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(54) Titre : METHODE DE LAVAGE D'UN TISSU AU MOYEN D'UNE ESTERASE DE LIPEDE, D'UN INGREDIENT ACTIF ASSOUPPLISSEUR DE TISSUS CHARGE DE MANIERE CATIONIQUE ET D'UNE COMPOSITION DE DETERGENT A LESSIVE

(54) Title: METHOD OF LAUNDERING A FABRIC WITH A LIPID ESTERASE, A CATIONICALLY CHARGED FABRIC SOFTENING ACTIVE AND A LAUNDRY DETERGENT COMPOSITION

(57) **Abrégé/Abstract:**

A method of laundering a fabric, comprising the steps of; (i) contacting a fabric with a lipid esterase; (ii) contacting the fabric from step (i) with a cationically charged fabric softening active, wherein the cationically charged fabric softening active is a substrate for the lipid esterase; and (iii) contacting the fabric from step (ii) with a laundry detergent composition, wherein the laundry detergent composition comprises an anionic surfactant, wherein the anionic surfactant is present at the ratio of anionic surfactant to fabric on a weight to weight basis of from 1:200 to 1:500.

ABSTRACT

A method of laundering a fabric, comprising the steps of; (i) contacting a fabric with a lipid esterase; (ii) contacting the fabric from step (i) with a cationically charged fabric softening active, wherein the cationically charged fabric softening active is a substrate for the lipid esterase; and (iii) contacting the fabric from step (ii) with a laundry detergent composition, wherein the laundry detergent composition comprises an anionic surfactant, wherein the anionic surfactant is present at the ratio of anionic surfactant to fabric on a weight to weight basis of from 1:200 to 1:500.

METHOD OF LAUNDERING A FABRIC WITH A LIPID ESTERASE,
A CATIONICALLY CHARGED FABRIC SOFTENING ACTIVE AND
A LAUNDRY DETERGENT COMPOSITION

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TECHNICAL FIELD

The present invention relates to methods of laundering fabrics.

BACKGROUND

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Fabric softening compositions are often added by consumers to the rinse step of a fabric washing operation. Fabric softeners impart a number of sensorial benefits that consumers enjoy, including softness and freshness. Oftentimes, softness is provided by esterified cationic surfactants.

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Laundry detergent compositions are used to provide fabric cleaning benefit in the wash step of a laundry operation. However, the ability of such compositions to effectively clean fabrics is reduced by the presence of cationic surfactant on fabrics that has been carried over from previous rinse steps. Anionic detergent surfactants present in the laundry detergent compositions provide stain removal benefits to fabrics, but they also work to strip carried-over cationic surfactant from the fabrics to allow other components of the laundry detergent composition to provide their benefit to the fabric. However, the cleaning performance of a laundry detergent composition is reduced because of the loss of available anionic surfactant due to the interaction of the cationic surfactant and anionic surfactant.

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This problem is exacerbated by a current market trend to compact laundry detergent products. In order to compact product, lower concentrations of ingredient including anionic surfactant are used. This results in a decrease in cleaning performance due to the higher ratio of cationic surfactant carry-over to anionic surfactant present in the wash liquor. Furthermore, environmental and energy consumption concerns mean that consumers tend to use colder wash temperatures and shorter wash cycle times. This again makes it harder for the anionic surfactant present to effectively strip the cationic surfactant from the fabric.

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Thus, there is a need in the art for a means to effectively remove carried-over cationic surfactant on fabric in the presence of low anionic surfactant levels. There is a further need in the art for a means to effectively remove carried-over cationic surfactant on fabrics at low wash temperatures and also at short wash cycles.

It was surprisingly found that the method of the present invention solved this problem. The present method comprises the steps of contacting a fabric with a lipid esterase followed by the step of contacting it with cationic surfactant. It was surprisingly found that the presence of the lipid esterase on the fabric prior to the addition of cationic surfactant improved cationic surfactant removal in a subsequent wash.

SUMMARY

Embodiments described herein relate to a method of laundering a fabric, comprising the steps of; (i) contacting a fabric with a lipid esterase; (ii) contacting the fabric from step (i) with a cationically charged fabric softening active, wherein the cationically charged fabric softening active is a substrate for the lipid esterase; and (iii) contacting the fabric from step (ii) with a laundry detergent composition, wherein the laundry detergent composition comprises an anionic surfactant, wherein the anionic surfactant is present at the ratio of anionic surfactant to fabric on a weight to weight basis of from 1:150 to 1:500.

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DETAILED DESCRIPTION

Exemplary embodiments relate to a method of laundering a fabric, comprising the steps of;

- i) contacting a fabric with a lipid esterase;
- 20 ii) contacting the fabric from step (i) with a cationically charged fabric softening active, wherein the cationically charged fabric softening active is a substrate for the lipid esterase; and
- iii) contacting the fabric from step (ii) with a laundry detergent composition, wherein the laundry detergent composition comprises an anionic deterative surfactant, wherein the anionic surfactant is present at the ratio of anionic deterative surfactant to fabric on a weight to weight basis of from 1:150 to 1:500.

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A fabric may be contacted with a lipid esterase in step (i) in the wash cycle of a washing operation, followed by being contacted with a cationically charged fabric softening active in step (ii) in a rinse step of a wash operation. The fabric may then be dried. The fabric may then be worn by a consumer or used in another way for its intended use. Following use of the fabric, the fabric may then be contacted with the laundry detergent composition in step (iii). Without wishing to be bound by theory, it is believed that the lipid esterase contacted to the fabric in step

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(i) acts 'out of the wash' to hydrolyse the cationically charged fabric softening active contacted in step (ii). Lipid esterases are a class of enzymes that hydrolyse lipid esters, i.e. split the ester into acid and alcohol. Thus, the cationically charged softening active provides softening benefit but since it is pre-hydrolysed out of the wash, it is more effectively stripped from the fabric in step (iii) even in the presence of low levels of anionic surfactant.

Step (i)

The method of the present invention comprises a step (i) of contacting a fabric with a lipid esterase.

The fabric may be any suitable fabric. The fabric may comprise natural or synthetic materials or a combination thereof. The fabric may comprise cotton, polycotton, polyester, or a combination thereof. The fabric may comprise cotton.

The lipid esterase may be any suitable lipid esterase. The lipid esterase may comprise at least a first and a second lipid esterase. The lipid esterase may comprise more than two lipid esterases. The lipid esterase may be a lipase, or a cutinase, or a combination thereof.

The lipid esterase may be selected from the following:

- (1) Triacylglycerol lipases (E.C. 3.1.1.3)
- (2) Carboxylic ester hydrolase (E.C. 3.1.1.1)
- (3) Cutinase (E.C. 3.1.1.74)
- (4) Sterol esterase (E.C. 3.1.1.13)
- (5) Wax-ester hydrolase (E.C. 3.1.1.50)

By 'E.C. class' we herein mean the Enzyme Commission class. The Enzyme Commission class is an international recognized enzyme classification scheme based on chemical reactions that the enzymes catalyse.

Suitable triacylglycerol lipases can be selected from variants of the *Humicola lanuginosa* (*Thermomyces lanuginosus*) lipase. Other suitable triacylglycerol lipases can be selected from variants of *Pseudomonas* lipases, e.g., from *P. alcaligenes* or *P. pseudoalcaligenes* (EP 218 272), *P. cepacia* (EP 331 376), *P. stutzeri* (GB 1,372,034), *P. fluorescens*, *Pseudomonas* sp. strain SD 705 (WO 95/06720 and WO 96/27002), *P. wisconsinensis* (WO 96/12012), *Bacillus* lipases, e.g., from *B. subtilis* (Dartois et al. (1993), Biochemica et Biophysica Acta, 1131, 253-360), *B. stearothermophilus* (JP 64/744992) or *B. pumilus* (WO 91/16422).

Suitable carboxylic ester hydrolases can be selected from wild-types or variants of carboxylic ester hydrolases endogenous to *B. gladioli*, *P. fluorescens*, *P. putida*, *B.*

acidocaldarius, *B. subtilis*, *B. stearothermophilus*, *Streptomyces chrysomallus*, *S. diastatochromogenes* and *Saccharomyces cerevisiae*.

Suitable cutinases can be selected from wild-types or variants of cutinases endogenous to strains of *Aspergillus*, in particular *Aspergillus oryzae*, a strain of *Alternaria*, in particular
 5 *Alternaria brassiciola*, a strain of *Fusarium*, in particular *Fusarium solani*, *Fusarium solani pisi*, *Fusarium oxysporum*, *Fusarium oxysporum cepa*, *Fusarium roseum culmorum*, or *Fusarium roseum sambucium*, a strain of *Helminthosporium*, in particular *Helminthosporium sativum*, a strain of *Humicola*, in particular *Humicola insolens*, a strain of *Pseudomonas*, in particular
 10 *Pseudomonas mendocina*, or *Pseudomonas putida*, a strain of *Rhizoctonia*, in particular *Rhizoctonia solani*, a strain of *Streptomyces*, in particular *Streptomyces scabies*, a strain of *Coprinopsis*, in particular *Coprinopsis cinerea*, a strain of *Thermobifida*, in particular *Thermobifida fusca*, a strain of *Magnaporthe*, in particular *Magnaporthe grisea*, or a strain of *Ulocladium*, in particular *Ulocladium consortiale*.

In a preferred embodiment, the cutinase is selected from variants of the *Pseudomonas mendocina* cutinase described in WO 2003/076580 (Genencor), such as the variant with three
 15 substitutions at I178M, F180V, and S205G.

In another preferred embodiment, the cutinase is a wild-type or variant of the six cutinases endogenous to *Coprinopsis cinerea* described in H. Kontkanen et al, App. Environ. Microbiology, 2009, p2148-2157

20 In another preferred embodiment, the cutinase is a wild-type or variant of the two cutinases endogenous to *Trichoderma reesei* described in WO2009007510 (VTT).

