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(54) Title: METHOD OF ACYLATING AMINO ACIDS AND USES OF N-ACYL AMINO ACID PRODUCTS

(57) Abstract: The present invention relates to a method of preparing *N*-acyl amino acids selected from *N*-acyl cysteine compounds, *N*-acyl serine compounds, *N*-acyl aspartic acid compounds and *N*-acyl glutamic acid compounds. The present invention also relates to the use of *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid and *N*-acyl glutamic acid surfactants, in removing per- and poly-fluoroalkyl substances (PFASs) from mixtures containing PFASs, such as soil and groundwater contaminated with PFASs and for use in cleaning compositions, detergent compositions and toothpaste compositions.



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Method of acylating amino acids and uses of *N*-acyl amino acid products

Field of the Invention

5 [0001] The present invention relates to a method of preparing *N*-acyl amino acids selected from *N*-acyl cysteine compounds, *N*-acyl serine compounds, *N*-acyl aspartic acid compounds and *N*-acyl glutamic acid compounds. The method finds particular, though not exclusive, application in the preparation of *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid and *N*-acyl glutamic acid surfactants and will primarily be described in this context.

10 As will be appreciated, the present invention may be applied to the preparation of a wide range of *N*-acyl cysteine compounds *N*-acyl serine compounds, *N*-acyl aspartic acid compounds and *N*-acyl glutamic acid compounds for a wide range of applications.

[0002] The present invention also relates to the use of *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid and *N*-acyl glutamic acid surfactants, including their use in removing
15 per- and poly-fluoroalkyl substances (PFASs) from mixtures containing PFASs, such as soil and groundwater contaminated with PFASs.

Background of the Invention

[0003] *N*-Acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid and *N*-acyl glutamic acid
20 products have many uses. For example, *N*-acyl cysteines having longer acyl chains have been useful as surfactants. In WO 2018/071985, *N*-acyl cysteines having longer acyl chains were useful for removing heavy metals from aqueous solutions.

[0004] One problem that has hindered wider commercial use of *N*-acyl cysteines is the difficulty in obtaining *N*-acyl cysteines on a commercially useful scale. Underlying this
25 problem is the low-yielding processes currently available for preparing *N*-acyl cysteines.

[0005] It would be advantageous to provide alternative methods of accessing *N*-acyl cysteines, *N*-acyl serines, *N*-acyl aspartic acids and *N*-acyl glutamic acids, especially methods that are efficient (i.e. high yielding), clean (i.e. minimal and/or easily removable by-products), operationally simple, and do not require difficult and/or time-consuming
30 purification steps to deliver *N*-acyl cysteines, *N*-acyl serines, *N*-acyl aspartic acids and *N*-acyl glutamic acids in a form pure enough for subsequent use.

[0006] Per- and poly-fluoroalkyl substances (PFASs) have been widely used over the last 60 years or more for a range of applications, including in non-stick cookware, as water

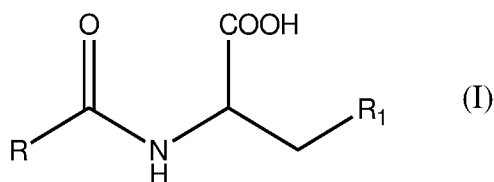
- 2 -

repellent coatings, stain resistant fabrics and carpets, some cosmetics and some products that resist grease, water, and oil. Notably, PFASs have been used in fire-fighting foams, especially for aviation fires, where they have been released into the environment, including into the groundwater surrounding airports. PFASs can travel long distances, move through soil, seep into groundwater or be carried through air. The release of PFASs into the environment has become an increasing concern as PFASs do not easily break down by natural processes. PFASs can persist for long periods in the environment and can be absorbed by humans or animals, where they may accumulate. Recent studies have reported that human exposure to PFASs is associated with adverse health effects. For example, studies have shown that PFASs affect growth, learning, and behavior of infants and older children; lower a woman's chance of getting pregnant; interfere with the body's natural hormones; increase cholesterol levels; affect the immune system; and increase the risk of cancer. The USEPA has recommended limits of 70ng/L for these compounds in drinking water.

[0007] It would be advantageous to provide methods of removing PFASs from mixtures containing PFASs, such as soil or groundwater contaminated with PFASs.

Summary of the Invention

[0008] In a first aspect, the present invention provides a method of preparing an *N*-acyl amino acid of Formula (I):



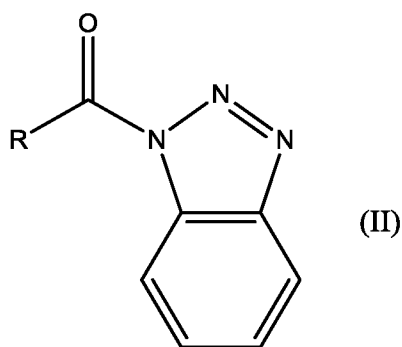
or a salt thereof,

where R is selected from H, C₁-C₁₅ alkyl groups, C₂-C₁₅ alkenyl groups, C₂-C₁₅ alkynyl groups, fluorinated C₁-C₁₅ alkyl groups, fluorinated C₂-C₁₅ alkenyl groups and fluorinated C₂-C₁₅ alkynyl groups, and

R₁ is selected from SH, OH, CO₂H and CH₂CO₂H,

the method comprising reacting cysteine, serine, aspartic acid or glutamic acid and an *N*-acylbenzotriazole of Formula (II):

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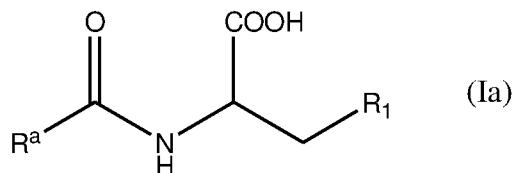


wherein R is as defined in Formula (I), in a solvent composed substantially of methanol.

[0009] In some embodiments, R is a linear or branched C₅-C₁₀ alkyl group. In some embodiments, R is a linear C₆-C₉ alkyl group. In some embodiments, R₁ is SH. In some
5 embodiments, the solvent is at least 99 %v/v methanol.

[0010] In a second aspect, the present invention provides an *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid or *N*-acyl glutamic acid of Formula (I) or a salt thereof prepared by the method of the first aspect.

[0011] In a third aspect, the present invention provides a method of removing one or
10 more PFASs from an aqueous phase comprising one or more PFASs, the method comprising dissolving in the aqueous phase a foaming agent of Formula (Ia):



or a salt thereof,

wherein R^a is a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group, a C₅-C₁₂ alkynyl group, a
15 fluorinated C₅-C₁₂ alkyl group, a fluorinated C₅-C₁₂ alkenyl group or a fluorinated C₅-C₁₂ alkynyl group;

R₁ is SH, OH, CO₂H or CH₂CO₂H;

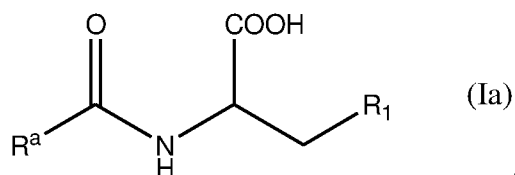
forming a foam from the aqueous phase and a gas; and
separating the foam from the aqueous phase.

[0012] In some embodiments, R^a is a C₆-C₉ alkyl group. In some embodiments, R₁ is
20 SH. In particular embodiments, the foaming agent of formula (Ia) is in a salt form. In some embodiments, the foaming agent of Formula (Ia) or salt thereof has a solubility of greater than 0.0001 M in water. In some embodiments, the Critical Micelle Concentration (CMC) of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is greater
25 than 0.0001 M. In some embodiments, the concentration of the foaming agent of Formula

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(Ia) or salt thereof in the aqueous phase is from about 0.01 mM to about 2 M, for example, from about 0.01 mM to about 0.02 M or from about 0.01 mM to about 0.2 M. In some embodiments, the concentration of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is from about 0.0001 M to about 2 M, for example, from about 0.0001 mM to about 0.02 M or from about 0.0001 mM to about 0.2 M. In some embodiments, the one or more PFASs comprises at least one PFAS capable of binding to a multivalent metal ion. In some embodiments, the aqueous phase has a pH within the range of from about pH 4 to about pH 12. In some embodiments, the aqueous phase comprises one or more metal ions selected from Ca^{2+} , Mg^{2+} , Al^{3+} and Fe^{3+} . In some embodiments, the aqueous phase comprises PFAS-contaminated soil or PFAS-contaminated groundwater.

[0013] In a fourth aspect, the present invention provides the use of a foaming agent of Formula (Ia):



or a salt thereof,

where R^a is a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group, a C₅-C₁₂ alkynyl group, a fluorinated C₅-C₁₂ alkyl group, a fluorinated C₅-C₁₂ alkenyl group or a fluorinated C₅-C₁₂ alkynyl group;

R₁ is SH, OH, CO₂H or CH₂CO₂H;

for removing one or more PFASs from an aqueous phase.

[0014] In some embodiments, the foaming agent of Formula (Ia) or salt thereof is used in a foam fractionation process to remove the one or more PFASs from the aqueous phase. In some embodiments, R^a is a C₆-C₉ alkyl group. In some embodiments, R₁ is SH. In particular embodiments, the foaming agent of formula (Ia) is in a salt form. In some embodiments, the foaming agent of Formula (Ia) or salt thereof has a solubility of greater than 0.0001 M in water. In some embodiments, the CMC of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is greater than 0.0001 M. In some embodiments, the aqueous phase comprises PFAS-contaminated soil or PFAS-contaminated groundwater.

[0015] In some embodiments, there is provided a cleaning composition, detergent composition or toothpaste composition comprising an *N*-acyl amino acid of Formula (I), or

a salt thereof, where R is selected from a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group and a C₅-C₁₂ alkynyl group and R₁ is SH, OH, CO₂H or CH₂CO₂H.,

Brief Description of the Figures

5 [0016] Particular embodiments of the present invention are described below, by way of example only, with reference to the accompanying drawings in which:

Figure 1 is a schematic representation of a column apparatus suitable for carrying out the method of the third aspect of the present invention.

10 **Figure 2** is a schematic representation of another example of a column apparatus suitable for carrying out the method of the third aspect of the present invention. An expanded view of the waste container is shown in Figure 7.

Figure 3 is a graph showing the electrical conductivities of the surfactant solutions at pH 8, used to calculate the CMC in Example 1.

15 **Figure 4** is an FT-IR spectra of *N*-octanoyl-cysteine obtained by Exemplary method 1, as described in Example 2.

Figure 5 is a schematic representation of the presumed orientation of the foaming agent around a bubble.

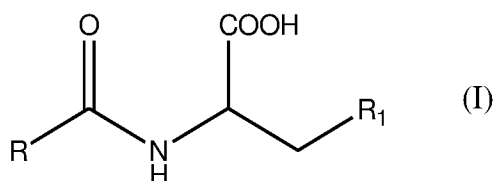
20 **Figure 6** is a pair of photos from the foaming test described in Example 2; the first photo (upper) is a photo of *N*-octanoyl cysteine solutions at: pH 3 (far left), pH 6 (middle left), pH 9 (middle right) and pH 12 (far right) and the second photo (lower) is a photo of *N*-octyl cysteine solutions at: pH 3 (far left), pH 6 (middle left), pH 9 (middle right) and pH 12 (far right). (C_{surfactant}=0.01M and the pH adjusted with 0.1 M NaCl).

25 **Figure 7** is a schematic diagram of the region in and adjacent to the surface of a bubble showing: a possible binding motif between a negatively charged soil particle, a metal ion and a PFAS (upper left); a possible binding motif between a negatively charged soil particle, a metal ion and a foaming agent of Formula (Ia) (lower left); and a possible interaction between the hydrophobic regions of a PFAS and a foaming agent of Formula (Ia) (right). Such a scenario may, for example, exist in the waste container depicted in Figure 2.

Detailed Description of the Invention

[0017] Particular embodiments of the present invention are described below, by way of example only.

[0018] In a first aspect, the present invention provides a method of preparing an *N*-acyl amino acid of Formula (I):

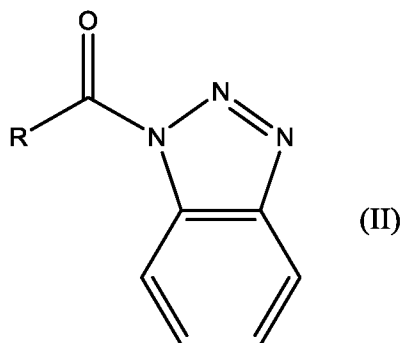


or a salt thereof,

where R is selected from H, C₁-C₁₅ alkyl groups, C₂-C₁₅ alkenyl groups, C₂-C₁₅ alkynyl groups, fluorinated C₁-C₁₅ alkyl groups, fluorinated C₂-C₁₅ alkenyl groups and fluorinated C₂-C₁₅ alkynyl groups, and

R₁ is SH, OH, CO₂H or CH₂CO₂H;

the method comprising reacting cysteine and an *N*-acylbenzotriazole of Formula (II):



wherein R is as defined in Formula (I), in a solvent composed substantially of methanol.

[0019] In some embodiments, R is H. In such embodiments, the reaction may be referred to as a “formylation” reaction. In other embodiments (e.g. where R is a C₁-C₁₅ alkyl group) the reaction may be referred to as an “acylation” reaction.

[0020] The term “alkyl” refers to a straight chain or branched chain saturated hydrocarbyl group. The term “C₁₋₁₅ alkyl” refers to an alkyl group having 1 to 15 carbon atoms. Similarly, “C₅₋₁₂ alkyl” refers to an alkyl group having 5 to 12 carbon atoms.

