and dispersion in fluoruous media

Exfoliation of graphite was effected by treating 0.2 mg of graphite with a tip sonicator (Soniprep 150 MSE, 24 kHz, amplitude 26 μ m, 150 W, sonication at 40% of maximum amplitude) for 15 minutes in an aqueous solution of F-HFBI (0.5 mg/ml in milli-Q water). Exfoliation was effected on 0.2 mg of graphite in a volume of 1 ml of a 0.5 mg/ml solution of protein in milli-Q water. After exfoliation, the larger, non-exfoliated fragments of graphite were separated from the exfoliated material by gentle centrifugation and the material that remained, consisting of a light fraction of ultrafine graphite and graphene, was lyophilized. A solution of poly(1H,1H,2H,2H-perfluorodecylacrylate), called P(AF8) hereinafter, in Galden SV90 (4 mg/ml) was added and the mixture was sonicated as described above.

The amount of graphite exfoliated, dispersed homogeneously in the fluorinated solvent, was determined by UV-Vis spectrophotometry of the solution after gentle centrifugation with a minicentrifuge, using the value of absorbance at wavelength of 660 nm as the indicative parameter. For comparison, the results are also shown for samples in which the graphite was treated with native protein HFBI or was not treated with any protein before attempting redispersion in the Galden SV90/F(AF8) mixture. The amount of graphene dispersed is significantly greater when the graphite had been treated beforehand with F-HFBI ($A=0.703$), compared with when the sample was treated with HFBI ($A=0.380$) or not previously exposed to any protein ($A = 0.029$), thus indicating a clear increase in affinity for the fluorinated phase due to F-HFBI.

The experimental tests demonstrate how F-HFBI combines the remarkable properties of surface activity of the fluorosurfactants with the unusual film-forming properties of the hydrophobic, which have been reported to possess the capacity to form elastic films and particular effectiveness in the stabilization of foams and emulsions.

The modified hydrophobins according to the invention contain hydrophilic, lipophilic and fluorophilic exposed surface regions, a characteristic that is distinctive relative to the known hydrophobins, which only contain hydrophilic and lipophilic sections.

In general, the fluorinated hydrophobins according to the invention are capable of dissolving in aqueous solutions, even containing small amounts of organic solvents. As shown by the image in Fig. 2, obtained for a drop of Galden SV90 immersed in 0.01 mM solution of F-HFBI in milli-Q water, in the presence of a fluorous/aqueous interphase, the protein takes up a position at the interface and forms a strong, elastic film within a few seconds. This film hampers coalescence of the coated droplets when these come in contact.

Owing to these characteristics, the fluorinated hydrophobins according to the invention are superior relative to the known fluorinated surfactants for their remarkable film-forming abilities, and display greater affinity for the fluorinated phases compared with the non-fluorinated hydrophobins, allowing them to be used as stabilizers and compatibilizing agents in systems containing highly fluorinated phases.

CLAIMS

1. Modified protein comprising a hydrophobin molecule and at least one fluorocarbon group covalently linked to an amino acid of the hydrophobin.

2. Modified protein according to Claim 1, characterized in that it is obtainable by reaction of a hydrophobin with a fluorinated compound of formula:

Y-RH-RF,

wherein

Y is a functional group capable of reacting with an amino acid residue of the hydrophobin forming a covalent bond between the fluorinated compound and said amino acid of the hydrophobin,

R_H is an optional aliphatic and/or aromatic hydrocarbon group, possibly including heteroatoms, and

R_F is a fluorinated or perfluorinated hydrocarbon residue, which can be linear, branched or cyclic, possibly including one or more heteroatoms.

3. Modified protein according to Claim 2, wherein the group Y is selected from the group consisting of maleimide, iodoacetamide, sulphydryl, carboxyl, reactive ester of carboxylic acid and amine.

4. Modified protein according to Claims 2 or 3, where R_H is an alkyl group having from 2 to 8 carbon atoms, having preferably 3 or 4 carbon atoms.

5. Modified protein according to any one of Claims 2 to 4, in which R_F is selected from a perfluoroalkyl group having preferably 6 to 20 carbon atoms, perfluoroether, or an alkyl group in which at least 30% of the hydrogen atoms is replaced with fluorine atoms.

6. Modified protein according to any of the preceding claims, in which the fluorinated compound is linked to an amino acid selected from cysteine, lysine, aspartic acid and glutamic

acid.

7. Modified protein according to any of the preceding claims, wherein the hydrophobin is a natural or genetically modified hydrophobin.
8. Modified protein according to any of the preceding claims, wherein the hydrophobin is a NCys-HFBI or HFBI-CysC and wherein the fluorinated compound is linked to a Cys amino acid of the hydrophobin and said fluorinated compound is a fluorinated maleimide.
9. Use of a modified protein according to any one of Claims 1 to 8, as a surfactant for the stabilization of foams and/or emulsions, formed by a fluorinated hydrophobic phase and a hydrophilic phase.
10. Use of a modified protein according to any one of Claims 1 to 8, as a stabilizer in the formulation of artificial blood comprising a perfluorocarbon/water emulsion.
11. Use of a modified protein according to any one of Claims 1 to 8, as a stabilizer of drops in microfluidic systems based on perfluorocarbon/ water and/or perfluorocarbon/organic solvent.
12. Use of a modified protein according to any one of Claims 1 to 8, as a compatibilizer in nanocomposite materials, including a fluoropolymer phase and a non-fluorinated dispersed phase.