In a most preferred embodiment the cutinase is derived from a strain of *Humicola insolens*, in particular the strain *Humicola insolens* DSM 1800. *Humicola insolens* cutinase is described in WO 96/13580. The cutinase may be a variant, such as one of the variants disclosed
 25 in WO 00/34450 and WO 01/92502. Preferred cutinase variants include variants listed in Example 2 of WO 01/92502.

Suitable sterol esterases may be derived from a strain of *Ophiostoma*, for example *Ophiostoma piceae*, a strain of *Pseudomonas*, for example *Pseudomonas aeruginosa*, or a strain of *Melanocarpus*, for example *Melanocarpus albomyces*.

30 In a most preferred embodiment the sterol esterase is the *Melanocarpus albomyces* sterol esterase described in H. Kontkanen et al, Enzyme Microb Technol., 39, (2006), 265-273.

Suitable wax-ester hydrolases may be derived from *Simmondsia chinensis*.

The lipid esterase may be selected from an enzyme in E.C. class 3.1 or 3.2 or a combination thereof. The lipid esterase may comprise an enzyme selected from E.C. class 3.1.1.1 or 3.1.1.3 or 3.1.1.74 or a combination thereof. The lipid esterase may comprise an enzyme selected from E.C. class 3.1.1.3. The lipid esterase may comprise a variant having at least 90% sequence
5 identity to wild-type lipase from *Thermomyces lanuginosus* and having sequence substitutions T231R and N233R, or a variant corresponding to Claim 5, part (u) of EP1290150B1, or a combination thereof. The variant described in EP 1290150B1 has substitutions G8D, N15D, S48E, A88H, N91H, A130V, and R189V compared to a wild-type *Humicola insolens* cutinase from strain DSM 1800. The lipid esterase may comprise a variant having at least 90% sequence
10 identity to wild-type lipase from *Thermomyces lanuginosus* and having sequence substitutions T231R and N233R.

The fabric may have been contacted with a lipid esterase at a concentration of between 30 and 55,000 ng enzyme/g fabric. The fabric may have been contacted with a lipid esterase at a concentration of between 30 and 2000 ng enzyme/g fabric. Alternatively, the fabric may have
15 been contacted with a lipid esterase at a concentration of between 50 and 1700ng enzyme/g fabric, or even 80 and 1600ng enzyme/g fabric. Alternatively, the fabric may have been contacted with a lipid esterase at a concentration of between 100 and 3000 ng enzyme/g fabric, or even 125 and 2500 ng enzyme/g fabric. Alternatively, the fabric may have been contacted with the lipid esterase at a concentration of between 100 and 35,000 ng enzyme/g fabric, or even
20 between 500 and 30,000 ng enzyme/g fabric. Without wishing to be bound by theory, it is believed that these concentrations are optimal for soil removal from the fabrics.

The lipid esterase may be contacted in a previous wash operation and the fabric subsequently dried. The lipid esterase may have been previously deposited by washing the fabric in a wash liquor comprising the lipid esterase. For example the lipid esterase may be formed in a wash
25 cycle of a machine wash operation. Alternatively, the lipid esterase may have been added to the fabric in the form of a pre-treater. For example it may have been deposited as a pre-treat stain remover composition. In this aspect, the pre-treat composition is added to a portion or all of the fabric at some point before it is subjected to a wash operation. Alternatively, the pre-treat composition is added to a specific stain on the fabric at some point before the fabric is subjected
30 to a wash operation. Alternatively, the lipid esterase may have been contacted to the fabric during fabric manufacture.

The lipid esterase in step (i) can be used in combination with any other known laundry detergent ingredients detailed below.

Step (ii)

The present invention comprises a step (ii) of contacting the fabric from step (i) with a cationically charged fabric softening active, wherein the cationically charged fabric softening active comprises a substrate for the lipid esterase.

5 The cationically charged fabric softening active may be present in a laundry treatment composition. The laundry treatment composition may be in any suitable form including granular, liquid or unitized dose. When in unitized dose form, it is preferred that the laundry treatment composition is enclosed with a water-soluble film, for example a polyvinyl alcohol-based film.

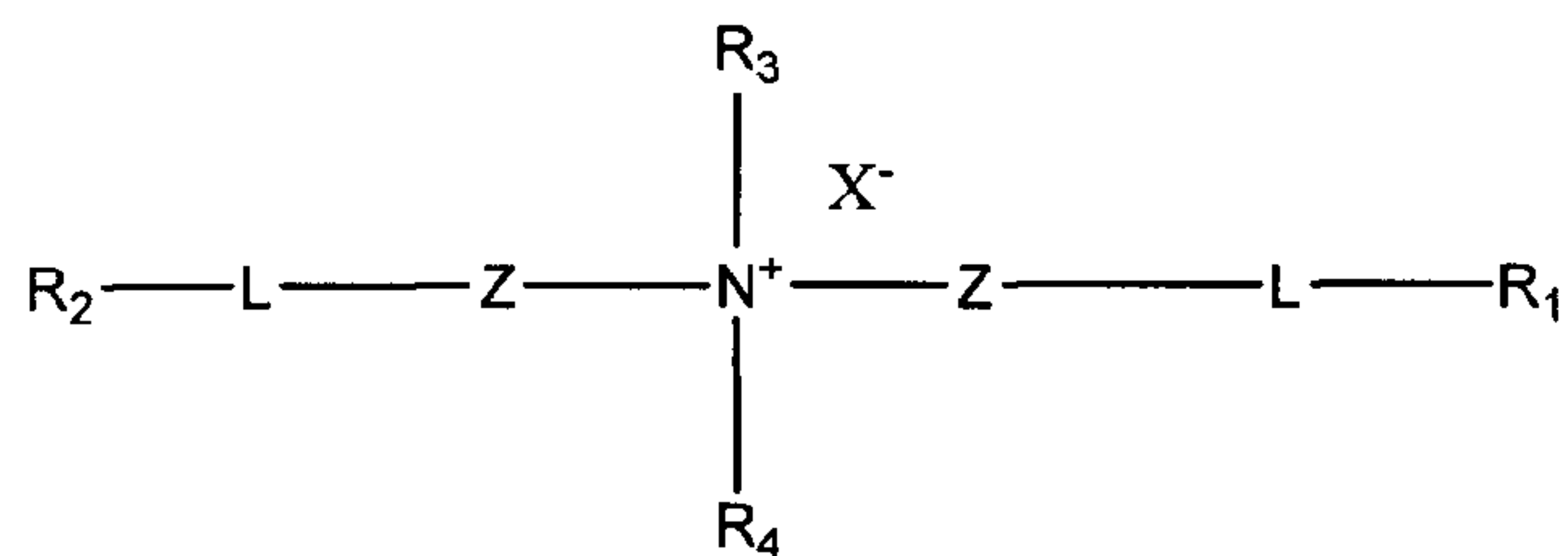
10 In the context of the present invention, the lipid esterase is believed to hydrolyse esters present in the cationically charged fabric softening active. Thus, the fabric softening active must comprise a substrate for the lipid esterase.

Preferably, the fabric softener active comprises an active selected from the group comprising, diester quaternary ammonium compounds, dialkyl quaternary ammonium
15 compounds, imidazolinium quaternary compounds, cationic starch, sucrose ester-based fabric care materials, and mixtures thereof.

In one aspect, said ester quat fabric softener active, monoester, diester, and triester quat fabric softener active and ion pair fabric softener actives are selected from the group consisting of:

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a) materials having Formula (1) below



(Formula 1)

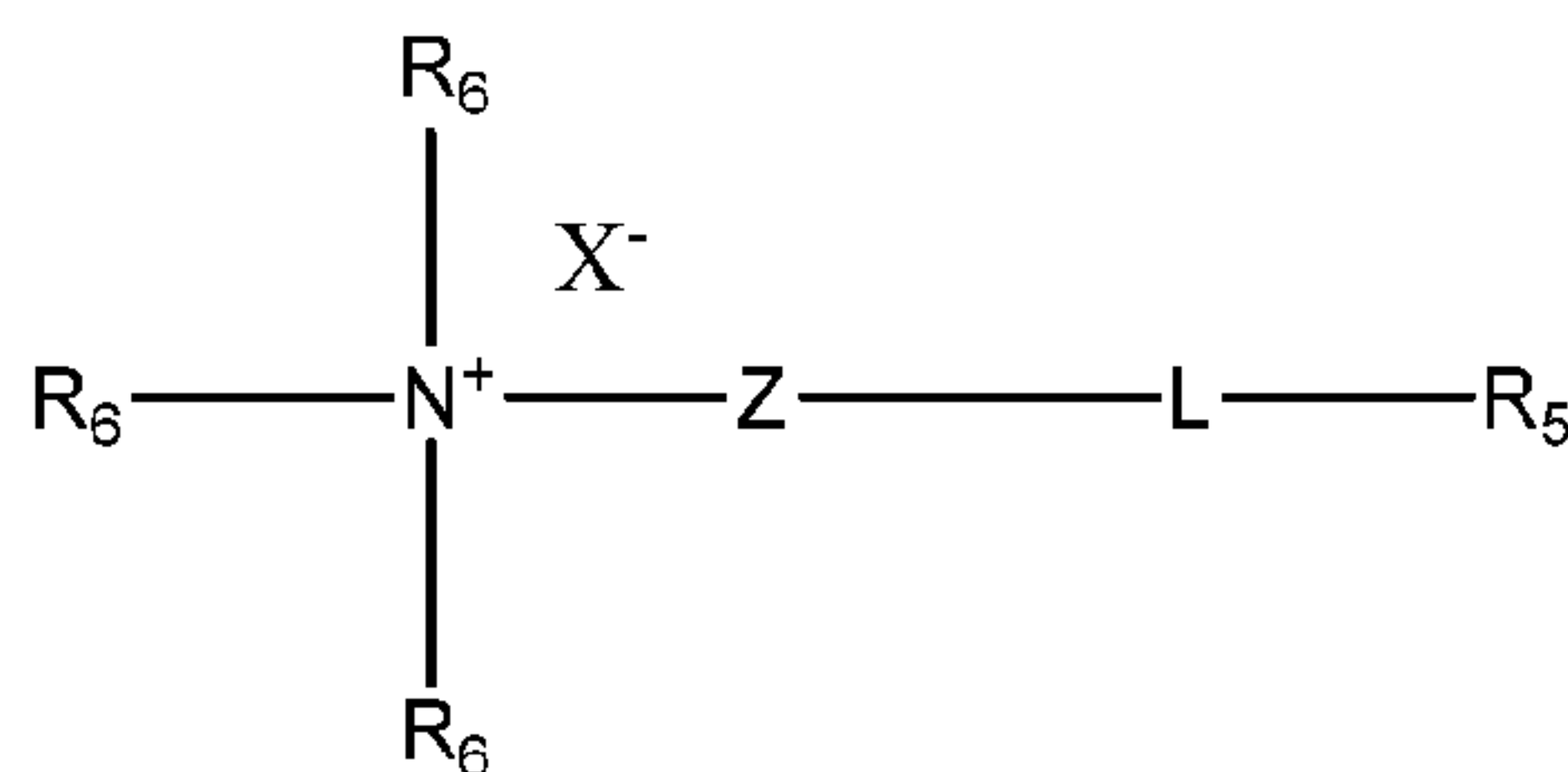
wherein:

- 25 (i) R_1 and R_2 are each independently a $C_5 - C_{23}$ hydrocarbon;
- (ii) R_3 and R_4 are each independently selected from the group consisting of $C_1 - C_4$ hydrocarbon, $C_1 - C_4$ hydroxy substituted hydrocarbon, benzyl, $-(C_2H_4O)_yH$ where y is an integer from 1 to 10;

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- (iii) L is selected from the group consisting of $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$;
- (iv) Z is selected from the group consisting of $-(\text{CH}_2)_n$, $-\text{CH}_2\text{C}(\text{CH}_3)\text{H}-$ where each n is independently an integer from 1 to 4
- (v) X^- is a softener-compatible anion;

5 b) materials having Formula (2) below

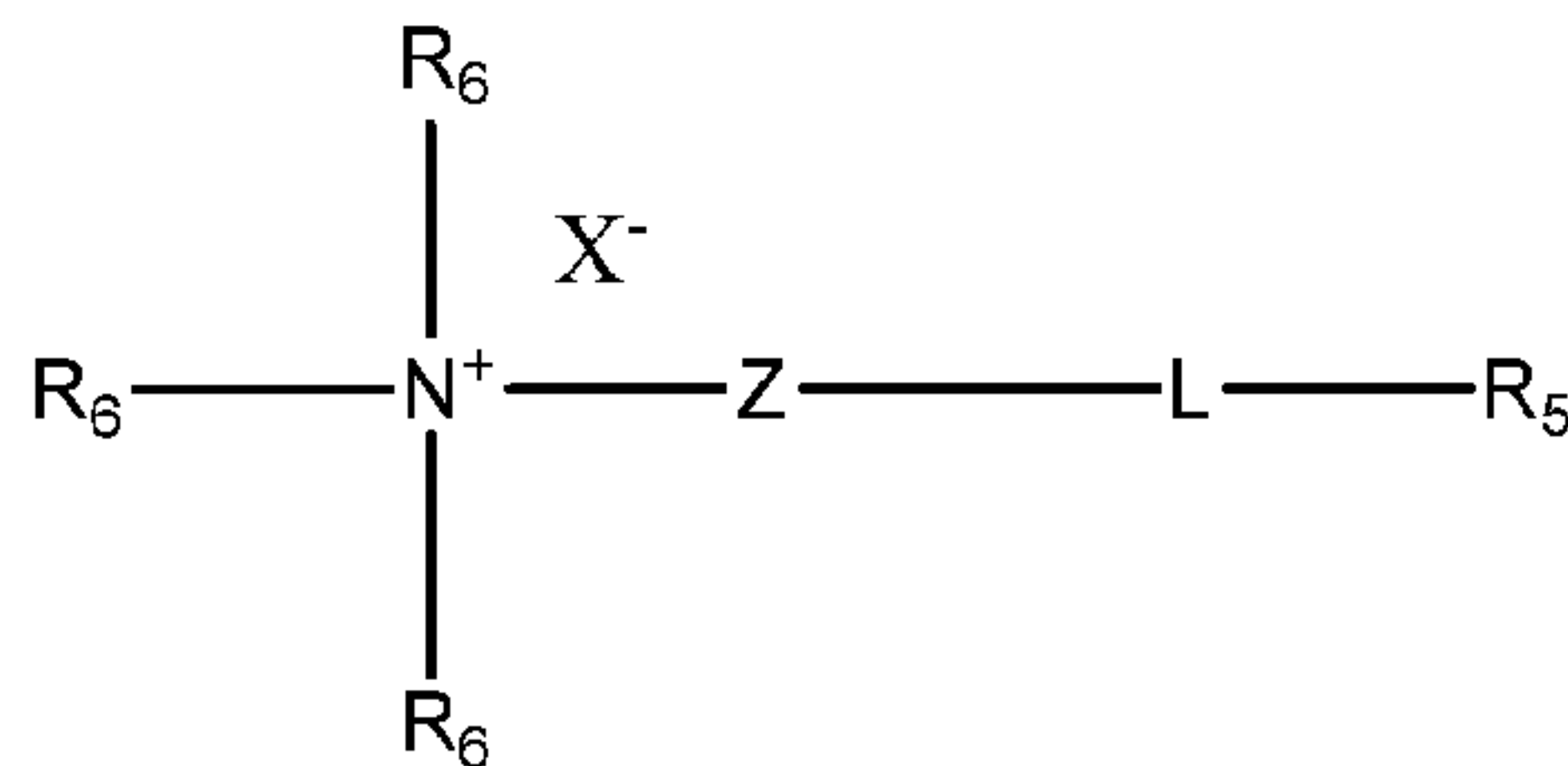


(Formula 2)

wherein

- 10 (i) R_5 is a $\text{C}_5 - \text{C}_{23}$ hydrocarbon;
- (ii) each R_6 is independently selected from the group consisting of $\text{C}_1 - \text{C}_4$ hydrocarbon, $\text{C}_1 - \text{C}_4$ hydroxy substituted hydrocarbon, benzyl, $-(\text{C}_2\text{H}_4\text{O})_y\text{H}$ where y is an integer from 1 to 10;
- (iii) L is selected from the group consisting of $-\text{C}(\text{O})\text{O}-$, $-\text{O}(\text{O})\text{C}-$,
- 15 (iv) Z is selected from the group consisting of $-(\text{CH}_2)_n$, $-\text{CH}_2\text{C}(\text{CH}_3)\text{H}-$ where each n is independently an integer from 1 to 4;
- (v) X^- is a softener-compatible anion;

c) materials having Formula (3) below



(Formula 3)

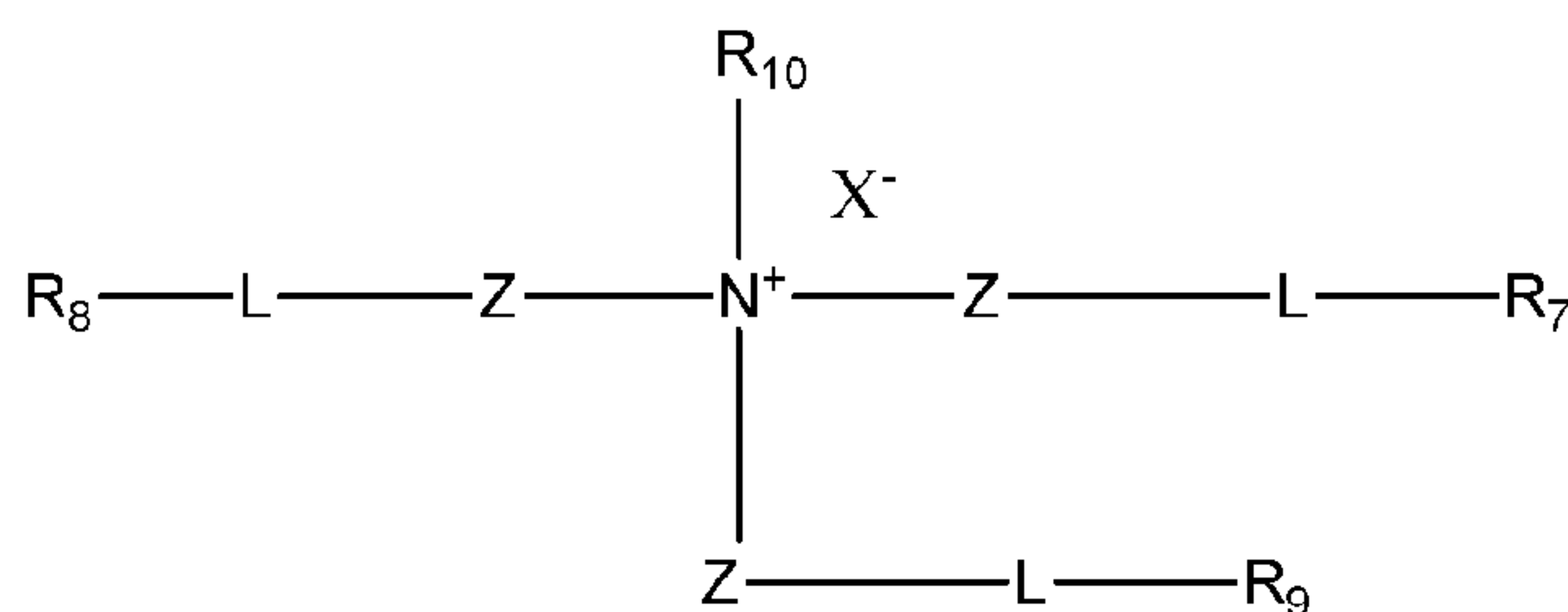
wherein

- (i) R_5 is a $\text{C}_5 - \text{C}_{23}$ hydrocarbon;