Examples of linear alkyl groups include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl and n-dodecyl and the like. Examples of branched alkyl groups include 2-methylpropyl, 2-methylbutyl, 3-methylbutyl, 2,2-dimethylpropyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2,2-dimethylbutyl, 2,3-

dimethylbutyl, 2-ethylbutyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 3,4-dimethylpentyl, 2-ethylpentyl, 3-ethylpentyl and the like.

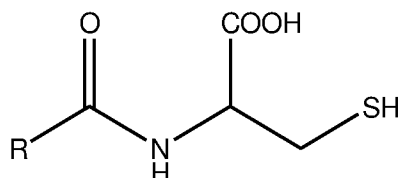
[0021] The term “alkenyl” refers to a straight chain or branched chain hydrocarbonyl group having at least one double bond of either *E*- or *Z*- stereochemistry where applicable. The term “C₂₋₁₅ alkenyl” refers to an alkenyl group having 2 to 15 carbon atoms. Similarly, “C₅₋₁₂ alkenyl” refers to an alkenyl group having 5 to 12 carbon atoms. In some embodiments, the alkenyl group contains more than one double bond, for example 2, 3, 4, 5 or 6 double bonds. The alkenyl group may, in addition to the one or more double bonds, also contain one or more triple bonds. Examples of alkenyl include vinyl, 1-propenyl, 1- and 2-butenyl, 2-methyl-2-propenyl, 2- and 3-butenyl, 2-, 3- and 4-pentenyl, 2-, 3-, 4- and 5-hexenyl, 2-, 3-, 4-, 5- and 6-heptenyl, 2-, 3-, 4-, 5-, 6- and 7-octenyl, 3-methyl-6-octenyl and the like.

[0022] The term “alkynyl” refers to a straight chain or branched chain hydrocarbonyl group having at least one triple bond. The term “C₂₋₁₅ alkynyl” refers to an alkynyl group having 2 to 15 carbon atoms. Similarly, “C₅₋₁₂ alkynyl” refers to an alkynyl group having 5 to 12 carbon atoms. In some embodiments, the alkynyl group contains more than one triple bond, for example 2, 3, 4, 5 or 6 triple bonds. The alkynyl group may, in addition to the one or more triple bonds, also contain one or more double bonds. Examples of alkynyl include ethynyl, 1-propynyl, 1- and 2-butyne, 2-, 3- and 4-pentyne, 2-, 3-, 4- and 5-hexynyl, 2-, 3-, 4-, 5- and 6-heptyne, 2-, 3-, 4-, 5-, 6- and 7-octynyl, 3-methyl-6-octynyl and the like.

[0023] The term “fluorinated” refers to a group such as an alkyl, alkenyl or alkynyl group defined above, in which one or more hydrogen atoms in the relevant group are replaced with one or more fluorine atoms and includes instances where all of the hydrogen atoms are replaced with fluorine atoms. Functional groups where all of the hydrogen atoms have been replaced with fluorine are referred to as “perfluorinated”. In some embodiments, the fluorinated group contains one fluorine atom. In other embodiments, the fluorinated group contains more than one fluorine atom (e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 fluorine atoms), as permitted by the valency of the relevant group. Examples of fluorinated alkyl groups include -CH₂F, -CHF₂, -(CH₂)₃CF₂CH₃, -(CH₂)₅CF₃ and -(CF₂)₆CH₃. Examples of perfluorinated alkyl groups include -CF₃, -C₆F₁₃, -C₇F₁₅ and -C₈F₁₇.

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[0024] In some embodiments, R₁ is SH and the compound of formula (I) is a N-acyl cysteine. In other embodiments, R₁ is OH and the compound of formula (I) is a N-acyl serine. In yet other embodiments, R₁ is CO₂H and the compound of formula (I) is a N-acyl aspartic acid. In yet other embodiments, R₁ is CH₂CO₂H and the compound of formula (I) is a N-acyl glutamic acid. In particular embodiments, R₁ is SH and the compound of formula (I) is a N-acyl cysteine having the formula:



[0025] In some embodiments, R is branched (e.g. a branched C₃-C₁₅ alkyl group, a branched C₃-C₁₅ alkenyl group or a branched C₄-C₁₅ alkynyl group). Due to steric considerations, when R is branched, the α-carbon (i.e. the carbon alpha to the carbonyl of the acyl group) is preferably CH₂ (i.e. the α-carbon is preferably not the site of branching in the branched group). As a person skilled in the art will appreciate, acyl groups having substituted α-carbons may be affected by steric effects during the reaction, leading to a less facile reaction.

[0026] In some embodiments, R contains from 1 to 15 carbon atoms (i.e. is a C₁-C₁₅ group). Examples include a C₂-C₁₀ group, a C₂-C₅ group, a C₃-C₁₅ group, a C₃-C₁₀ group, a C₃-C₅ group, a C₄-C₁₅ group, a C₄-C₁₀ group, a C₄-C₈ group, a C₄-C₆ group, a C₅-C₁₅ group, a C₅-C₁₀ group or a C₅-C₈ group. In some embodiments, R contains 1 carbon atom. In some embodiments, R contains 2 carbon atoms. In some embodiments, R contains 3 carbon atoms. In some embodiments, R contains 4 carbon atoms. In some embodiments, R contains 5 carbon atoms. In some embodiments, R contains 6 carbon atoms. In some embodiments, R contains 7 carbon atoms. In some embodiments, R contains 8 carbon atoms. In some embodiments, R contains 9 carbon atoms. In some embodiments, R contains 10 carbon atoms. In some embodiments, R contains 11 carbon atoms. In some embodiments, R contains 12 carbon atoms. In some embodiments, R contains 13 carbon atoms. In some embodiments, R contains 14 carbon atoms. In some embodiments, R contains 15 carbon atoms.

[0027] In some embodiments, R is selected from linear C₁-C₁₅ alkyl groups, linear C₂-C₁₅ alkenyl groups, linear C₂-C₁₅ alkynyl groups, fluorinated linear C₁-C₁₅ alkyl groups, fluorinated linear C₂-C₁₅ alkenyl groups, fluorinated linear C₂-C₁₅ alkynyl groups,

branched C₃-C₁₅ alkyl groups, branched C₃-C₁₅ alkenyl groups, branched C₄-C₁₅ alkynyl groups, fluorinated branched C₃-C₁₅ alkyl groups, fluorinated branched C₃-C₁₅ alkenyl groups, fluorinated branched C₄-C₁₅ alkynyl groups. In some particular embodiments, R is a linear C₆-C₉ alkyl group, especially the C₇ alkyl group. In some particular embodiments, R is n-heptyl (-C₇H₁₅), and the product is therefore *N*-octyl cysteine, *N*-octyl serine, *N*-octyl aspartic acid or *N*-octyl glutamic acid.

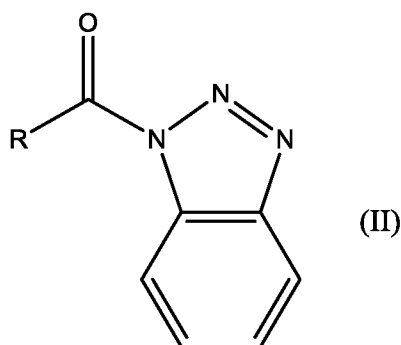
[0028] In some particular embodiments, R is methyl (-CH₃), and the product is therefore *N*-acetyl cysteine.

[0029] In some embodiments, the R group of the *N*-acyl amino acid of Formula (I) is hydrophobic. In such embodiments, the *N*-acyl amino acid of Formula (I) may perform as an *N*-acyl amino acid surfactant. Advantageously, when the R group is sufficiently hydrophobic, the product *N*-acyl amino acid has poor solubility in solvents composed substantially on methanol. As a result, the product may precipitate from the solution and be collected by filtration techniques. Advantageously, the reaction typically produces minimal by-products, so the precipitated product may not need further purification or only need washing/rinsing with a suitable solvent (e.g. ethanol or acetone) to provide *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid or *N*-acyl glutamic acid product having a useful purity. In some embodiments, the yield is greater than 90% (e.g. greater than 95%, greater than 97% or greater than 98%). Also advantageously, the benzotriazole by-product typically remains in solution after the reaction and may be recycled to prepare more *N*-acylbenzotriazole for use in the method. For example, the benzotriazole-containing by-product may be soluble in methanol or acetone. Therefore, rinsing the product *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid or *N*-acyl glutamic acid with acetone may keep the benzotriazole-containing by-product in solution, allowing the separation, for example, filtration, from the *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid or *N*-acyl glutamic acid product. The dissolved benzotriazole-containing by-product may then be recovered, for example, by removal of solvent under reduced pressure, and subsequently used.

[0030] Cysteine, serine, aspartic acid and glutamic acid are four of the twenty common amino acids and are readily commercially available in both stereoisomers. As will be appreciated, the natural stereoisomer of cysteine (i.e. L-cysteine), serine (L-serine), aspartic acid (L-aspartic acid) and glutamic acid (L-glutamic acid) may be more readily available and are typically cheaper to purchase from commercial suppliers. The stereochemistry of

the cysteine, serine, aspartic acid and glutamic acid in the present invention is not particularly limited, and the choice may be dependent on the desired use of the *N*-acyl amino acid product. In some embodiments, the amino acid is L-cysteine, L-serine, L-aspartic acid or L-glutamic acid. In other embodiments, the amino acid is D-cysteine, D-serine, D-aspartic acid or D-glutamic acid. For large scale applications where stereochemistry is not important, L-cysteine, L-serine, L-aspartic acid or L-glutamic acid may be preferred to minimize costs. In some embodiments, the amino acid may be in the form of a racemic mixture (D/L)

[0031] The method of the present invention comprises reacting an amino acid selected from cysteine, serine, aspartic acid and glutamic acid, and an *N*-acylbenzotriazole of Formula (II):



[0032] For the avoidance of doubt, the R group of the *N*-acylbenzotriazole of Formula (II) corresponds to the R group of the *N*-acyl amino acid product of Formula (I). Therefore, R in Formula (II) is selected from H, linear C₁-C₁₅ alkyl groups, C₂-C₁₅ alkenyl groups, C₂-C₁₅ alkynyl groups, fluorinated C₁-C₁₅ alkyl groups, fluorinated C₂-C₁₅ alkenyl groups and fluorinated C₂-C₁₅ alkynyl groups as defined above for Formula (I). Accordingly, the R group of the *N*-acylbenzotriazole of Formula (II) will be selected according to the R group of the desired *N*-acyl amino acid product of Formula (I).

[0033] Various *N*-acylbenzotriazoles are commercially available. Various syntheses of various *N*-acylbenzotriazoles have also been reported. For example, a convenient procedure to access a variety of *N*-acylbenzotriazoles, from the corresponding carboxylic acid, has been reported (Katritzky et al., *Synthesis*, 2003, **3**, 2795-2798). As a person skilled in the art will appreciate, a wide range of *N*-acylbenzotriazoles may be purchased or prepared using this, and/or other, reported procedures.

[0034] The reaction between an amino acid selected from cysteine, serine, aspartic acid and glutamic acid, and the *N*-acylbenzotriazole takes place “in a solvent composed

substantially of methanol". In this context, "in a solvent" refers to the medium in which the reaction occurs (i.e. where the cysteine, serine, aspartic acid or glutamic acid and *N*-acylbenzotriazole are able to make contact and react). In the present context, "composed substantially of methanol" refers to the composition of the solvent, which is at least 80 %v/v methanol (e.g. at least 90 %v/v, at least 95 %v/v, at least 98 %v/v, at least 99 %v/v or at least 99.5 %v/v methanol). The balance of the solvent may be a co-solvent selected from suitable inert solvents that allow the cysteine to dissolve, and do not react with the *N*-acylbenzotriazole to any appreciable extent. Examples of co-solvents include ethanol, propanol and isopropanol. The reaction may, in some embodiments, tolerate small amounts of contaminants in the solvent. The methanol does not necessarily need to be rigorously dried. However, the methanol typically contains less than about 1 wt.% water as the presence of water may affect the reaction yield. The reaction typically performs better (e.g. higher yield, less by-products) when there is no, or at least minimal, water in the solvent. Accordingly, in some embodiments, the solvent contains less than 1 wt.% water, for example, less than 0.5 wt.%, less than 0.2 wt.%, less than 0.1 wt.%, less than 0.05 wt.%, less than 0.02 wt.% or less than 0.01 wt.% water. In some embodiments, methanol is used (as the solvent or co-solvent) as supplied without any further purification or drying. In some embodiments, methanol is dried prior to use as the solvent or co-solvent (e.g. by passage or storage over 3 angstrom molecular sieves). Persons skilled in the art are aware of various methods of drying methanol.

[0035] It is believed that it is important that at least a portion of the amino acid selected from cysteine, serine, aspartic acid and glutamic acid (preferably a significant portion of the cysteine, serine, aspartic acid and glutamic acid, if not all of the cysteine, serine, aspartic acid and glutamic acid) dissolves in the solvent for the reaction to proceed at a practical rate. It is believed that a major drawback to previous methods was that in previous methods the cysteine was not sufficiently soluble in the solvent, which would slow or inhibit any reaction from occurring. Previously (e.g. WO 2018/071985), appreciable amounts of water were required to facilitate the dissolution of cysteine; however, solvents having appreciable water content proved to be incompatible with the acylating agents used (e.g. acid chlorides), leading to low yields of the desired product.

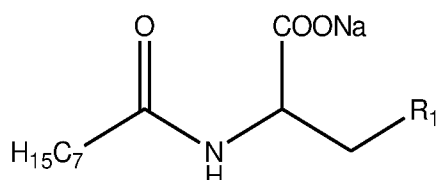
[0036] The present invention is predicated, at least in part, on the discovery of cysteine being sufficiently soluble in a solvent/solvent system which is simultaneously compatible with a class of acylating agents that do not react to any appreciable extent with the

solvent/solvent system, and that are also soluble (at least to a useful extent) in the solvent/solvent system.