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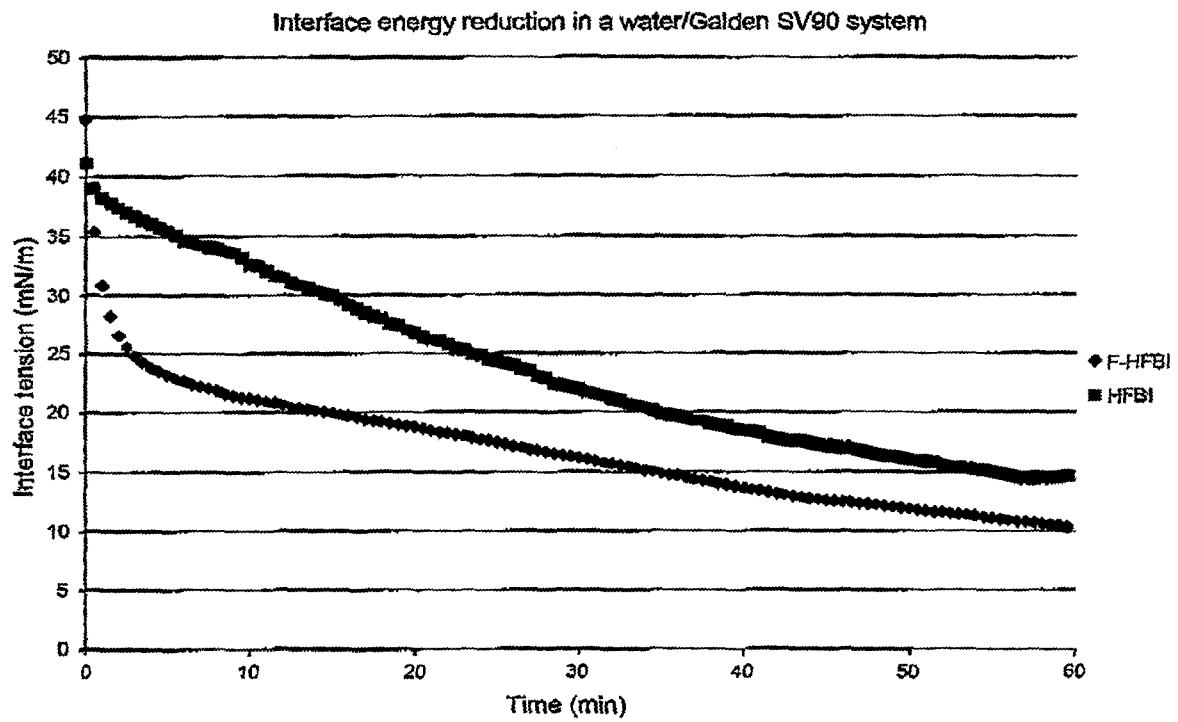


FIG. 1

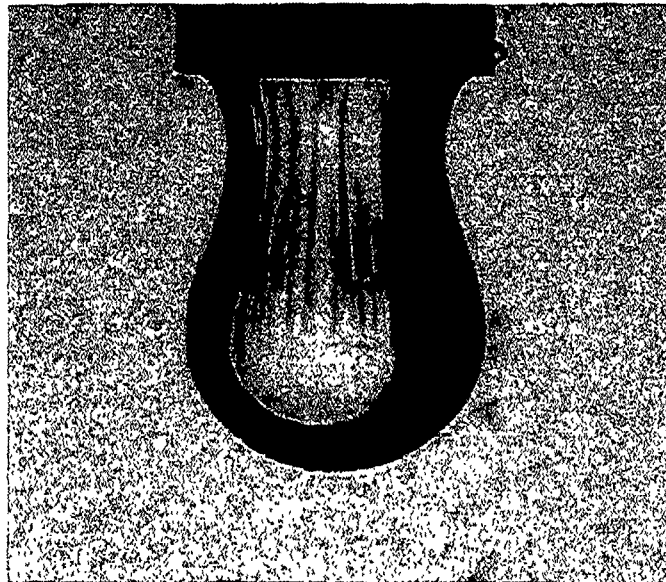


FIG. 2

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2012/056785

A. CLASSIFICATION OF SUBJECT MATTER
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 ADD.

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)

A61K C07K CUD

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 practicable, search terms used)

EPO-Internal , CHEM ABS Data, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/136433 AI (SUBKOWSKI THOMAS [DE] ET AL) 28 May 2009 (2009-05-28)	1
A	[0030] seqq, [0040] , [0059] seqq, [0090] seqq	2-12
A	----- RAMANATHAN M ET AL: "Basic properties of foam films stabilized with tetraethyl ammonium salt of perfluorooctane sulfonate (PFOS) ", COLLOIDS AND SURFACES. A, PHYSICOCHEMICAL AND ENGINEERING ASPECTS, ELSEVIER, AMSTERDAM, NL, vol . 354, no. 1-3 , 5 February 2010 (2010-02-05) , pages 1-7 , XP026824889 , ISSN : 0927-7757 [retrieved on 2009-11-24] abstract -----	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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US 2009136433 A1	28-05-2009	CA 2612458 A1	28-12-2006
		CN 101203207 A	18-06-2008
		DE 102005029704 A1	11-01-2007
		EP 1898871 A2	19-03-2008
		JP 2009501701 A	22-01-2009
		US 2009136433 A1	28-05-2009
		WO 2006136607 A2	28-12-2006