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- (ii) each R_6 is independently selected from the group consisting of C_1 - C_4 hydrocarbon, C_1 - C_4 hydroxy substituted hydrocarbon, benzyl, $-(C_2H_4O)_yH$ where y is an integer from 1 to 10;
- (iii) L is selected from the group consisting of $-C(O)O-$, $-O-(O)C-$,
- (iv) Z is selected from the group consisting of $-(CH_2)_n$, $-CH_2C(CH_3)H-$ where each n is independently an integer from 1 to 4;
- (v) X^- is an anionic surfactant comprising a C_6 - C_{24} hydrocarbon.

d) materials having Formula (4) below



(Formula 4)

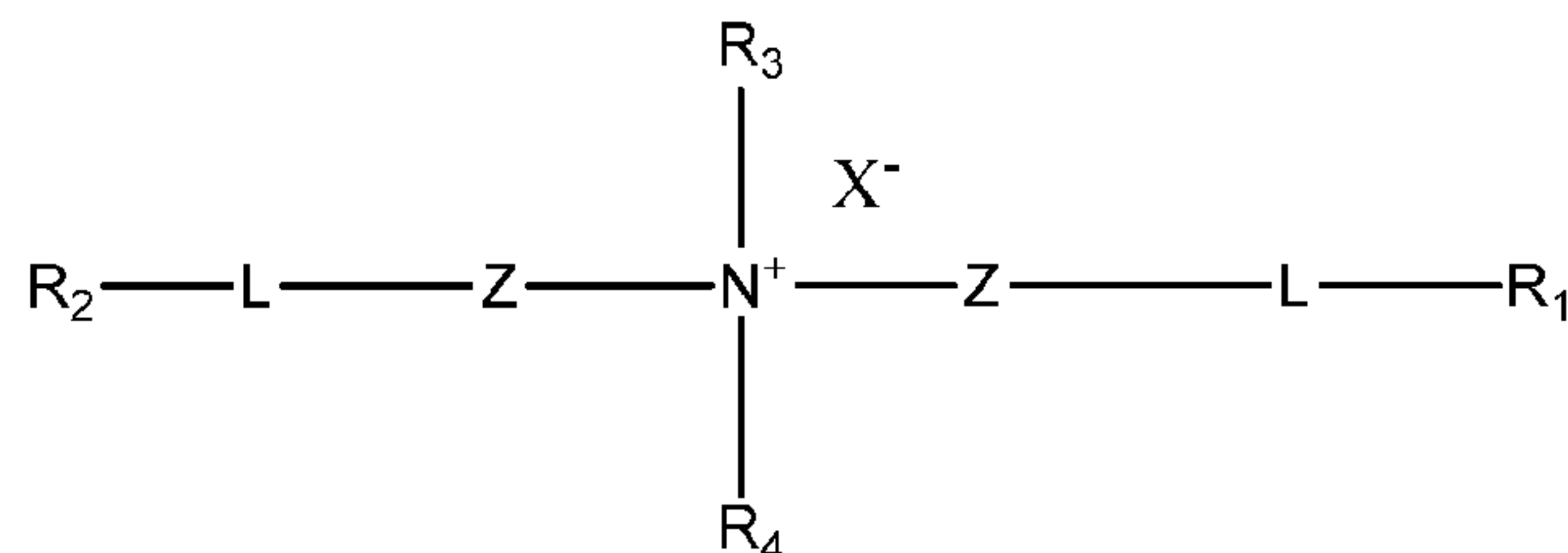
wherein:

- (i) R_7 , R_8 and R_9 are each independently a $C_5 - C_{23}$ hydrocarbon;
- (i) R_{10} is selected from the group consisting of C_1 - C_4 hydrocarbon, C_1 - C_4 hydroxy substituted hydrocarbon, benzyl, $-(C_2H_4O)_yH$ where y is an integer from 1 to 10;
- (ii) L is selected from the group consisting of $-C(O)O-$, $-O-(O)C-$;
- (iii) Z is selected from the group consisting of $-(CH_2)_n$, $-CH_2C(CH_3)H-$ where each n is independently an integer from 1 to 4;
- (iv) X^- is a softener-compatible anion;

In one aspect, said di-tail fabric softener active, mono-tail fabric softener active and ion pair fabric softener actives are selected from the group consisting of:

- a) materials having Formula (1) below

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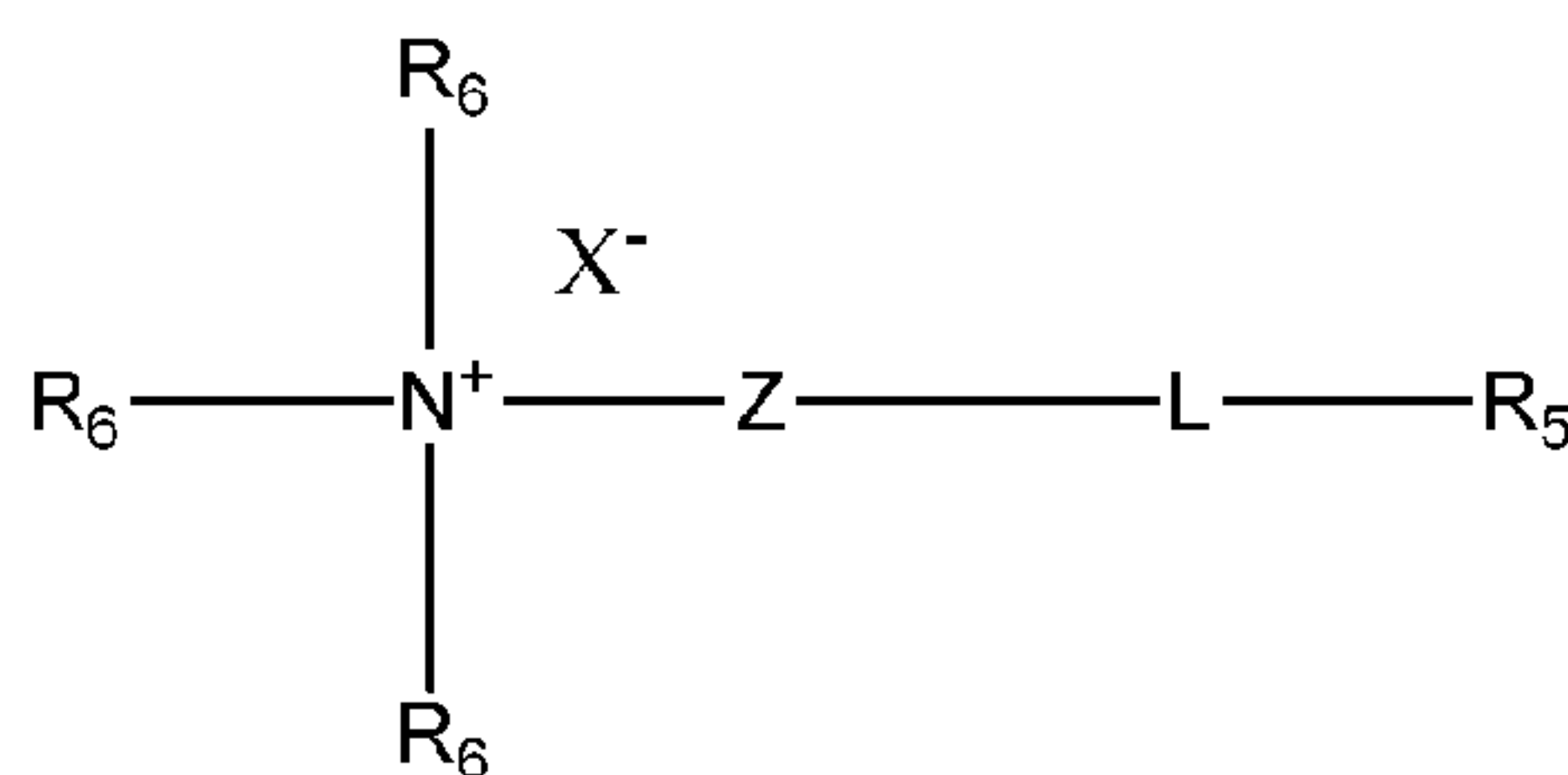


(Formula 1)

wherein:

- 5 (i) R_1 and R_2 are each independently a $C_{11} - C_{17}$ hydrocarbon;
- (ii) R_3 and R_4 are each independently selected from the group consisting of $C_1 - C_2$ hydrocarbon, $C_1 - C_2$ hydroxy substituted hydrocarbon;
- (iii) Z is selected from the group consisting of $-(CH_2)_n$, $-CH_2C(CH_3)H-$ where each n is independently an integer from 1 to 2;
- (iv) L is selected from the group consisting of $-C(O)O-$, $-O-(O)C-$;
- 10 (v) X^- is a softener-compatible anion, selected from the group consisting of halides, sulfonates, sulfates, and nitrates.

b) materials having Formula (2) below



(Formula 2)