[0037] The method of the present invention does not need to be performed at any particular temperature, so long as the solvent is in the liquid state. A person skilled in the art will be able to choose an appropriate temperature, and may adjust the temperature to take account of differing reaction rates (e.g. depending on the nature of the *N*-acylbenzotriazole). The temperature does not need to be static and can be changed during the reaction. In some embodiments, the reaction of cysteine, serine, aspartic acid or glutamic acid, and *N*-acylbenzotriazole is performed at a temperature in the range of from about 10 °C to about 65 °C (the boiling point of methanol), from about 10 °C to about 50 °C, from about 15 °C to about 30 °C or from about 20 °C to about 25 °C. Advantageously, the reaction may, in some embodiments, proceed at a practical rate at ambient temperature, avoiding the need for heating or cooling and thereby reducing manufacturing costs.

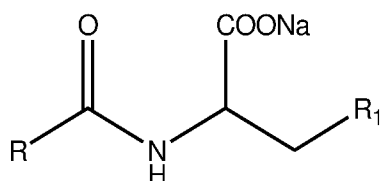
[0038] The method comprises reacting cysteine, serine, aspartic acid or glutamic acid and an *N*-acylbenzotriazole in a solvent composed substantially of methanol. In some embodiments, the *N*-acylbenzotriazole is added neat to a solution of cysteine, serine, aspartic acid or glutamic acid. In some embodiments, the *N*-acylbenzotriazole is in a solution which is added to a solution of cysteine, serine, aspartic acid or glutamic acid. In some embodiments, the cysteine, serine, aspartic acid or glutamic acid is added neat to a solution of *N*-acylbenzotriazole. In some embodiments, the cysteine, serine, aspartic acid or glutamic acid is in a solution which is added to a solution of *N*-acylbenzotriazole. In some embodiments, the addition is in a single batch. In other embodiments, the addition is portion-wise (e.g. dropwise) over a period of time. In some embodiments, the reaction mixture is mechanically agitated (e.g. stirred) before, during and/or after the cysteine, serine, aspartic acid and glutamic acid and the *N*-acylbenzotriazole are combined.

[0039] The *N*-acyl amino acid of Formula (I) may be prepared as a salt, where an H atom (H⁺ ion) of the α-COOH group is replaced by another cation, for example Na⁺. For example, the sodium salt of *N*-octanoyl cysteine, *N*-octanoyl serine, *N*-octanoyl aspartic acid or *N*-octanoyl glutamic acid has the following structure:



wherein R₁ is SH, OH, CO₂H or CH₂CO₂H.

[0040] In some embodiments, where the amino acid is aspartic acid or glutamic acid, the side chain carboxylic acid may also be in the form of a salt, for example, a disodium salt having the following structure:



wherein R₁ is CO₂Na or CH₂CO₂Na.

[0041] Examples of suitable salts also include salts of Na, K, Li or Cs. Methods of converting carboxylic acids to carboxylate salts are known (e.g. selective precipitation, cation exchange columns etc.) and a person skilled in the art will be able to determine an appropriate method. Salt forms of formula (I) are particularly useful in surfactants.

[0042] In a second aspect, the present invention provides an *N*-acyl amino acid of Formula (I) or a salt thereof prepared by the method of the first aspect.

[0043] In some embodiments, the present invention provides the use of an *N*-acyl amino acid of Formula (I) or a salt thereof prepared by the method of first aspect.

[0044] In some embodiments, the *N*-acyl amino acid of formula (I) is *N*-acyl cysteine, *N*-acyl serine or *N*-acyl aspartic acid. In some embodiments, the *N*-acyl amino acid of formula (I) is *N*-acyl cysteine or *N*-acyl serine. In some embodiments, the *N*-acyl amino acid of formula (I) is *N*-acyl cysteine.

Applications of *N*-acyl amino acids of Formula (I)

[0045] Advantageously, the method of the present invention provides access to *N*-acyl cysteines of Formula (I) having free thiol groups (-SH). Free thiol groups are reported to have antioxidant effects and are reportedly useful in the harvesting of free radicals in cells, leading to a number of potential therapeutic uses of *N*-acyl cysteines of Formula (I) where R₁ is SH, prepared by the method of the first aspect of the present invention. In some embodiments, the *N*-acyl amino acid is in free acid form. For example, *N*-acetyl cysteine has been reported to participate in general antioxidant activities of the body (Sansone et al. *Innov. Clin. Neurosci.* 2011;8(1): 10-14) and may be implicated in a number of diseases. Accordingly, in some embodiments there is provided a method of treating psychiatric disorders allegedly related to oxidative stress (e.g. schizophrenia, bipolar disorder) and/or

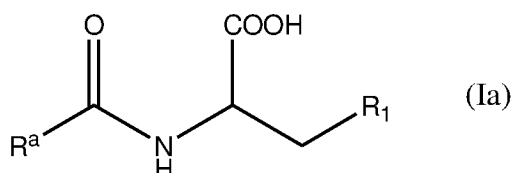
psychiatric syndromes characterized by impulsive/compulsive symptoms (e.g. trichotillomania, pathological nail biting, gambling, substance misuse), by administration to a patient in need of treatment of an *N*-acyl cysteine of Formula (I) where R₁ is SH (e.g. *N*-acetyl cysteine) prepared by the method of the first aspect of the present invention.

[0046] *N*-acyl amino acids of Formula (I) including *N*-acyl cysteines, *N*-acyl serines, *N*-acyl aspartic acids or *N*-acyl glutamic acids, and salts thereof, are, in some embodiments, believed to be biocompatible. Further, in some embodiments, at least some of the decomposition products are also believed to be biocompatible. As such, in some embodiments, the present invention provides the use of an *N*-acyl cysteine, *N*-acyl serine, *N*-acyl aspartic acid or *N*-acyl glutamic acid of Formula (I), especially foaming agents of Formula (Ia), more especially where R is a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group or a C₅-C₁₂ alkynyl group, as a replacement for surfactants such as sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS) in detergents/cleaners or toothpastes. In this regard, SDS and SLS have reported health and environmental concerns. Accordingly, in some embodiments there is provided a cleaning composition, detergent composition or toothpaste composition comprising an *N*-acyl amino acid of Formula (I) or a salt thereof, where R is selected from a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group and a C₅-C₁₂ alkynyl group. In some particular embodiments, the *N*-acyl amino acid of Formula (I) or a salt thereof, where R is selected from a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group and a C₅-C₁₂ alkynyl group, is prepared by the method of the first aspect of the present invention.

[0047] In some embodiments, the *N*-acyl amino acid is a *N*-acyl cysteine. In other embodiments, the *N*-acyl amino acid is *N*-acyl serine. In yet other embodiments, the *N*-acyl amino acid is *N*-acyl aspartic acid. In still other embodiments, the *N*-acyl amino acid is *N*-acyl glutamic acid. In particular embodiments, the *N*-acyl amino acid is *N*-acyl cysteine. In some embodiments, the *N*-acyl amino acid is in a salt form.

Methods of removing PFAS

[0048] In a third aspect, the present invention provides a method of removing one or more PFASs from an aqueous phase containing one or more PFASs, the method comprising dissolving in the aqueous phase a foaming agent of Formula (Ia):



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or a salt thereof,

wherein R_1 is SH, OH, CO_2H or CH_2CO_2H ; and

R^a is a C_5 - C_{12} alkyl group, a C_5 - C_{12} alkenyl group, a C_5 - C_{12} alkynyl group, a fluorinated C_5 - C_{12} alkyl group, a fluorinated C_5 - C_{12} alkenyl group or a fluorinated C_5 - C_{12} alkynyl group;

forming a foam from the aqueous phase and a gas; and
separating the foam from the aqueous phase.

[0049] As used herein, “PFASs” refers to per- and poly-fluoroalkyl substances.

Examples of PFASs include perfluorooctane sulfonate, also known as “PFOS”; and

perfluorooctanoic acid, also known as “PFOA”. Perfluorohexane sulfonate (PFHxS) is another chemical of the PFAS group and is present in some fire-fighting foams. In some embodiments, the PFAS is a PFAS capable of binding to a multivalent cation, such as Ca^{2+} , Mg^{2+} , Al^{3+} and Fe^{3+} . Examples include per- or polyfluoro C_1 - C_{20} alkanolic acids, per- or polyfluoro C_1 - C_{20} alkyl sulfonates.

[0050] As used herein, “removing one or more PFASs” refers to the removal of at least a portion of at least one PFAS, thus reducing the amount or concentration of at least one PFAS in the aqueous phase. In some embodiments, at least 50 % (e.g. at least 80 %, at least 90 %, at least 95 %, at least 98 %, or at least 99 %) of one type or species of PFAS is removed. In some embodiments, at least 50 % (e.g. at least 80 %, at least 90 %, at least 95 %, at least 98 %, or at least 99 %) of the entire content of PFASs is removed. In some embodiments, the one or more PFASs comprises at least one PFAS capable of binding to a multivalent metal ion, such as Ca^{2+} , Mg^{2+} , Al^{3+} or Fe^{3+} .

[0051] The method comprises dissolving a foaming agent of Formula (Ia) or a salt thereof in an aqueous phase containing one or more PFASs. A reference herein to an

“aqueous phase” refers to a phase in which water is the only solvent or is at least 50 % (e.g. at least 80 %, at least 90 %, at least 95 %, at least 98 %, or at least 99 %) by weight of the total solvent. As will be appreciated, the aqueous phase may contain other solid components (such as soil) and/or contaminants. Preferably, water comprises at least 90 %, at least 95 %, at least 98 %, or at least 99%, by weight of the total solvents in the aqueous phase.

[0052] The aqueous phase may be any aqueous phase comprising one or more PFASs. The aqueous phase may, for example, be groundwater or waste water from an industrial process, mining or other human activity, comprising one or one or more PFASs. In some

embodiments, metal ions are added prior to performing the method. In some embodiments, the aqueous phase is or comprises PFAS-contaminated groundwater. The aqueous phase may comprise particulates. In some embodiments, the aqueous phase comprises PFAS-contaminated soil. In such embodiments, the soil may, for example, represent from about 1% to about 95 %w/w of the aqueous phase, e.g. from about 10% to about 50 %w/w of the aqueous phase. When forming a foam, the gas may, for example, occupy from about 1 % to about 95 %v/v of the total volume (the total volume including the gas, the aqueous phase and the soil). For example, in some embodiments, the gas occupies from about 20 % to about 90 %v/v of the total volume. In some embodiments, the soil represents from about 5 % to about 90%v/v of the total volume, such as from about 10 % to about 80%v/v or from about 20 % to about 60%v/v of the total volume. In some embodiments, the aqueous phase is an aqueous phase that has been used to rinse contaminated soil (e.g. an aqueous phase that has been in contact with contaminated soil). In some embodiments, the aqueous phase contains soil contaminated with one or more PFASs. Typically, the aqueous phase does not contain large amounts of materials (e.g. insoluble oils, polydimethylsiloxanes and other silicones, certain alcohols, stearates and glycols) which will inhibit the foaming agent of Formula (Ia) from facilitating the formation of foam from the aqueous phase.

[0053] Advantageously, in some embodiments, foaming agents of Formula (Ia) are capable of forming foams over a broad range of pHs. For example, *N*-octanoyl cysteine may form a foam at a pH of from about 3 to about 12. Accordingly, in some embodiments, the pH of the aqueous phase to be treated is in the range of from about pH 3 to about pH 12 (e.g. from about pH 4 to about pH 10, from about pH 4 to about pH 8, from about pH 5 to about pH 7). Advantageously, foaming agents of Formula (Ia) are typically capable of forming foams at pHs resembling groundwater.

[0054] In some embodiments, the aqueous phase comprises one or more metal ions in addition to the one or more PFASs. Examples of metal ions include multivalent metal ions such as Ca^{2+} , Mg^{2+} , Al^{3+} or Fe^{3+} . In some embodiments, the metal ions are added to the aqueous phase prior to treatment. In some embodiments, the metal ions are already present in the aqueous phase to be treated. In some embodiments, additional metal ions are added, before or during treatment, to an aqueous phase which already contains metal ions. In such embodiments, the added metal ions may be the same or different to those already in the aqueous phase. Examples of metal ions useful in the present invention include multi-valent

cations capable of forming bridges, such as cations of Ca, Mg, Al and Fe (e.g. Ca^{2+} , Mg^{2+} , Al^{3+} and Fe^{3+}), especially Ca. Advantageously, Ca is prevalent in soil and groundwater, which can obviate the need to add additional metal ions to the aqueous phase before treatment.

5 **[0055]** The concentration of metal ions in the aqueous phase is not particularly limited. In some embodiments, the concentration of metal ions is equal to or greater than the concentration of the foaming agent of Formula (Ia) dissolved in the aqueous phase. In some embodiments, the concentration of metal ions is selected to minimise the wastage of the foaming agent of Formula (Ia). In some embodiments, the concentration of metal ion in
10 the aqueous phase is greater than 0.0001 M, e.g. greater than 0.001 M, 0.01 M or 0.1 M. For example, from about 0.0001 M to about 2 M or from about 0.0001 M to about 0.02 M.

[0056] In some embodiments, the foaming agent of Formula (Ia) or salt thereof is prepared by the method of the first aspect or is derived from a compound of Formula (I) prepared by the method of the first aspect.

15 **[0057]** In some embodiments of the foaming agent of Formula (Ia), R_1 is SH, In other embodiments, R_1 is OH. In yet other embodiments, R_1 is CO_2H . In still other embodiments, R_1 is $\text{CH}_2\text{CO}_2\text{H}$. In particular embodiments, R_1 is SH, OH or CO_2H , especially SH or OH and more especially SH.

[0058] In the foaming agent of Formula (Ia), R^a is preferably hydrophobic. In some
20 embodiments, R^a is linear. In some embodiments, R^a is branched. In some embodiments, R^a is an alkyl group. In some embodiments, R^a is an alkenyl group. In some embodiments, R^a is an alkynyl group. In some embodiments, R^a is a fluorinated alkyl group. In some
25 embodiments, R^a is a fluorinated alkenyl group. In some embodiments, R^a is a fluorinated alkynyl group. In some embodiments, R^a is C_6 - C_9 group. In some embodiments, R^a is a C_6 - C_8 group. In some embodiments, R^a is a C_7 group. In some particular embodiments, R^a is a
30 linear C_5 - C_{10} alkyl group, especially a linear C_6 - C_8 alkyl group, more especially n-heptyl (- C_7H_{15}). In some particular embodiments, R^a is a fluorinated linear C_5 - C_{10} alkyl group, especially a fluorinated linear C_6 - C_8 alkyl group, more especially a fluorinated n-heptyl (e.g. perfluorinated n-heptyl, $-\text{C}_7\text{F}_{15}$).

[0059] As used herein, a “foaming agent” is a material, typically a surfactant, which facilitates the formation of foam from an aqueous phase. When gas passes through the aqueous phase, typically as small bubbles, the foaming agent facilitates the formation of a foam. The “foaming agent of Formula (Ia) or salt thereof” is therefore a compound of

Formula (Ia) or a salt thereof which facilitates the formation of a foam from an aqueous phase. In particular embodiments of foaming agent, the *N*-acyl amino acid is in salt form. In some embodiments, the foaming agent of Formula (Ia) or salt thereof contains a cysteine group and acts as a surfactant. It is therefore sometimes referred to as a “cysteine surfactant”. In other embodiments, the foaming agent of Formula (Ia) or salt thereof contains a serine group and acts as a surfactant. It is therefore sometimes referred to as a “serine surfactant”. In yet other embodiments, the foaming agent of Formula (Ia) or salt thereof contains an aspartic acid group and acts as a surfactant. It is therefore sometimes referred to as a “aspartic acid surfactant”. In still other embodiments, the foaming agent of Formula (Ia) or salt thereof contains a glutamic acid group and acts as a surfactant. It is therefore sometimes referred to as a “glutamic acid surfactant”.

[0060] Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. Surfactants are usually organic compounds that are amphiphilic, meaning they contain both a hydrophobic group (their tail) and a hydrophilic group (their head). Therefore, a surfactant contains both a water-insoluble or oil-soluble component and a water-soluble component (typically a polar group and/or a group capable of carrying a charge). Surfactants will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil. The water-insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase. In some embodiments, the PFAS may act as a surfactant. In such embodiments, the PFAS and the foaming agent of Formula (Ia) or salt thereof may each act as co-surfactants.

[0061] The foaming agent is capable of facilitating the formation of a foam from the aqueous phase, that is, facilitating the formation of a foam comprising pockets of a gas encapsulated by the aqueous phase. For example, after the gas is passed through the aqueous phase, typically in the form of small bubbles (e.g. having a diameter of about 1 mm to about 10 mm), bubbles of the gas move to the surface of the aqueous phase to form a foam on the surface. Typically, the gas bubbles are allowed to rise to the top of the aqueous phase to form a foam layer. The foam layer phase can then be separated from the aqueous phase thereby isolating the PFASs (present in the foam layer) from the aqueous phase. The foam may be separated by any means which separates the foam (containing the adsorbed PFASs) from the bulk aqueous phase. In some embodiments, the foam forms a

foam layer on top of the bulk aqueous phase and the foam layer is wiped away (e.g. displaced with a paddle or similar). In some embodiments, the foam is removed under negative pressure (e.g. by suction through a tube). In some embodiments, the foam is removed using a positive pressure (e.g. by being pushed or extruded through an outlet).

5 The foam can, for example, be separated from the liquid phase using an outlet tube to collect the foam in a waste container (e.g. exiting the bubble column in Figure 2 through the "Foam outlet" and into the "Waste"). The collected foam comprising the PFAS may be collapsed prior to disposal, for example, using silicone or ethanol sprays. In some embodiments, a foam breaker is used to collapse the foam. In some embodiments, a
10 mechanical foam breaker, such as a defoaming pump or ultrasonic foam breaker, is used to collapse the foam. In some embodiments, the PFAS is isolated from the foam and/or the collapsed foam. In some embodiments, the foam may be treated to isolate the PFAS from the foam.

[0062] The method of the present invention may, for example, comprise aeration at a
15 low flow-rate (e.g. 3 L/min through a sinter of about 7 cm² in area) of an aqueous phase containing the PFAS and the foaming agent of Formula (Ia) or a salt thereof in a suitable vessel, e.g. a bubble column. In such embodiments, as the aeration is performed, bubbles rise to the surface of the aqueous phase forming a foam on the surface which is then separated from the aqueous phase. Additional volume of an aqueous solution containing
20 the PFAS, with or without the cysteine surfactant, may be introduced into the vessel. The additional aqueous phase introduced into the vessel (the feed solution) may be introduced into the vessel in a manner such that the feed solution is initially contacted with the foam so that some amount of separation of the PFAS from the feed solution takes place while it is passing through the foam. Alternatively, the feed solution could also be added directly to
25 the aqueous phase below the interface of the aqueous phase and the foam, e.g. just below the foam.

[0063] The foaming agent of Formula (Ia) or salt thereof dissolved in the aqueous phase forms a monolayer around the bubbles of the gas. The orientation of the foaming agent of Formula (Ia) or salt thereof around the bubble is such that the hydrophobic group
30 (i.e. R^a) orients towards the gas and the polar head-group orients towards the aqueous phase (as shown for cysteine in Figure 5). Bubbles passing through the aqueous phase provide a continuous supply of amino acid coated monolayers, where the surfactants and head-groups will be relatively mobile at room temperature (e.g. at 25 °C). It is believed that

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collisions between the PFAS species and the *N*-acyl amino acid coated bubbles provide a mechanism by which the cysteine can capture the PFAS, allowing the capture and removal of PFAS in a one-step treatment process. In embodiments where the aqueous phase comprises one or more metal ions, collisions between the metal ions, PFAS species and the *N*-acyl amino acid coated bubbles provide a mechanism by which the *N*-acyl amino acid such as *N*-acyl cysteine can assist in the capture and removal of PFAS. In this system, the polar head groups effectively chelate with metal ions in the aqueous phase, and facilitate the adsorption of PFASs into the bubble surface as a surfactant or co-surfactant. Without wishing to be bound by theory, it is believed that the fluid nature of the adsorbed surfactant layer at a bubble surface, which moves through the aqueous solution, supports an efficient spatial arrangement of groups to incorporate PFAS molecules, optionally via chelation with one or more metal ions. Once adsorbed, the PFAS and/or PFAS/metal ion complex can be effectively removed by the bubbles into a foam on the surface of the aqueous phase.

[0064] In the bulk aqueous phase, surfactants form aggregates, such as micelles, at concentrations above the critical micelle concentration (CMC), where the hydrophobic tails form the core of the aggregate and the hydrophilic heads are in contact with the surrounding liquid. The CMC of a surfactant in an aqueous phase can be determined by a person skilled in the art using techniques known in the art. For example, the CMC of a surfactant can be determined by measuring changes in the conductance or surface tension of the solution at different concentrations of the surfactant.

[0065] Preferably the foaming agent of Formula (Ia) or salt thereof has a solubility of greater than 0.0001 M in water, e.g. greater than 0.001 M or 0.01 M, at 25 °C. For foaming agents of Formula (Ia) or salts thereof having a solubility in water below 0.0001 M, only low concentrations can be dissolved in an aqueous phase and this may limit the rate at which the PFASs can be removed from the aqueous phase.

[0066] Preferably, the CMC of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is greater than 0.0001 M, e.g. greater than 0.001 M or 0.01 M. In some embodiments, the CMC of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is from 0.0001 M to 1 M. In some embodiments, the CMC of the foaming agent of Formula (Ia) or salt thereof in water at 25 °C and atmospheric pressure is greater than 0.0001 M, e.g. greater than 0.001 M or 0.01 M. In some embodiments, the CMC of the foaming agent of Formula (Ia) or salt thereof in water at 25 °C and atmospheric pressure is from 0.0001 M to 1 M. Preferably, the foaming agent of Formula (Ia) or a salt thereof, is

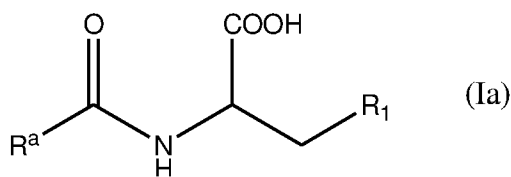
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dissolved in the aqueous solution in an amount up to the CMC, that is, it is preferred that the foaming agent is dissolved in the aqueous phase in an amount to provide a concentration of the foaming agent below the CMC of the foaming agent. This is preferred as, if the concentration of the foaming agent exceeds the CMC, the foaming agent may form aggregates, such as micelles, and some of the PFASs and metal ions (if present) may become bound with the aggregates rather than the cysteine groups in the mono-layer around the bubbles of the gas passing through the aqueous phase. This may reduce the efficiency of the method of the present invention in removing the PFASs from the aqueous phase. However, in some embodiments, it may be possible to use amino acid surfactants such as cysteine surfactants at concentrations well above their CMC, for example, up to 1-2M or more, limited only by its solubility.

[0067] The method comprises forming a foam from the aqueous phase and a gas. When the aqueous phase comprises the dissolved foaming agent of Formula (Ia), the foaming agent will facilitate the formation of a foam. The foam may be formed by introducing the gas into the aqueous phase. The gas may be any gas that is substantially non-reactive with the foaming agent and the aqueous phase. The gas may, for example, be selected from the group consisting of air such as dry air or humidified air, carbon dioxide, nitrogen, oxygen, helium or argon.

[0068] In some embodiments, the aqueous phase is agitated to form the foam. In some embodiments the agitation is mechanical mixing (e.g. with a blade). In some embodiments the agitation is by aeration, such as by spraying a portion of the aqueous phase into another portion of the aqueous phase.

[0069] In some particular embodiments, the foam is formed by passing a gas through the aqueous phase. Accordingly, the present invention provides a method for removing PFASs from an aqueous phase comprising one or more PFASs, the method comprising dissolving in the aqueous phase a foaming agent of Formula (Ia):



or a salt thereof,

wherein R_1 is SH, OH, CO_2H or $\text{CH}_2\text{CO}_2\text{H}$; and

R^a is a C5-C12 alkyl group, a C5-C12 alkenyl group, a C5-C12 alkynyl group, a fluorinated

C₅-C₁₂ alkyl group, a fluorinated C₅-C₁₂ alkenyl group or a fluorinated C₅-C₁₂ alkynyl group;

passing a gas through the aqueous phase to form a foam; and

separating the foam from the aqueous phase.

5 **[0070]** In some embodiments, the gas is passed through the aqueous solution to form a foam by bubbling the gas through the aqueous phase. In some embodiments, the gas is introduced into the aqueous phase near the base of the aqueous phase (e.g. near the floor of a vessel holding the aqueous phase) to form bubbles (typically having a diameter in the range of about 1 mm to about 10 mm) which rise through the aqueous phase to form a
10 foam on the upper surface of the aqueous phase. The gas is typically passed through a porous material, for example, a porous glass or metal sinter, to form multiple bubbles of the gas in the aqueous phase. In some embodiments, the aqueous phase comprises one or more metal ions in addition to the one or more PFASs.

15 **[0071]** Without wishing to be bound by theory, it is believed that as the bubbles of the gas pass through the aqueous phase, a mono-layer of the foaming agent of Formula (Ia) is formed around each bubble of the gas. It is believed that, in some embodiments, it is the interaction between one or more of the cysteine groups, which extend from the surface of the bubble, with a metal ion, such as Ca²⁺ (present in abundance in soil and groundwater), which results in the metal ion being bound by the cysteine group. In relation to
20 contaminated soils and groundwater, it has been reported that PFASs, especially those that are negatively charged, may be bound to charged particles of sand, clay and humic acid coated materials and microorganisms via bridging multi-valent ions (e.g. Ca²⁺). It is reasoned that, in some embodiments, the foaming agents of Formula (Ia) bind the multi-valent metal ions and displace the PFASs which are bound, via bridging multi-valent ions,
25 to the charged particles of sand, clay and humic acid coated materials. The displaced PFASs may then associate (e.g. through hydrophobic attraction forces, fluorine-fluorine interactions, fluorine- π interactions, London dispersion forces, etc.) with the foaming agent of Formula (Ia), and thus facilitate the removal of the PFAS from the aqueous phase. Additionally, when a metal ion is captured by the cysteine group of the foaming agent of
30 Formula (Ia), it could also foreseeably associate with a PFAS, especially a PFAS comprising a functional group which associates with metal ions (such as a carboxylate, sulfonate or other organic acid). Having associated with one PFAS (comprising a functional group which associates with a metal ion), the associated PFAS compound may

then associate with other PFASs which do not contain a functional group known to bind to metal ions (for example through fluorine-fluorine interactions, fluorine- π interactions, London dispersion forces, hydrophobic attraction forces etc.). Accordingly, a wide range of PFASs may be removed by this method.