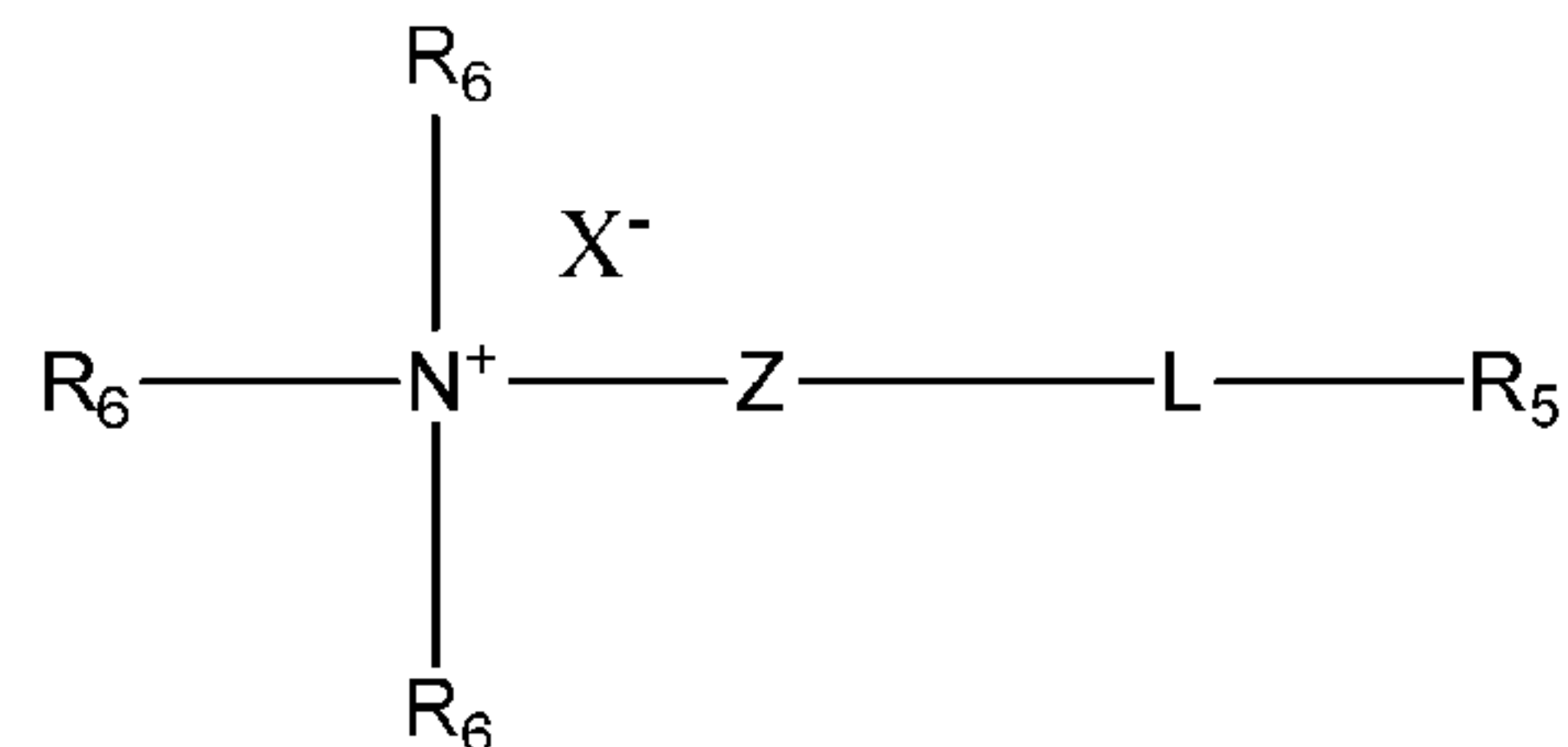
15 wherein

- (i) R_5 is a $C_{11} - C_{17}$ hydrocarbon;
- (ii) each R_6 is independently selected from the group consisting of $C_1 - C_2$ hydrocarbon, $C_1 - C_2$ hydroxy substituted hydrocarbon;
- (iii) Z is selected from the group consisting of $-(CH_2)_n$, $-CH_2C(CH_3)H-$ where each n is independently an integer from 1 to 4;
- 20 (iv) L is selected from the group consisting of $-C(O)O-$, $-O-(O)C-$;

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(v) X⁻ is a softener-compatible anion, selected from the group consisting of halides, sulfonates, sulfates, and nitrates;

c) materials having Formula (3) below



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(Formula 3)

wherein

(i) R₅ is a C₁₁ – C₁₇ hydrocarbon;

(ii) each R₆ is independently selected from the group consisting of C₁-C₄ hydrocarbon, C₁-C₄ hydroxy substituted hydrocarbon, benzyl, -(C₂H₄O)_yH where y is an integer from 1 to 10;

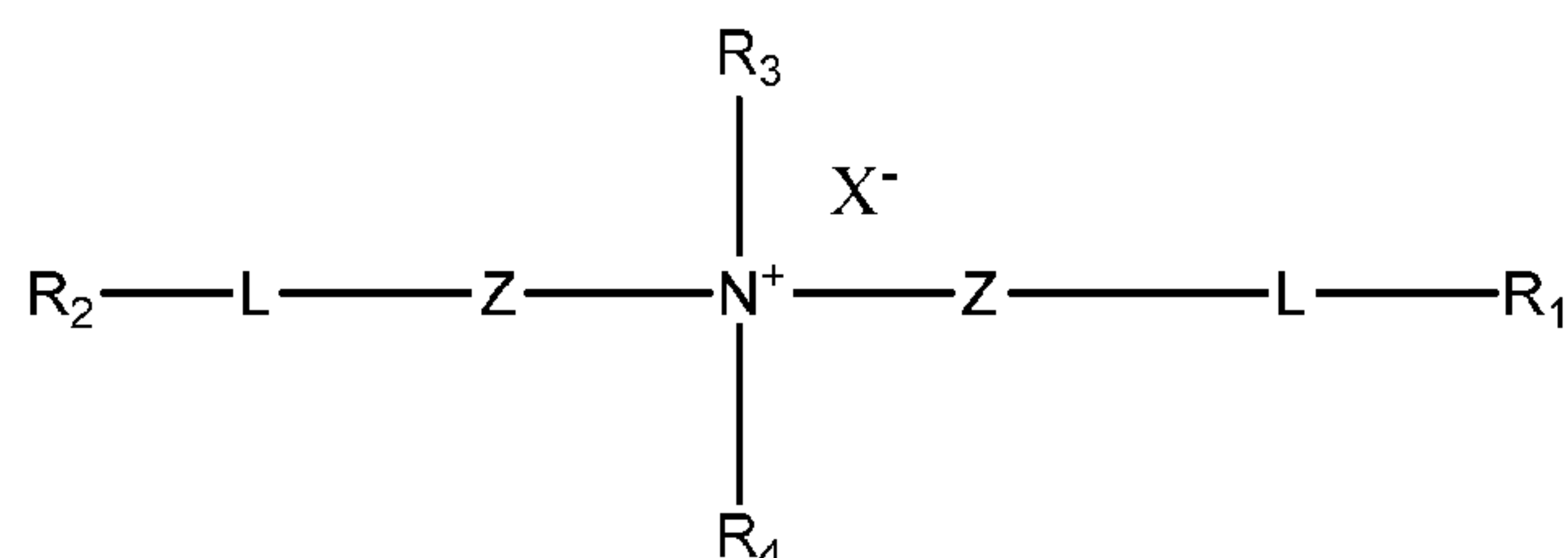
(iii) L is selected from the group consisting of -C(O)O-, -O-(O)C-;

(iv) Z is selected from the group consisting of -(CH₂)_n, -CH₂C(CH₃)H- where each n is independently an integer from 1 to 4;

(v) X⁻ is an anionic surfactant comprising a C₆-C₂₄ hydrocarbon.

In one aspect, said di-tail fabric softener active, mono-tail fabric softener active and ion pair fabric softener actives are selected from the group consisting of:

b) materials having Formula (1) below



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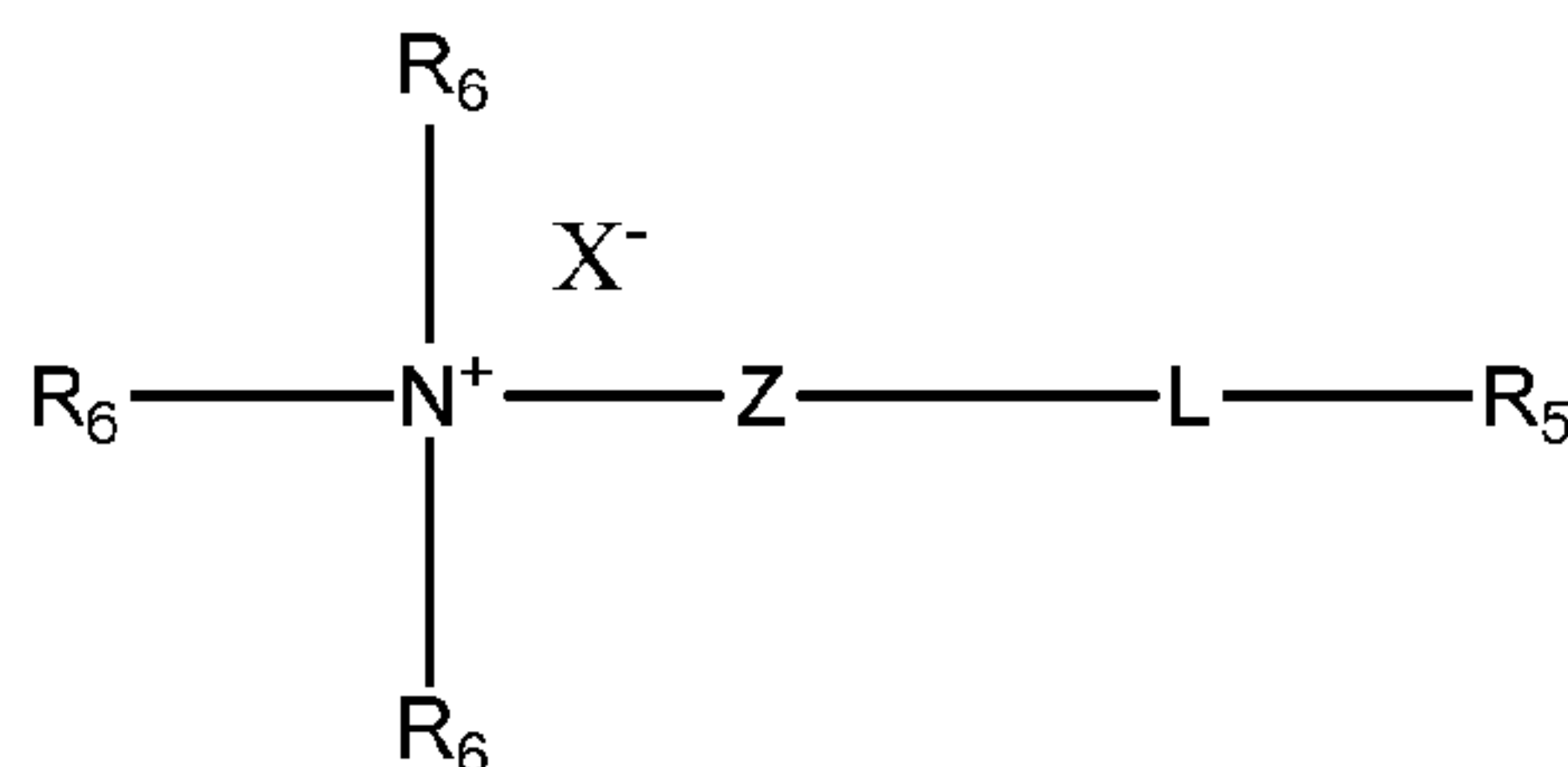
(Formula 1)

wherein:

(i) R₁ and R₂ are each independently a C₁₁ – C₁₇ hydrocarbon;

- (ii) R_3 and R_4 are each independently selected from the group consisting of C_1 - C_2 hydrocarbon, C_1 - C_2 hydroxy substituted hydrocarbon;
- (iii) Z is selected from the group consisting of $-(CH_2)_n$, $-CH_2C(CH_3)H$ - where each n is independently an integer from 1 to 2;
- 5 (iv) L is selected from the group consisting of $-C(O)O-$, $-O-(O)C-$;
- (v) X^- is a softener-compatible anion, selected from the group consisting of chloride, bromide, methylsulfate, ethylsulfate, and methyl sulfonate.

b) materials having Formula (2) below



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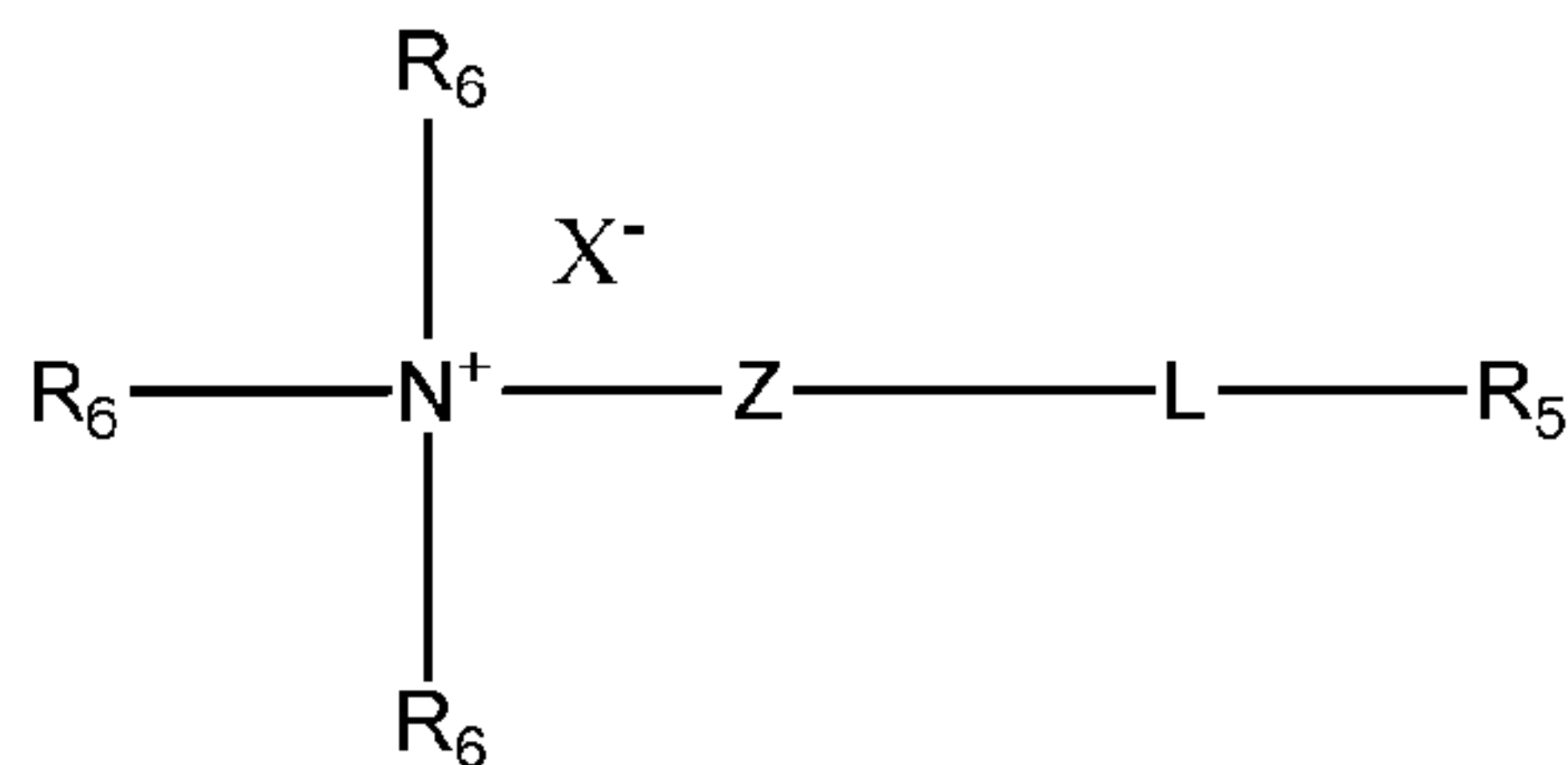
(Formula 2)

wherein

- (i) R_5 is a $C_{11} - C_{17}$ hydrocarbon;
- (ii) each R_6 is independently selected from the group consisting of C_1 - C_2 hydrocarbon, C_1 - C_2 hydroxy substituted hydrocarbon;
- 15 (iii) Z is selected from the group consisting of $-(CH_2)_n$, $-CH_2C(CH_3)H$ - where each n is independently an integer from 1 to 4;
- (iv) L is selected from the group consisting of $-C(O)O-$, $-O-(O)C-$;
- (v) X^- is a softener-compatible anion, selected from the group consisting of chloride, bromide, methylsulfate, ethylsulfate, and methyl sulfonate or anionic surfactant comprising a C_6 - C_{18} hydrocarbon
- 20

c) materials having Formula (3) below

12



(Formula 3)

wherein

- (i) R_5 is a $\text{C}_{11} - \text{C}_{17}$ hydrocarbon;
- 5 (ii) each R_6 is independently selected from the group consisting of $\text{C}_1 - \text{C}_2$ hydrocarbon, $\text{C}_1 - \text{C}_2$ hydroxy substituted hydrocarbon;
- (iii) Z is selected from the group consisting of $-(\text{CH}_2)_n-$, $-\text{CH}_2\text{C}(\text{CH}_3)\text{H}-$ where each n is independently an integer from 1 to 4;
- (iv) L is selected from the group consisting of $-\text{C}(\text{O})\text{O}-$, $-\text{O}(\text{O})\text{C}-$;
- 10 (v) X^- is a softener-compatible anion, selected from the group consisting of chloride, bromide, methylsulfate, ethylsulfate, and methyl sulfonate or anionic surfactant comprising a $\text{C}_6 - \text{C}_{18}$ hydrocarbon.

In one aspect, for Formula 2, X^- is a $\text{C}_6 - \text{C}_{24}$ hydrocarbon that is an anionic surfactant.

- 15 In one aspect, said fabric care active comprises a fabric softening active selected from the group consisting of N,N-di(hydrogenated tallowoyloxyethyl)-N,N-dimethylammonium chloride; N,N-di(tallowoyloxyethyl)-N,N-dimethylammonium chloride; N,N-di(hydrogenated tallowoyloxyisopropyl)-N,N-dimethylammonium chloride; N,N-di(tallowoyloxyisopropyl)-N,N-dimethylammonium chloride; N,N-di(stearoyloxyisopropyl)-N,N-dimethylammonium chloride;
- 20 N,N-di(palmoyloxyisopropyl)-N,N-dimethylammonium chloride; bis-(2-hydroxypropyl)-dimethylammonium chloride stearic acid diester; partially hydrogenated bis-(2-hydroxypropyl)-dimethylammonium chloride palmitic acid diester; and mixtures thereof.

In the cationic nitrogenous salts herein, the anion A^- , which is any softener compatible anion, provides electrical neutrality. Most often, the anion used to provide electrical neutrality in

25 these salts is from a strong acid, especially a halide, such as chloride, bromide, or iodide.

However, other anions can be used, such as methylsulfate, ethylsulfate, acetate, formate, sulfate, carbonate, and the like. Chloride and methylsulfate are preferred herein as anion A. The anion can also, but less preferably, carry a double charge in which case A^- represents half a group.

It was surprisingly found that deposition of the lipid esterase onto the fabric was improved wherein the fabric had been previously been treated with a cationically charged fabric softening active.

Step (iii)

The present invention comprises a step (iii) of contacting the fabric from step (ii) with a laundry detergent composition, wherein the laundry detergent composition comprises an anionic
10 detersive surfactant, wherein the anionic detersive surfactant is present at the ratio of anionic surfactant to fabric on a weight to weight basis of from 1:150 to 1:500.

The composition may be in any suitable form including granular, liquid or unitized dose. When in unitized dose form, it is preferred that the composition is enclosed with a water-soluble
15 film, for example a polyvinyl alcohol-based film.