5 **[0072]** In most soils, Ca^{2+} constitutes over 90% of total cation (Rieuwerts et al., *Chemical Speciation & Bioavailability*, 1998, **10**, 61-75). Without wishing to be bound by theory, it is believed that Ca^{2+} attaches to PFAS compounds (which may be present as contamination in soil), particularly PFAS compounds that are negatively charged. *N*-octanoyl-amino acid surfactants such as *N*-octanoyl cysteine surfactant may release PFAS
10 compounds attached to the contaminated soil and act as a co-surfactant in foam cleaning/removal.

[0073] As gas bubbles pass through an aqueous phase, the PFASs (including PFASs that are bound to metal ions) become adsorbed onto the polar head groups of the monolayer of the foaming agent of Formula (Ia) formed around each rising bubble. The bubbles
15 then form a foam comprising the adsorbed PFASs (including PFASs that are bound to metal ions), acting as a surfactant or co-surfactant, which can then be separated from the aqueous phase. Continuation of sparging until foaming ceases can be used to remove residual foaming agent from the aqueous phase as the presence of even low levels of the foaming agent causes foaming.

20 **[0074]** Advantageously, the foaming agent of Formula (Ia) may be broken down/decomposed to environmentally benign components, such as cysteine, serine, aspartic acid and glutamic acid, and C_6 - C_{13} alkanolic acids. The R^a group may be chosen such that the resultant alkanolic acid has minimal environmental impact. This may be important in embodiments where complete removal of the foaming agent of Formula (Ia)
25 may be difficult and/or not feasible (e.g. where method is used to treat large amounts of soil/groundwater). Accordingly, it may be viable to return the treated wastewater to the environment without completely removing the foaming agent of Formula (Ia) and without having environmental consequences.

[0075] Also advantageously, the method can be carried out with low energy
30 consumption and small space requirements, which does not require the use of expensive technology, and which can be conducted in a one step process and/or at the point of access to the contaminated water or soil. Further still, the method can be carried out in a batch process or, advantageously, a continuous process. A continuous process can be

advantageous as a continuous process is generally more energy and cost efficient for treating large volumes of an aqueous phase than a batch process.

[0076] Figure 1 is a schematic diagram of the column apparatus used in the experiments described in Example 2. The same or similar apparatus can, for example, be used to perform the method of the present invention in a batch process or a continuous process. For example, in a batch process, a sample of the aqueous phase may be treated in the column depicted in Figure 1 and then the treated aqueous phase is removed from the column through the outlet. Following this, a new sample of the aqueous phase and the foaming agent is introduced to the column through the inlet for treatment by the method of the present invention. Similar apparatus can also be used in a continuous process. Figure 2 depicts one example of suitable apparatus for carrying out the method of the present invention in a continuous process. Using the apparatus depicted in Figure 2, the aqueous phase (feed solution) is introduced to the bubble column in a continuous flow through the inlet, and the treated aqueous solution removed through the outlet in a continuous flow. A concentrated solution of the foaming agent is introduced in a continuous flow through a separate inlet (labeled "Surfactant" in Figure 2), positioned near to the sinter of the column. To provide a longer period of contact between the rising bubbles and the aqueous solution, the height of the column, relative to its other dimensions, will typically be greater than that depicted in Figure 2. The outlet in Figure 2 is orientated in a downward direction to avoid bubbles rising through the aqueous solution moving through the outlet as the aqueous solution is removed from the column. As a person skilled in the art will appreciate, various other apparatuses are possible for performing the method of the present invention.

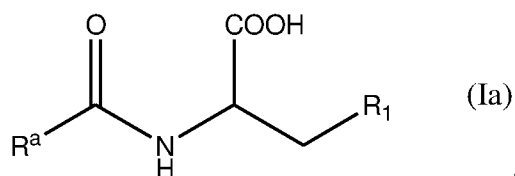
[0077] Adsorptive bubble separation techniques have been used to remove various substances from wastewaters. There are several separation techniques employing adsorption on gas bubbles; these methods are divided into two categories, foam separation and non-foaming adsorptive bubble separation techniques. Foam separation techniques can be subdivided into foam fractionation and flotation methods. Flotation methods include ion flotation, ore flotation, macro flotation, colloidal flotation and precipitate flotation. Ion flotation is a separation technology for recovering and removing metal ions from aqueous phases based on the association between the ions and a surfactant species. The ion and surfactant are adsorbed onto the surface of rising bubbles and carried into a foam on the surface, which is then removed from the solution. Foam fractionation methods

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preferentially separate hydrophobic molecules from a liquid solution using columns of foam.

[0078] The present invention may therefore provide a novel foam fractionation technique for removing PFASs from an aqueous phase.

- 5 **[0079]** In a fourth aspect, the present invention provides the use of a foaming agent of Formula (Ia):



or a salt thereof,

wherein R₁ is SH, OH, CO₂H or CH₂CO₂H; and

- 10 **R^a** is a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group, a C₅-C₁₂ alkynyl group, a fluorinated C₅-C₁₂ alkyl group, a fluorinated C₅-C₁₂ alkenyl group or a fluorinated C₅-C₁₂ alkynyl group;

for removing one or more PFASs from an aqueous phase.

- 15 **[0080]** In some particular embodiments, the foaming agent of Formula (Ia) or salt thereof is used in an foam fractionation process to remove one or more metal ions and one or more PFASs from the aqueous phase.

- [0081]** In some embodiments of the foaming agent of Formula (Ia), R₁ is SH. In other embodiments, R₁ is OH. In yet other embodiments, R₁ is CO₂H. In still other embodiments, R₁ is CH₂CO₂H. In particular embodiments, R₁ is SH, OH or CO₂H,
20 especially SH or OH and more especially SH.

- [0082]** In some particular embodiments, R^a is a C₆-C₉ alkyl group. In some particular embodiments, the foaming agent of Formula (Ia) or salt thereof has a solubility of greater than 0.0001 M in water. In some particular embodiments, the CMC of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is greater than 0.0001 M. In some
25 particular embodiments, the aqueous phase comprises PFAS-contaminated soil. In some particular embodiments, the aqueous phase is or comprises PFAS-contaminated groundwater.

EXAMPLES

Materials

- [0083] L-cysteine (97%), octanoyl chloride, octyl bromide, triethylamine, thionylchloride, cadmium standard solutions, calcium chloride dihydrate, sodium hydroxide, THF, methanol, acetone and ethanol were all purchased from Sigma-Aldrich (Australia) and used as received. 1-octanoylbenzotriazole (97%) was purchased from Amadis Chemical (Zhejiang, China). Milli-Q water was used in these experiments.

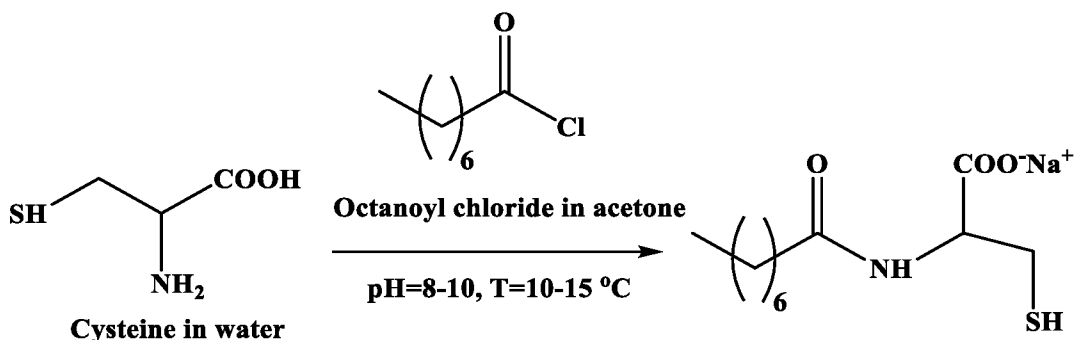
Abbreviations

[0084]

Abbreviation	Explanation
CMC	Critical Micelle Concentration
Cys	Cysteine
EA	Elemental Analysis
FT-IR	Fourier transform infrared spectroscopy
HM	Heavy metal
ICP-MS	Inductively Coupled Plasma/Mass Spectrometry
MP	Melting Point
PFAS	Poly- and perfluoralkyl substances
ppm	Parts per million
<i>N</i> -octanoyl-cys	<i>N</i> -octanoyl-cysteine
<i>N</i> -octyl-cys	<i>N</i> -octyl-cysteine
SDS	Sodium dodecyl sulfate
SLS	Sodium lauryl sulfate
WHO	World Health Organization

Example 1 – Preparation of *N*-octanoyl- cysteineComparative method 1

[0085]



Scheme 1. Synthesis of *N*-octanoyl-cys using cysteine and octanoyl chloride

[0086] The synthetic route for the synthesis by Comparative method 1 of *N*-octanoyl-cys is shown in Scheme 1. This method is a water-based method and has been reported previously (M. Taseidifar et al., *Environmental Technology & Innovation*, 2017, **8**, 182-190).

[0087] 0.06mol of NaOH and 0.06mol of L-cysteine were dissolved into 20mL water at room temperature, followed by adding (dropwise, from a 250mL separatory funnel) a mixture of 0.08mol octanoyl chloride and 20mL acetone, while stirring at 10-15°C. The pH of the solution was kept at about 8-10 by adding sodium hydroxide solution (10%) before adding the mixture of octanoyl chloride and acetone. Then 100 ml acetone was added to the resulting mixture and then the precipitate was filtered and washed with acetone. Finally, the precipitate was recrystallised from ethanol:water (V:V, 50:50). The yield of *N*-octanoyl-cys from this process was found to be less than 5%, which is too low to support efficient large-scale commercial production.

[0088] The melting point of *N*-octanoyl-cys prepared by this method was determined to be 128°C (using an Electrothermal IA9100 melting apparatus at a heating rate of 1°C min⁻¹).

[0089] The CMC of *N*-octanoyl-cys prepared by this method was determined to be 0.1M. The CMC was determined using a Bench-Top water quality meter, SPER SCIENTIFIC Instrument model 860033 to measure the conductivity and pH of the surfactant solution. The CMC value of the surfactant was measured by plotting conductivity values versus different surfactant concentrations at pH 9 by adding NaOH

solution (0.1 mol L⁻¹) and 25.0°C. The CMC was obtained as the point of sharp transition in the slope of conductivity with increasing surfactant concentration (data not shown).

Comparative method 2

[0090] Cysteine and octanoic acid were dissolved and refluxed in thionylchloride (SOCl₂) to form *N*-octanoyl cysteine. To trap the formed HCl, the weak base triethylamine (TEA) was used. This route failed.

Comparative method 3

[0091] A solution of cysteine and the base TEA in THF was stirred for 30 min and then a stoichiometric amount of octanoyl chloride was added, followed by a catalytic amount of 4-dimethylaminopyridine (DMAP). Then the mix was refluxed for 18 hours. This method was based on a previous method (*J. Phys. Org. Chem.*, 2017, **30**, e3675) reported to be successful for the synthesis of *N*-acyl phenylalanine and tyrosine. However, in this case octanoyl chloride appeared to react with TEA rather than cysteine (which has a very low solubility in THF). This reaction only produced octanoyl TEA as a white precipitate, which is soluble in acidic solution, whereas, by comparison, octanoyl cysteine should precipitate out.

Comparative method 4

[0092] The 'Schotten Baumann' conditions are based on keeping the reactants (i.e. acyl chloride and amine) in the organic phase (e.g. THF), so that the HCl produced goes into the alkaline water phase – to take up the product acid and so prevents protonation of the amine, which would stop the reaction. Unfortunately, however, the reaction did not proceed after numerous attempts. It is believed that the reaction did not proceed due to the poor solubility of cysteine in the organic phase (THF).

Comparative method 5

[0093] A reaction was carried out in which octanoic acid was used as both a reactant and the solvent. Cysteine was heated in octanoic acid to near reflux at 200 °C to try to obtain *N*-octanoyl cysteine, with the continuous removal of the produced water. However, this reaction was unsuccessful, presumably because the liquid octanoic acid dehydrated on heating to produce octanoic anhydride and this dominated the reaction, rather than reacting with cysteine, which was in solid form and hard to dissolve in the less-polar solvent.

Comparative method 6

[0094] Ionic liquids (ILs) were investigated in an attempt to increase the yield of *N*-octanoyl-cys because cysteine should have a high solubility in ILs and octanoyl chloride

should not be hydrolysed in this choice of solvent. Two ILs: 1-butyl-3-methylimidazolium triflate, 99% and 1-butyl-3-methylimidazolium hexafluorophosphate, 99%, were supplied from IoLiTec Ionic Liquids Technologies GmbH, Germany. However, use of both ILs failed to produce the desired *N*-octanoyl-cys, even in a water-free system, using continuous evacuation to remove the HCl product, and with heating to 40 °C.

Comparative method 7

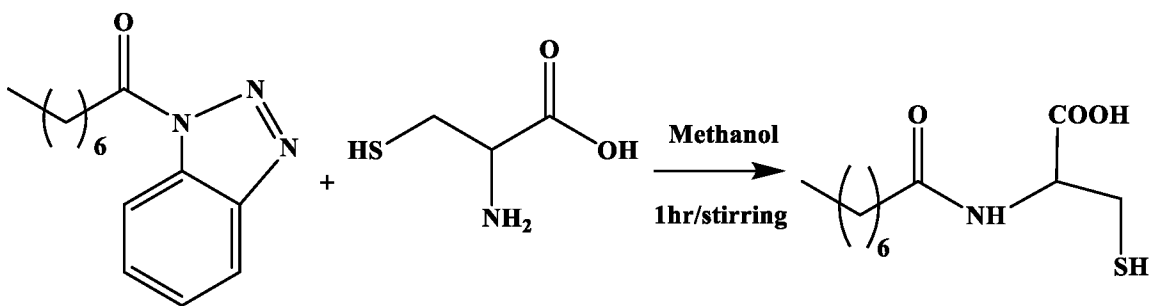
[0095] An alternative synthetic route based on a published procedure was trialed (A. R. Katritzky et al. *The Journal of Organic Chemistry*, 2000, **65**, 8210-8213). Using THF as solvent, 1-octanoylbenzotriazole failed to deliver the desired the *N*-octanoylcysteine.

Comparative method 8

[0096] Following a reported method (A. R. Katritzky et al. *The Journal of Organic Chemistry*, 2009, **74**, 7165-7167), 2mmol 1-octanoylbenzotriazole was added to a solution of cysteine (2mmol) and TEA (2mmol) in acetonitrile:water (3:1, 8mL). The mixture was stirred for 2h at room temperature. However, the reaction failed to produce the desired *N*-octanoyl-cys product.

Exemplary method 1

[0097] In a typical batch, 1-octanoylbenzotriazole (0.03 mol, 1eq) was added to a solution of cysteine (0.03 mol, 1eq) in methanol (20 mL, used as supplied) and stirred for 1 h at room temperature. A white precipitate formed which was collected by filtration and washed with ethanol. The yield was generally in the range of about 95-98%. Scheme 2 shows the synthetic route.



Scheme 2. Synthesis of *N*-octanoyl cysteine

[0098] The CMC of the *N*-octanoyl-cys surfactant prepared by Exemplary method 1, in Na⁺ form at pH 8, was determined (as reported above for Comparative method 1 above from measurements of the electrical conductivity with surfactant concentration) to be about 0.09 mol/L, see Figure 3. The melting point was found to be about 122 °C.

Discussion

[0099] Using the method reported above for Comparative method 1, based on reacting octanoyl chloride with cysteine in an aqueous environment, a low yield was obtained. The dominant peak in mass spectrum of the product was 353.27 g/mol, which is inconsistent with the calculated value of 247.35 g/mol. The peak corresponding to the desired product was comparatively minor. It is suspected that some portion of the product may have formed the oxidised dimer S-S (i.e. cystine form); especially as there was no peak found that could be ascribed to the -SH group in the Fourier transform infrared spectroscopy (FT-IR) spectrum. It has been reported that cysteine can be oxidised to cystine whilst in a 1mM solution within few days at pH 7 if exposed to the atmosphere. In our work, the cysteine concentration was kept around 3 M which is relatively concentrated and difficult to become oxidised at normal experimental conditions.

[00100] Mass spectroscopy analysis shows that the molecular weight of product obtained by Exemplary method 1 was 248.13 g/mol, consistent with the expected value (in acid form).

[00101] The FT-IR spectrum was obtained for *N*-octanoyl-cys prepared by Exemplary method 1, which showed values ($\nu_{\max}$, cm^{-1}) at 3420 (NH), 2921, 2853 (CH), 2550 (SH), 1685 (C=O), 1580, 1415 (CO_2^-) and 721 ($-\text{CH}_2$ -skeletal). This spectral analysis indicated the presence of NH, SH, COO, CO and CH groups.

[00102] An elemental analysis was performed on a sample of *N*-octanoyl-cys (prepared by Exemplary method 1). The results are presented in Table 1 below.

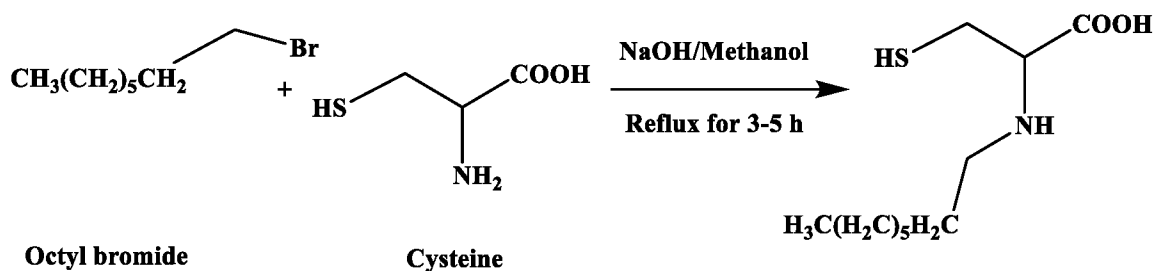
Table 1. Total Elemental Analysis of the synthesised *N*-octanoyl-cys (prepared by Exemplary method 1) in acid form

Sample	%C	%H	%N	%S
Theory	53.41	8.56	5.66	12.96
Found	55.86	8.52	7.62	10.37

Example 2 – Comparison of *N*-octyl cysteine and *N*-octanoyl cysteine in ion flotation Synthesis of *N*-octyl cysteine

[00103] A similar surfactant, *N*-octyl cysteine, was synthesised and purified according to a previously reported method (Aslam et al., *ACS Omega*, 2017, **2**, 5691-5707). Octyl bromide (0.08 mol) was added to a solution of cysteine (0.06 mol) and thymolphthaleine (as pH indicator) in methanol containing NaOH and stirred at reflux for 5 hours. The

presence of the blue colour (from the thymolphthaleine indicator) was monitored to maintain the pH at above about 10. After 5 h, the pH was adjusted to 5 by adding 1M HCl and then the white-coloured precipitate was filtered and washed with a mixture of acetone and water. Finally, the precipitate was crystallised two times in a mixture of ethanol:water (V:V, 50:50). Scheme 3 shows the synthetic route.



Scheme 3. Reaction to obtain *N*-octyl cysteine (*N*-octyl-cys) surfactant.

[00104] The same protocols as described above were used to determine the CMC and melting point of the twice-recrystallised *N*-octyl-cysteine surfactant. The values measured were 0.1M and 145 °C, respectively. These values are consistent with literature values.

Ion flotation system - Cd

[00105] In a model experiment, 0.01M of the *N*-octanoyl-cys surfactant was dissolved (with heating around 65°C while stirring) in a 5 mg L⁻¹ solution (i.e. 5ppm) of Cd containing 0.1 M NaOH and finally made up to 100 ml using Milli-Q water, with the pH of around 8.

[00106] The solution was then poured into a column (with 30 cm height and 3 cm diameter) while a 2 L/min flow of air gas was passed through it with air pump (Hiblow HP40, Philippines). Two samples were taken after each of 30 and 60 minutes from around 2 cm top of the sinter, and the Cd concentrations of each sample was determined by ICP-MS analysis. The upper-outlet foam was also collected in a waste container using an outlet tube. A schematic diagram of the column setup is shown in Figures 1 and 2.

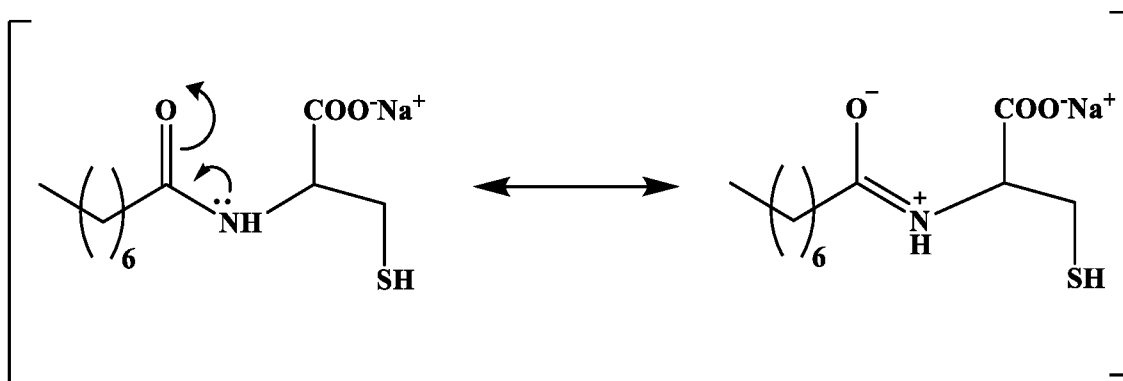
[00107] The same procedure was used with the *N*-octyl-cys surfactant in the flotation experiment, but where the pH of the solution was increased to 12, to enable this surfactant to successfully produce a foam (this difference is discussed later).

[00108] The results of the removal of 5 mg L⁻¹ of Cd solution using the *N*-octanoyl-cys and the *N*-octyl-cys surfactants presented in Table 2 show that *N*-octanoyl-cys surfactant is noticeably more efficient for removing Cd.

Table 2. Ion flotation results for 100 mL of 5 mg L⁻¹ Cd solution using *N*-octanoyl-cys and *N*-octyl-cys surfactants with air inlet gas. In the brackets, the relative standard deviations (RSD) for concentrations are given. ($C_{\text{surfactant}}=0.01\text{M}$)

Surfactant (prepared by)	Operating pH	Concentration of Cd after 30min (RSD%)	Concentration of Cd after 60min (RSD%)	Adsorption (%) After 60min
<i>N</i> -octanoyl-cys (Comparative method 1)	8	0.269 mg L ⁻¹ (14.38)	0.041 mg L ⁻¹ (36.63)	99.2
<i>N</i> -octanoyl-cys (Exemplary method 1)	8	0.43 mg L ⁻¹ (19.5)	0.01 mg L ⁻¹ (23.8)	99.8
<i>N</i> -octanoyl-cys (Exemplary method 1)	12	4.928 mg L ⁻¹ (0.77)	4.383 mg L ⁻¹ (5.8)	12.3
<i>N</i> -octyl-cys (described above)	12	4.727 mg L ⁻¹ (3.14)	4.263 mg L ⁻¹ (4.29)	14.2

[00109] A likely explanation for the better performance of the *N*-octanoyl-cys surfactant compared with the *N*-octyl-cys surfactant in the flotation experiments is that protonation of the amide group in the *N*-octanoyl-cys surfactant only occurs at low pH (i.e. in strong acid), since the pK_a is around -0.5. However, the amine group in the *N*-octyl-cys surfactant more readily protonated, having a pK_a value of about 10.7. Hence, at medium pH (of around 8) the *N*-octyl-cys surfactant may be protonated, forming the observed agglomeration phase. Increasing pH to 12 allows the *N*-octyl-cys surfactant to dissolve and can then foam in the flotation system. By comparison, at a pH of around 8, the *N*-octanoyl-cys surfactant readily foams.



Scheme 4. Resonance contributor of the amide group in *N*-octanoyl-cys means that sharing the electrons on the nitrogen deters its protonation.

[00110] Foaming tests of the two surfactants (*N*-octanoyl-cys and *N*-octyl-cys) show that *N*-octanoyl-cys surfactant starts foaming at pH ~ 6 while for the *N*-octyl-cys surfactant foaming point was obtained to be more than pH 12 (see Figure 6).

[00111] The wide active pH range for the *N*-octanoyl-cys surfactant is also useful for soil remediation because rainwater (pH of ~5.7) has a lower pH than typical river water (pH of ~8) and during heavy rainfall the soil can become saturated and the water then has a reduced pH, sometimes in the pH 6-7 range. Hence, this surfactant may still be used for soil foam fractionation cleaning under these flooded conditions.

10 Ion flotation system - Ca

[00112] In the second series of ion flotation experiments, *N*-octanoyl-cys surfactant (obtained by Exemplary method 1) was used for removing calcium ions using air in the ion flotation process. Table 3 shows the analysis of the ion flotation solutions using ICP-MS. The results show that the *N*-octanoyl-cys had a high performance in removing calcium, at high level of 97% in the ion flotation process. This surfactant successfully reduced the 5 mg L⁻¹ calcium content to a level lower than 0.13 mg L⁻¹ in a single stage, physicochemical process. The same procedure using *N*-octyl-cys surfactant had a removal efficiency of 14% at pH 12.

20 **Table 3.** Flotation results using an air bubbles for 100 mL of 5 mg L⁻¹ calcium using *N*-octanoyl-cys and *N*-octyl-cys surfactants ($C_{\text{calcium}} = 5 \text{ mg L}^{-1}$ and $C_{\text{surfactant}} = 0.01 \text{ M}$)

Surfactant	pH	C(mg L ⁻¹) (RSD%)	C(mg L ⁻¹) (RSD%)	Adsorption (%)
		After 30min	After 60min	After 60min
<i>N</i> -octanoyl-cys	8	0.72 (42.02)	0.13 (29.01)	97.3
<i>N</i> -octyl-cys	12	4.89 (0.87)	4.27 (3.31)	14.6

Observations concerning Examples 1 and 2

[00113] In view of the above examples, the surfactant *N*-octanoyl-cys proved itself as a useful surfactant which has excellent metal-binding and foaming properties over a wide range of pH values. It is also likely to be environmentally acceptable, as it readily breaks down into octanoic acid and cysteine, both of which have little to no toxicity concerns and are even present in some foods. For example, octanoic acid is found naturally in the milk of various mammals and as a minor constituent of coconut oil and palm kernel oil. The chain length is also similar to many common PFAS compounds and may offer co-

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surfactant and chelation properties useful for soil treatment using foam fractionation and/or foam flotation. *N*-octanoyl-cys may be biodegraded by enzymes to produce natural products. *N*-octanoyl-cys surfactant shows promising commercial applications in various industries, including detergents, cleaning products, toothpaste and also as natural soaps.