The fabric may be contacted with the composition in step (iii) in the form of a wash liquor, or even a wash liquor in a machine wash cycle. Alternatively, the fabric may be contacted with the composition in the form of a wash pre-treat composition. In this aspect, the pre-treat composition is added to a portion or all of the fabric at some point before it is contacted
20 with a wash liquor. Alternatively, the pre-treat composition may be added to a specific stain on the fabric at some point before the fabric is contacted with a wash liquor.

The fabric may be contacted with the composition in step (iii) at a temperature of 60°C or less, or even 40°C or less. The fabric may be contacted with the composition at a temperature of between 5°C and 50°C, preferably between 10°C and 30°C. The fabric may be contacted at these
25 temperatures in the wash cycle of a domestic washing machine.

The fabric may be contacted with a laundry detergent composition in step (iii) in a wash cycle of an automatic washing machine and the length of the wash cycle may be at least 30 seconds, or even at least 3 mins, or even at least 6 mins, but no more than 30 mins, or even no more than 45 mins, or even no more than 1 hour.

30 The laundry detergent composition comprises an anionic detersive surfactant. Suitable anionic detersive surfactants include linear alkyl benzene sulfonate, alkoxyated anionic

surfactant, or a combination thereof. Suitable anionic deterative surfactants include sulphate and sulphonate deterative surfactants.

Suitable sulphonate deterative surfactants include alkyl benzene sulphonate, such as C₁₀₋₁₃ alkyl benzene sulphonate. Suitable alkyl benzene sulphonate (LAS) is obtainable, or even
5 obtained, by sulphonating commercially available linear alkyl benzene (LAB); suitable LAB includes low 2-phenyl LAB, such as those supplied by Sasol under the tradename Isochem® or those supplied by Petresa under the tradename Petrelab®, other suitable LAB include high 2-phenyl LAB, such as those supplied by Sasol under the tradename Hyblene®. Another suitable anionic deterative surfactant is alkyl benzene sulphonate that is obtained by DETAL catalyzed
10 process, although other synthesis routes, such as HF, may also be suitable.

Suitable sulphate deterative surfactants include alkyl sulphate, such as C₈₋₁₈ alkyl sulphate, or predominantly C₁₂ alkyl sulphate. The alkyl sulphate may be derived from natural sources, such as coco and/or tallow. Alternative, the alkyl sulphate may be derived from synthetic sources such as C₁₂₋₁₅ alkyl sulphate.

15 Another suitable sulphate deterative surfactant is alkyl alkoxyated sulphate, such as alkyl ethoxyated sulphate, or a C₈₋₁₈ alkyl alkoxyated sulphate, or a C₈₋₁₈ alkyl ethoxyated sulphate. The alkyl alkoxyated sulphate may have an average degree of alkoxylation of from 0.5 to 20, or from 0.5 to 10. The alkyl alkoxyated sulphate may be a C₈₋₁₈ alkyl ethoxyated sulphate, typically having an average degree of ethoxylation of from 0.5 to 10, or from 0.5 to 7, or from
20 0.5 to 5 or from 0.5 to 3.

The alkyl sulphate, alkyl alkoxyated sulphate and alkyl benzene sulphonates may be linear or branched, substituted or un-substituted.

The anionic deterative surfactant may be a mid-chain branched anionic deterative surfactant, such as a mid-chain branched alkyl sulphate and/or a mid-chain branched alkyl benzene
25 sulphonate. The mid-chain branches are typically C₁₋₄ alkyl groups, such as methyl and/or ethyl groups.

Another suitable anionic deterative surfactant is alkyl ethoxy carboxylate.

The anionic deterative surfactants are typically present in their salt form, typically being complexed with a suitable cation. Suitable counter-ions include Na⁺ and K⁺, substituted
30 ammonium such as C₁-C₆ alkanolammonium such as mono-ethanolamine (MEA) tri-ethanolamine (TEA), di-ethanolamine (DEA), and any mixture thereof.

The deterative surfactant may comprise linear alkylbenzene sulfonate and a co-surfactant, wherein, the co-surfactant is selected from a non-ionic surfactant, an alkoxyated anionic

surfactant, or a combination thereof. Suitable alkoxyated anionic surfactants are described above. Suitable non-ionic deterative surfactants are selected from the group consisting of: C₈-C₁₈ alkyl ethoxylates, such as, NEODOL® non-ionic surfactants from Shell; C₆-C₁₂ alkyl phenol alkoxyates wherein optionally the alkoxyate units are ethyleneoxy units, propyleneoxy units or a mixture thereof; C₁₂-C₁₈ alcohol and C₆-C₁₂ alkyl phenol condensates with ethylene oxide/propylene oxide block polymers such as Pluronic® from BASF; C₁₄-C₂₂ mid-chain branched alcohols; C₁₄-C₂₂ mid-chain branched alkyl alkoxyates, typically having an average degree of alkoxylation of from 1 to 30; alkylpolysaccharides, such as alkylpolyglycosides; polyhydroxy fatty acid amides; ether capped poly(oxyalkylated) alcohol surfactants; and mixtures thereof.

The anionic deterative surfactant is present at the ratio of anionic surfactant to fabric on a weight to weight basis of from 1:150 to 1:500, or even from 1:200 to 1:500, or even from 1:300 to 1:500.

The laundry detergent composition in step (iii) may also comprise a lipid esterase. Suitable lipid esterases are as detailed above.

Other ingredients

The laundry detergent composition of step (iii) may comprise further laundry detergent ingredients. The laundry detergent composition of step (iii) may comprise a hueing agent, a polymer or a combination thereof. Suitable detergent ingredients include: hueing agent; deterative surfactants including anionic deterative surfactants, non-ionic deterative surfactants, cationic deterative surfactants, zwitterionic deterative surfactants, amphoteric deterative surfactants, and any combination thereof; polymers including carboxylate polymers, polyethylene glycol polymers, polyester soil release polymers such as terephthalate polymers, amine polymers, cellulosic polymers, dye transfer inhibition polymers, dye lock polymers such as a condensation oligomer produced by condensation of imidazole and epichlorhydrin, optionally in ratio of 1:4:1, hexamethylenediamine derivative polymers, and any combination thereof; builders including zeolites, phosphates, citrate, and any combination thereof; buffers and alkalinity sources including carbonate salts and/or silicate salts; fillers including sulphate salts and bio-filler materials; bleach including bleach activators, sources of available oxygen, pre-formed peracids, bleach catalysts, reducing bleach, and any combination thereof; chelants; photobleach; hueing agents; brighteners; enzymes including proteases, amylases, cellulases, lipases, xylogucanases, pectate lyases, mannanases, bleaching enzymes, cutinases, and any

combination thereof; fabric softeners including clay, silicones, quaternary ammonium fabric-softening agents, and any combination thereof; flocculants such as polyethylene oxide; perfume including starch encapsulated perfume accords, perfume microcapsules, perfume loaded zeolites, schif base reaction products of ketone perfume raw materials and polyamines, blooming
5 perfumes, and any combination thereof; aesthetics including soap rings, lamellar aesthetic particles, gelatin beads, carbonate and/or sulphate salt speckles, coloured clay, and any combination thereof: and any combination thereof.

Fabric Hueing Agents - The composition may comprise a fabric hueing agent (sometimes referred to as shading, bluing or whitening agents). Typically the hueing agent
10 provides a blue or violet shade to fabric. Hueing agents can be used either alone or in combination to create a specific shade of hueing and/or to shade different fabric types. This may be provided for example by mixing a red and green-blue dye to yield a blue or violet shade. Hueing agents may be selected from any known chemical class of dye, including but not limited to acridine, anthraquinone (including polycyclic quinones), azine, azo (e.g., monoazo, disazo,
15 trisazo, tetrakisazo, polyazo), including premetallized azo, benzodifurane and benzodifuranone, carotenoid, coumarin, cyanine, diazahemicyanine, diphenylmethane, formazan, hemicyanine, indigoids, methane, naphthalimides, naphthoquinone, nitro and nitroso, oxazine, phthalocyanine, pyrazoles, stilbene, styryl, triarylmethane, triphenylmethane, xanthenes and mixtures thereof. Suitable fabric hueing agents include dyes, dye-clay conjugates, and organic and inorganic
20 pigments. Suitable dyes include small molecule dyes and polymeric dyes. Suitable small molecule dyes include small molecule dyes selected from the group consisting of dyes falling into the Colour Index (C.I.) classifications of Acid, Direct, Basic, Reactive or hydrolysed Reactive, Solvent or Disperse dyes for example that are classified as Blue, Violet, Red, Green or Black, and provide the desired shade either alone or in combination. In another aspect, suitable
25 small molecule dyes include small molecule dyes selected from the group consisting of Colour Index (Society of Dyers and Colourists, Bradford, UK) numbers Direct Violet dyes such as 9, 35, 48, 51, 66, and 99, Direct Blue dyes such as 1, 71, 80 and 279, Acid Red dyes such as 17, 73, 52, 88 and 150, Acid Violet dyes such as 15, 17, 24, 43, 49 and 50, Acid Blue dyes such as 15, 17, 25, 29, 40, 45, 75, 80, 83, 90 and 113, Acid Black dyes such as 1, Basic Violet dyes such as 1, 3,
30 4, 10 and 35, Basic Blue dyes such as 3, 16, 22, 47, 66, 75 and 159, Disperse or Solvent dyes such as those described in US 2008/034511 A1 or US 8,268,016 B2, or dyes as disclosed in US 7,208,459 B2, and mixtures thereof. In another aspect, suitable small molecule dyes include small molecule dyes selected from the group consisting of C. I. numbers Acid Violet 17, Direct

Blue 71, Direct Violet 51, Direct Blue 1, Acid Red 88, Acid Red 150, Acid Blue 29, Acid Blue 113 or mixtures thereof.