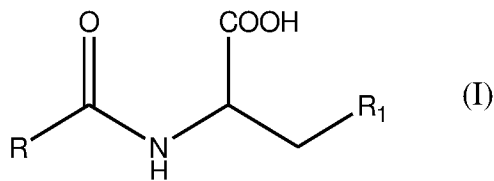
5 **[00114]** It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

10 **[00115]** In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

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The claims defining the invention are as follows:

1. A method of preparing an *N*-acyl cysteine of Formula (I):

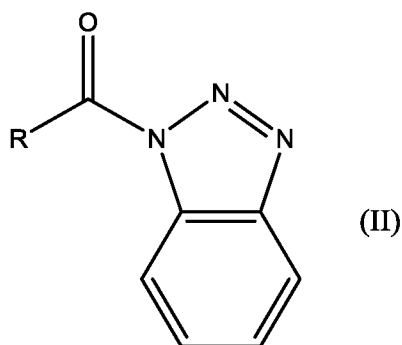


or a salt thereof,

wherein R_1 is SH, OH, CO_2H or $\text{CH}_2\text{CO}_2\text{H}$; and

R is selected from H, C_1 - C_{15} alkyl groups, C_2 - C_{15} alkenyl groups, C_2 - C_{15} alkynyl groups, fluorinated C_1 - C_{15} alkyl groups, fluorinated C_2 - C_{15} alkenyl groups and fluorinated C_2 - C_{15} alkynyl groups,

- the method comprising reacting cysteine and an *N*-acylbenzotriazole of Formula (II):



wherein R is as defined in Formula (I), in a solvent composed substantially of methanol.

2. The method according to claim 1 wherein R_1 is SH or OH.

3. The method according to claim 2, wherein R_1 is SH.

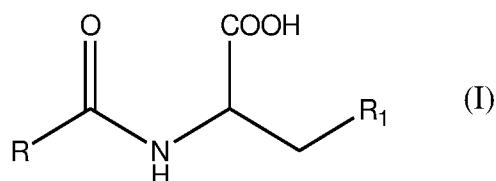
4. The method according to any one of claims 1 to 3, wherein R is a linear or branched C_5 - C_{10} alkyl group.

5. The method according to claim 4, wherein R is a linear C_6 - C_9 alkyl group.

6. The method according to any one of claims 1 to 5, wherein the solvent is at least 99 %v/v methanol.

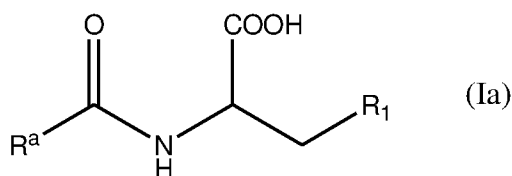
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7. An *N*-acyl amino acid of Formula (I):



or a salt thereof prepared by the method of any one of claims 1 to 6.

8. A method of removing one or more PFASs from an aqueous phase comprising one or more PFASs, the method comprising dissolving in the aqueous phase a foaming agent of Formula (Ia):



or a salt thereof,

wherein R₁ is SH, OH, CO₂H or CH₂CO₂H; and

R^a is a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group, a C₅-C₁₂ alkynyl group, a fluorinated C₅-C₁₂ alkyl group, a fluorinated C₅-C₁₂ alkenyl group or a fluorinated C₅-C₁₂ alkynyl group;

forming a foam from the aqueous phase and a gas; and
separating the foam from the aqueous phase.

9. The method according to claim 8, wherein R₁ is OH or SH.

10. The method according to claim 9, wherein R₁ is SH.

11. The method according to any one of claims 8 to 10, wherein R^a is a C₆-C₉ alkyl group.

12. The method according to any one of claims 8 to 11, wherein the foaming agent is in salt form.

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13. The method according to any one of claims 8 to 12, wherein the foaming agent of Formula (Ia) or salt thereof has a solubility of greater than 0.0001 M in water.

14. The method according to any one of claims 8 to 13, wherein the CMC of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is greater than 0.0001 M.

15. The method according to any one of claims 8 to 14, wherein the concentration of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is from about 0.01 mM to about 2 M.

16. The method according to any one of claims 8 to 15, wherein the concentration of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is from about 0.0001 M to about 2 M.

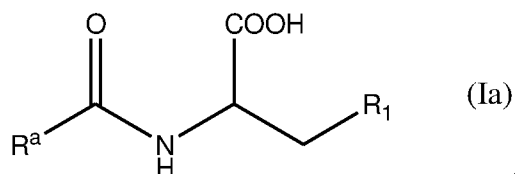
17. The method according to any one of claims 8 to 16, wherein the one or more PFASs comprises at least one PFAS capable of binding to a multivalent metal ion.

18. The method according to any one of claims 8 to 17, wherein the aqueous phase has a pH within the range of from about pH 4 to about pH 12.

19. The method according to any one of claims 8 to 18, wherein the aqueous phase comprises one or more metal ions selected from Ca^{2+} , Mg^{2+} , Al^{3+} and Fe^{3+} .

20. The method according to any one of claims 8 to 19, wherein the aqueous phase comprises PFAS-contaminated soil or PFAS-contaminated groundwater.

21. Use of a foaming agent of Formula (Ia):



or a salt thereof,

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wherein R₁ is SH, OH, CO₂H or CH₂CO₂H; and

R^a is a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group, a C₅-C₁₂ alkynyl group, a fluorinated C₅-C₁₂ alkyl group, a fluorinated C₅-C₁₂ alkenyl group or a fluorinated C₅-C₁₂ alkynyl group;

5 for removing one or more PFASs from an aqueous phase.

22. Use according to claim 21, wherein the foaming agent of Formula (Ia) or salt thereof is used in a foam fractionation process to remove the one or more PFASs from the aqueous phase.

10

23. Use according to claim 21 or 22, wherein R₁ is OH or SH.

24. Use according to claim 23 wherein R₁ is SH.

15

25. Use according to any one of claims 21 to 24, wherein the foaming agent is in salt form.

26. Use according to any one of claims 21 to 25, wherein R^a is a C₆-C₉ alkyl group.

20

27. Use according to any one of claims 21 to 26, wherein the foaming agent of Formula (Ia) or salt thereof has a solubility of greater than 0.0001 M in water.

28. Use according to any one of claims 21 to 27, wherein the CMC of the foaming agent of Formula (Ia) or salt thereof in the aqueous phase is greater than 0.0001 M.

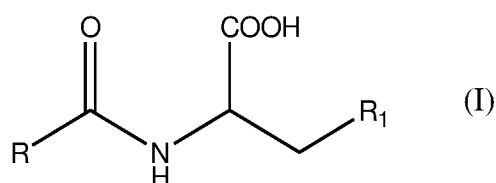
25

29. Use according to any one of claims 21 to 28, wherein the aqueous phase comprises PFAS-contaminated soil or PFAS-contaminated groundwater.

30. A cleaning composition, detergent composition or toothpaste composition

30 comprising an *N*-acyl amino acid of Formula (I):

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or a salt thereof,

wherein R₁ is SH, OH, CO₂H or CH₂CO₂H; and

R is selected from a C₅-C₁₂ alkyl group, a C₅-C₁₂ alkenyl group and a C₅-C₁₂ alkynyl group.

31. The cleaning composition, detergent composition or toothpaste composition according to claim 30, wherein R₁ is SH or OH.

32. The cleaning composition, detergent composition or toothpaste composition according to claim 31, wherein R₁ is SH.

33. The cleaning composition, detergent composition or toothpaste composition according to claim 31, wherein the compound of formula (I) is in the salt form.

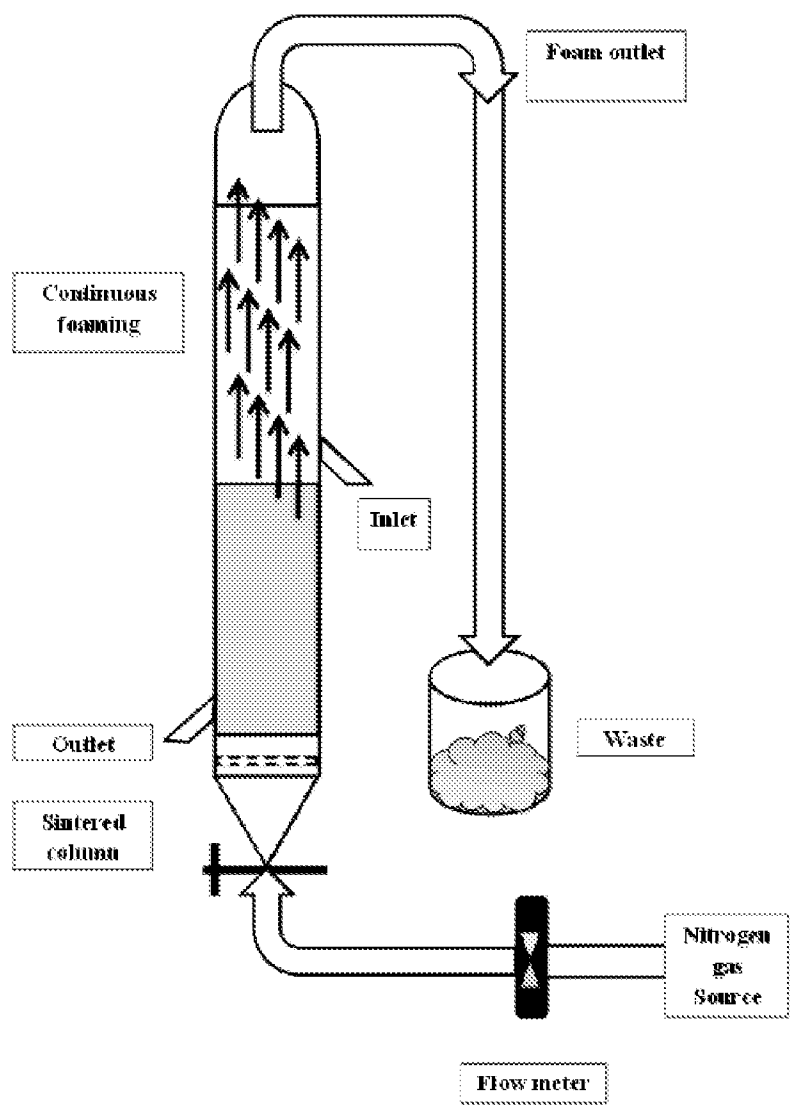


Figure 1

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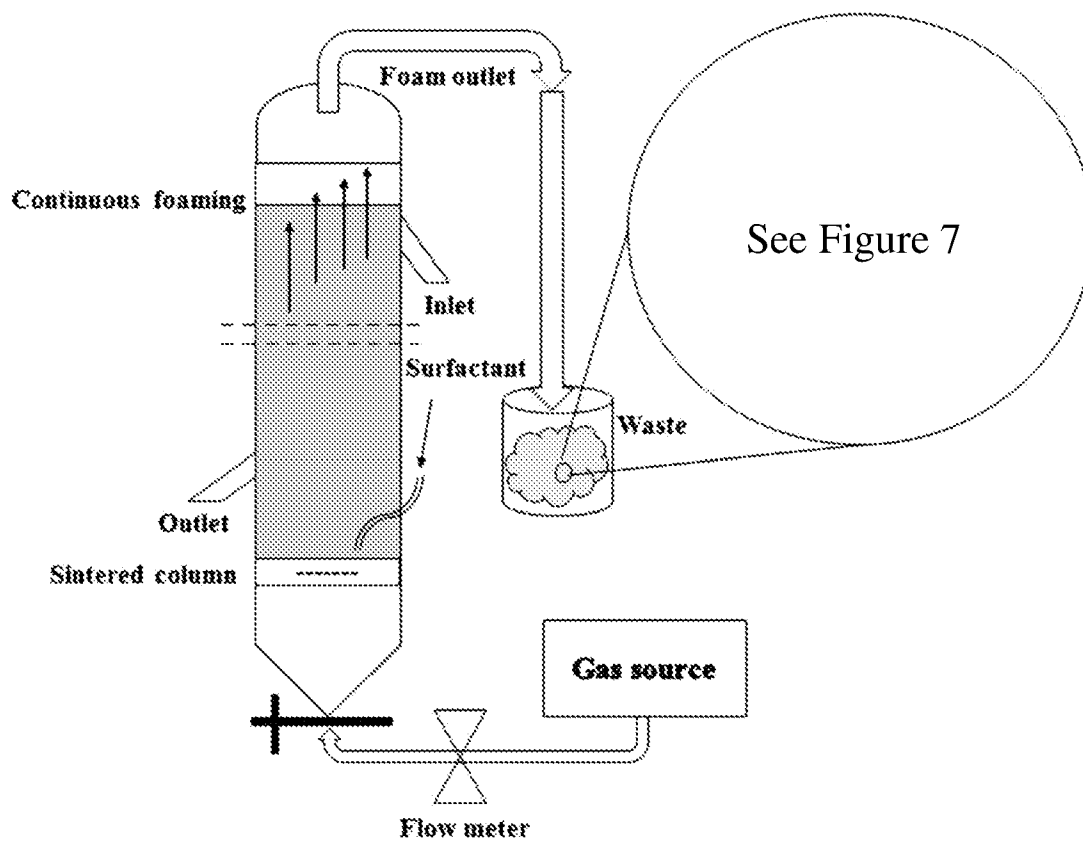


Figure 2

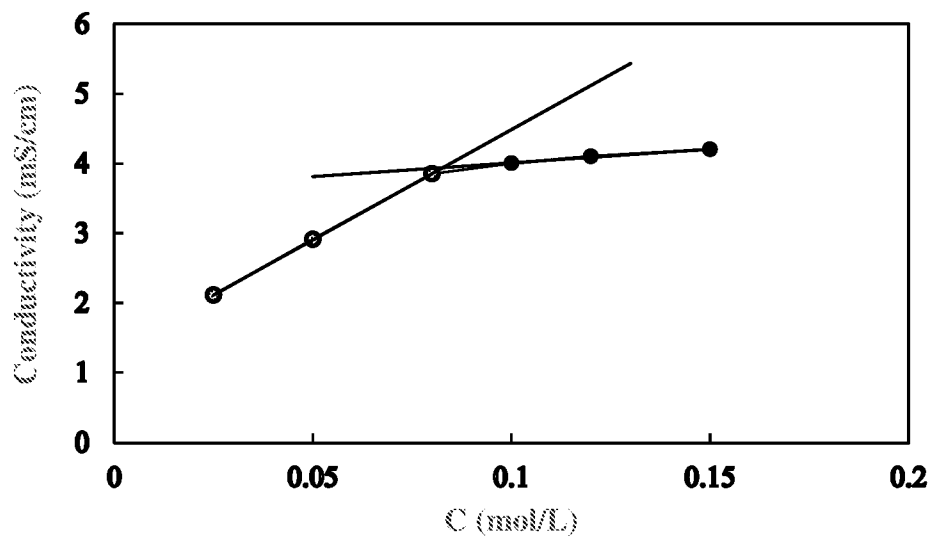


Figure 3

- 3/5 -

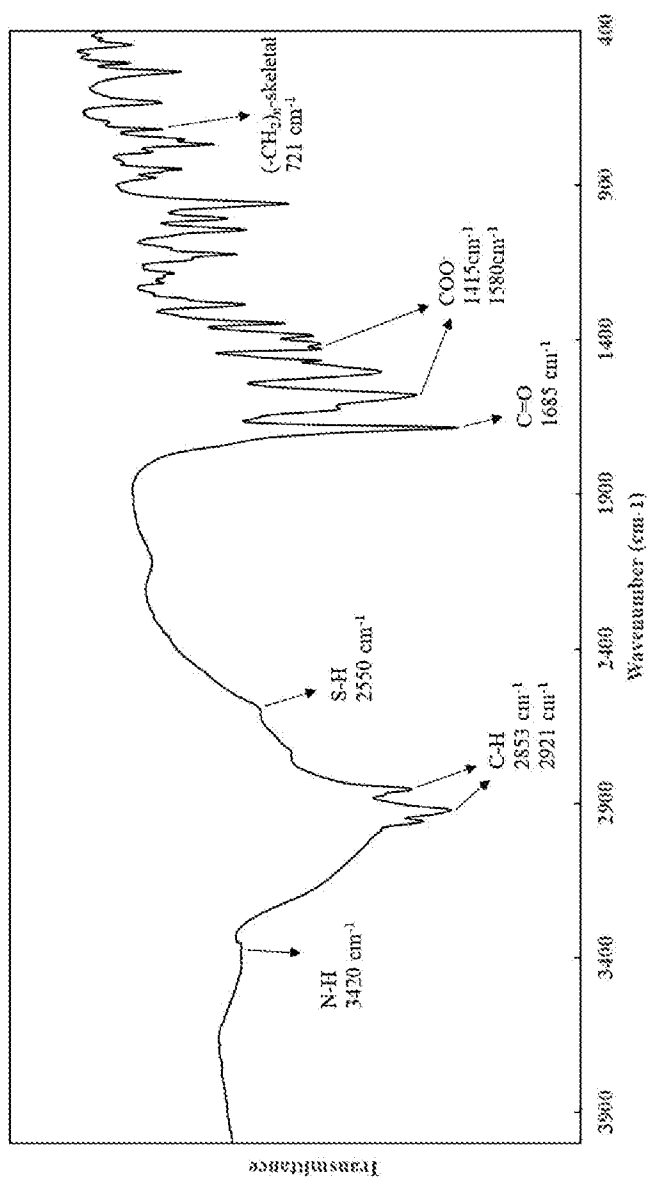


Figure 4

- 4/5 -

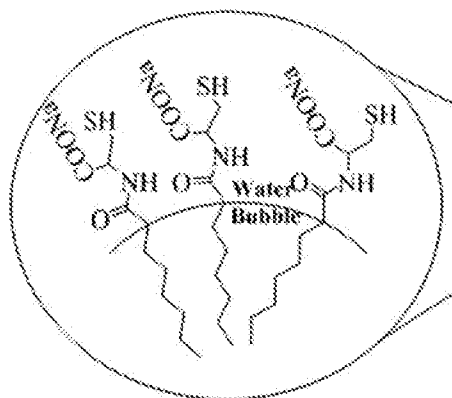
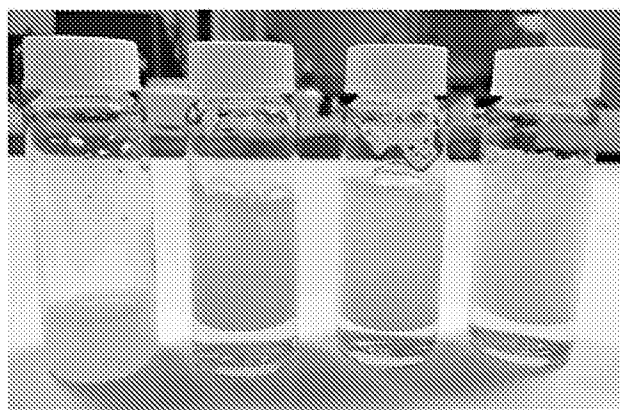
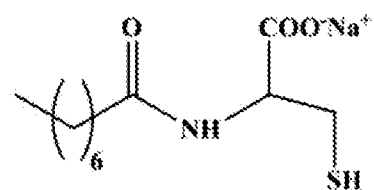


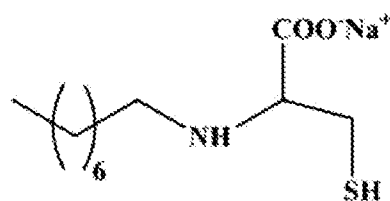
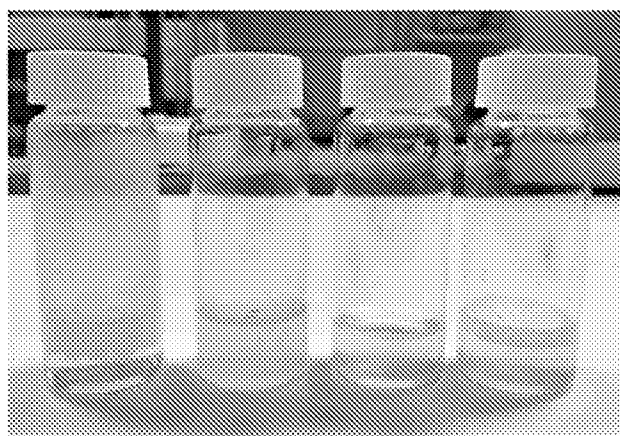
Figure 5



pH 3 6 9 12



octanoyl-cys surfactant



octyl-cys surfactant

Figure 6

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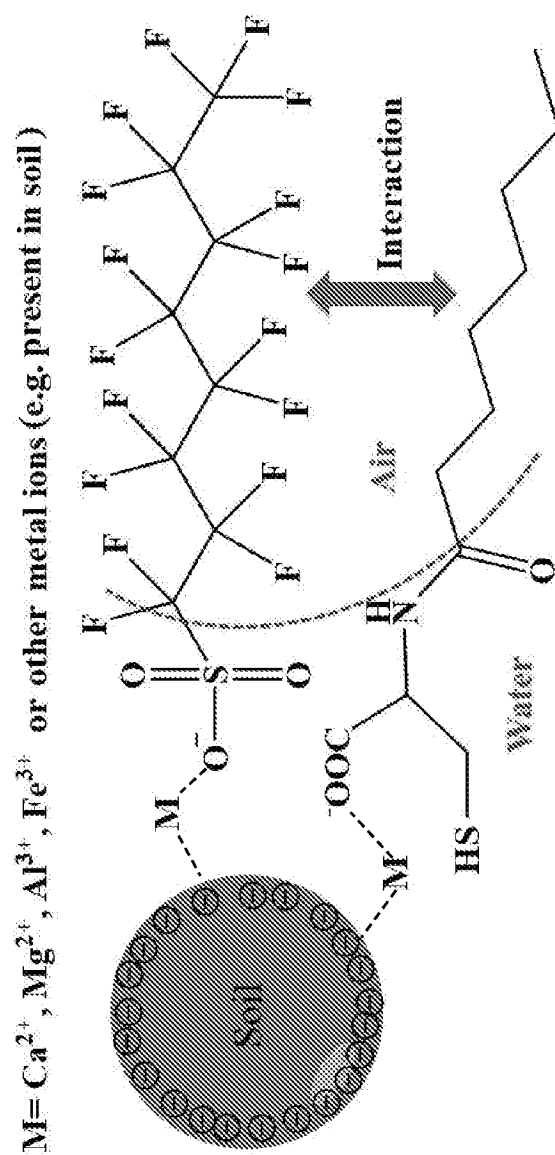


Figure 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2020/050142

A. CLASSIFICATION OF SUBJECT MATTER

C07C 231/00 (2006.01) **C07C 233/47 (2006.01)** **C07C 323/59 (2006.01)** **C07C 319/12 (2006.01)** **A61K 8/44 (2006.01)**
A61Q 11/00 (2006.01) **C02F 1/24 (2006.01)** **B03D 1/01 (2006.01)** **B03D 1/012 (2006.01)** **B03D 1/02 (2006.01)**
C11D 1/10 (2006.01)

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)

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)

CHEMICAL ABSTRACTS REGISTRY, CAPLUS: Structure search based on structures of claim 1.

Esp@cenet: NEWSOUTH as applicant.

Applicant name searched in internal databases provided by IP Australia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"D" document cited by the applicant in the international application	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search 3 April 2020	Date of mailing of the international search report 03 April 2020
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Matthew Francis AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262832424

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2020/050142
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5017364 A (MITSUTAKE ET AL) 21 May 1991 abstract; examples	7, 30-33
X	US 5981450 A (FABRY ET AL) 09 November 1999 column 2, lines 36-57; examples 1, 2; claims	7, 30-33
X	US 9987207 B1 (L'OREAL) 05 June 2018 example 1; claims	7, 30-33
X A	WO 2018071985 A1 (NEWSOUTH INNOVATIONS PTY LIMITED) 26 April 2018 page 3, line 17-page 4, line 30; Figure 1; claims whole document	7 8-29
X	Erol, K. et al, "Use of amino acid-based polymeric material for isolation of a protein from poison", Journal of Molecular Structure (2017), 1130, 753-759, CODEN: JMOSB4; ISSN: 0022-2860, DOI 10.1016/j.molstruc.2016.11.004 page 754, 3.1, Process 1	1-6
X	Jalilzadeh, M. et al, "Specific heavy metal ion recovery with ion-imprinted cryogels", Journal of Applied Polymer Science (2016), 133(10), CODEN: JAPNAB; ISSN: 0021-8995, DOI 10.1002/app.43095 page 2 of 9, "Preparation of Amino Acid Based Functional Monomers"	1-6
X	Katritzky, A. et al, "Selective synthesis and structural elucidation of S-acyl- and N-acylcysteines", Journal of Organic Chemistry (2009), 74(18), 7165-7167, CODEN: JOCEAH; ISSN: 0022-3263, DOI 10.1021/jo900853s page 7166, SCHEME 2 and TABLE 2.	1-6
X	Hur, D. et al, "N-acylbenzotriazole-mediated synthesis of some methacrylamido amino acids", Letters in Organic Chemistry (2007), 4(8), 585-587, CODEN: LOCEC7; ISSN: 1570-1786, DOI 10.2174/157017807782795556 page 586, Scheme 2; "RESULTS AND DISCUSSION"	1-6
A	Bordes, R. et al, "Amino acid-based surfactants – do they deserve more attention?", Advances in Colloid and Interface Science 222 (2015) 79–91 Fig. 1; page 80, column 2; 6. The cysteine polar head-group can be useful	1-33

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-7 are directed to methods of preparing N-acyl cysteine(s) of Formula (I). The feature of methods of preparing N-acyl cysteine(s) of Formula (I) is specific to this group of claims.
- Claims 8-29 are directed to methods of removing PFASs from an aqueous phase using a foaming agent of Formula (I). The feature of methods of removing PFASs from an aqueous phase using a foaming agent of Formula (I) is specific to this group of claims.
- Claims 30-33 are directed to cleaning compositions, detergents or toothpastes comprising an N-acyl amino acid of Formula (I). The feature of cleaning compositions, detergents or toothpastes comprising an N-acyl amino acid of Formula (I) is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is N-acyl cysteine(s) of Formula (I)

However this feature does not make a contribution over the prior art because it is disclosed in:

D4, which discloses a range of acylated amino acids (see claim 1, examples) that fall within the scope of present Formula (I) when R1 of D4 is hydrogen.

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2020/050142	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 5017364 A	21 May 1991	US 5017364 A	21 May 1991
		EP 0364928 A2	25 Apr 1990
		EP 0364928 B1	06 Apr 1994
		JP H02256608 A	17 Oct 1990
		JP 2845245 B2	13 Jan 1999
		KR 900005955 A	07 May 1990
US 5981450 A	09 November 1999	US 5981450 A	09 Nov 1999
		EP 0781319 A1	02 Jul 1997
		EP 0781319 B1	16 Aug 2000
		JP H10506417 A	23 Jun 1998
		WO 9608551 A1	21 Mar 1996
US 9987207 B1	05 June 2018	US 9987207 B1	05 Jun 2018
WO 2018071985 A1	26 April 2018	WO 2018071985 A1	26 Apr 2018
		AU 2017346939 A1	30 May 2019
		BR 112019008068 A2	02 Jul 2019
		CA 3041360 A1	26 Apr 2018
		CL 2019001080 A1	27 Sep 2019
		CN 110022984 A	16 Jul 2019
		EP 3528958 A1	28 Aug 2019
		KR 20190086677 A	23 Jul 2019
		SG 11201903525R A	30 May 2019
		US 2019248682 A1	15 Aug 2019
		End of Annex	
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.			