Preferred dyes include dye polymers, wherein a dye group is bound to a polymeric group, optionally via a linking group. Suitable polymeric groups include (1) alkoxyated
 5 polyethyleneimine (for example as disclosed in WO2012119859), (2) polyvinyl alcohol (for example as disclosed in WO2012130492), or (3) diamine derivative of an alkylene oxide capped polyethylene glycol (for example as disclosed in WO2012126665, especially figure 24), or polyalkoxyated alcohol, for example as described in WO2011/011799, WO2012/054058, WO2012/166699 or WO2012/166768. One preferred class of dye polymers is obtainable by
 10 reacting a blue or violet dye containing an NH₂ group with a polymer to form a covalent bond via the reacted NH₂ group of the blue or violet dye and the dye polymer has an average of from 0 to 30, preferably 2 to 20, most preferably 2 to 15 repeating same units. In a preferred embodiment the monomeric units are selected from alkylene oxides, preferably ethylene oxides. Typically dye polymers will be in the form of a mixture of dye polymers in which there is a
 15 mixture of molecules having a distribution of number of monomer groups in the polymer chains, such as the mixture directly produced by the appropriate organic synthesis route, for example in the case of alkylene oxide polymers, the result of an alkoxylation reaction. Such dye polymers are typically blue or violet in colour, to give to the cloth a hue angle of 230 to 345, more preferably 250 to 330, most preferably 270 to 300. In the synthesis of dye polymers unbound
 20 blue or violet organic dyes may be present in a mixture with the final dye-polymer product. The chromophore of the blue or violet dye is preferably selected from the group consisting of: azo; anthraquinone; phthalocyanine; triphendioxazine; and, triphenylmethane. In one aspect the dye polymer is obtainable by reacting a dye containing an NH₂ group with a polymer or suitable monomer that forms a polymer in situ. Preferably the NH₂ is covalently bound to an aromatic
 25 ring of the dye. Unbound dye is formed when the dye does not react with polymer. Preferred dyes containing -NH₂ groups for such reactions are selected from: acid violet 1 ; acid violet 3; acid violet 6; acid violet 11 ; acid violet 13; acid violet 14; acid violet 19; acid violet 20; acid violet 36; acid violet 36:1 ; acid violet 41 ; acid violet 42; acid violet 43; acid violet 50; acid violet 51 ; acid violet 63; acid violet 48; acid blue 25; acid blue 40; acid blue 40:1; acid blue 41 ;
 30 acid blue 45; acid blue 47; acid blue 49; acid blue 51 ; acid blue 53; acid blue 56; acid blue 61 ; acid blue 61 :1 ; acid blue 62; acid blue 69; acid blue 78; acid blue 81 :1 ; acid blue 92; acid blue 96; acid blue 108; acid blue 111 ; acid blue 215; acid blue 230; acid blue 277; acid blue 344; acid blue 117; acid blue 124; acid blue 129; acid blue 129:1 ; acid blue 138; acid blue 145;

direct violet 99; direct violet 5; direct violet 72; direct violet 16; direct violet 78; direct violet 77;
direct violet 83; food black 2; direct blue 33; direct blue 41 ; direct blue 22; direct blue 71 ;
direct blue 72; direct blue 74; direct blue 75; direct blue 82; direct blue 96; direct blue 1 10;
direct blue 1 1 1 ; direct blue 120; direct blue 120:1 ; direct blue 121 ; direct blue 122; direct blue
5 123; direct blue 124; direct blue 126; direct blue 127; direct blue 128; direct blue 129; direct blue
130; direct blue 132; direct blue 133; direct blue 135; direct blue 138; direct blue 140; direct blue
145; direct blue 148; direct blue 149; direct blue 159; direct blue 162; direct blue 163; food black
2; food black 1 wherein the acid amide group is replaced by NH[2]; Basic Violet 2; Basic Violet
5; Basic Violet 12; Basic Violet 14; Basic Violet 8; Basic Blue 12; Basic Blue 16; Basic Blue 17;
10 Basic Blue 47; Basic Blue 99; disperse blue 1 ; disperse blue 5; disperse blue 6; disperse blue 9;
disperse blue 1 1 ; disperse blue 19; disperse blue 20; disperse blue 28; disperse blue 40; disperse
blue 56; disperse blue 60; disperse blue 81 ; disperse blue 83; disperse blue 87; disperse blue
104; disperse blue 1 18; disperse violet 1 ; disperse violet 4, disperse violet 8, disperse violet 17,
disperse violet 26; disperse violet 28; solvent violet 26; solvent blue 12; solvent blue 13; solvent
15 blue 18; solvent blue 68. Further preferred dyes are selected from mono-azo dyes which contain
a phenyl group directly attached to the azo group, wherein the phenyl group has an NH[2] groups
covalent bound to it. For example a mono-azo thiophene dye. The polymer chain may be
selected from polyalkylene oxides. The polymer chain and/or the dye chromophore group may
optionally carry anionic or cationic groups. Examples of polyoxyalkylene oxide chains include
20 ethylene oxide, propylene oxide, glycidol oxide, butylene oxide and mixtures thereof.

Suitable polymeric dyes include polymeric dyes selected from the group consisting of
polymers containing covalently bound (sometimes referred to as conjugated) chromogens, (dye-
polymer conjugates), for example polymers with chromogens co-polymerized into the backbone
of the polymer and mixtures thereof. Polymeric dyes include those described in WO2011/98355,
25 US 2012/225803 A1, US 2012/090102 A1, US 7,686,892 B2, and WO2010/142503.

In another aspect, suitable polymeric dyes include polymeric dyes selected from the
group consisting of fabric-substantive colorants sold under the name of Liquitint® (Milliken,
Spartanburg, South Carolina, USA), dye-polymer conjugates formed from at least one reactive
dye and a polymer selected from the group consisting of polymers comprising a moiety selected
30 from the group consisting of a hydroxyl moiety, a primary amine moiety, a secondary amine
moiety, a thiol moiety and mixtures thereof. In still another aspect, suitable polymeric dyes
include polymeric dyes selected from the group consisting of Liquitint® Violet CT,
carboxymethyl cellulose (CMC) covalently bound to a reactive blue, reactive violet or reactive

red dye such as CMC conjugated with C.I. Reactive Blue 19, sold by Megazyme, Wicklow, Ireland under the product name AZO-CM-CELLULOSE, product code S-ACMC, alkoxylated triphenyl-methane polymeric colourants, alkoxylated thiophene polymeric colourants, and mixtures thereof.

5 Preferred hueing dyes include the whitening agents found in WO 08/87497 A1, WO2011/011799 and US 2012/129752 A1. Preferred hueing agents for use in the present invention may be the preferred dyes disclosed in these references, including those selected from Examples 1-42 in Table 5 of WO2011/011799. Other preferred dyes are disclosed in US 8,138,222B2, especially claim 1 of US 8,138,222B2. Other preferred dyes are disclosed in US
10 7,909,890 B2.

Suitable dye clay conjugates include dye clay conjugates selected from the group comprising at least one cationic/basic dye and a smectite clay, and mixtures thereof. In another aspect, suitable dye clay conjugates include dye clay conjugates selected from the group consisting of one cationic/basic dye selected from the group consisting of C.I. Basic Yellow 1
15 through 108, C.I. Basic Orange 1 through 69, C.I. Basic Red 1 through 118, C.I. Basic Violet 1 through 51, C.I. Basic Blue 1 through 164, C.I. Basic Green 1 through 14, C.I. Basic Brown 1 through 23, CI Basic Black 1 through 11, and a clay selected from the group consisting of Montmorillonite clay, Hectorite clay, Saponite clay and mixtures thereof. In still another aspect, suitable dye clay conjugates include dye clay conjugates selected from the group consisting of:
20 Montmorillonite Basic Blue B7 C.I. 42595 conjugate, Montmorillonite Basic Blue B9 C.I. 52015 conjugate, Montmorillonite Basic Violet V3 C.I. 42555 conjugate, Montmorillonite Basic Green G1 C.I. 42040 conjugate, Montmorillonite Basic Red R1 C.I. 45160 conjugate, Montmorillonite C.I. Basic Black 2 conjugate, Hectorite Basic Blue B7 C.I. 42595 conjugate, Hectorite Basic Blue B9 C.I. 52015 conjugate, Hectorite Basic Violet V3 C.I. 42555 conjugate, Hectorite Basic
25 Green G1 C.I. 42040 conjugate, Hectorite Basic Red R1 C.I. 45160 conjugate, Hectorite C.I. Basic Black 2 conjugate, Saponite Basic Blue B7 C.I. 42595 conjugate, Saponite Basic Blue B9 C.I. 52015 conjugate, Saponite Basic Violet V3 C.I. 42555 conjugate, Saponite Basic Green G1 C.I. 42040 conjugate, Saponite Basic Red R1 C.I. 45160 conjugate, Saponite C.I. Basic Black 2 conjugate and mixtures thereof.

30 Suitable pigments include pigments selected from the group consisting of flavanthrone, indanthrone, chlorinated indanthrone containing from 1 to 4 chlorine atoms, pyranthrene, dichloropyranthrene, monobromodichloropyranthrene, dibromodichloropyranthrene, tetrabromopyranthrene, perylene-3,4,9,10-tetracarboxylic acid diimide, wherein the imide groups

may be unsubstituted or substituted by C1-C3 -alkyl or a phenyl or heterocyclic radical, and wherein the phenyl and heterocyclic radicals may additionally carry substituents which do not confer solubility in water, anthrapyrimidinecarboxylic acid amides, violanthrone, isoviolanthrone, dioxazine pigments, copper phthalocyanine which may contain up to 2 chlorine atoms per molecule, polychloro-copper phthalocyanine or polybromochloro-copper phthalocyanine containing up to 14 bromine atoms per molecule and mixtures thereof.

In another aspect, suitable pigments include pigments selected from the group consisting of Ultramarine Blue (C.I. Pigment Blue 29), Ultramarine Violet (C.I. Pigment Violet 15) and mixtures thereof.

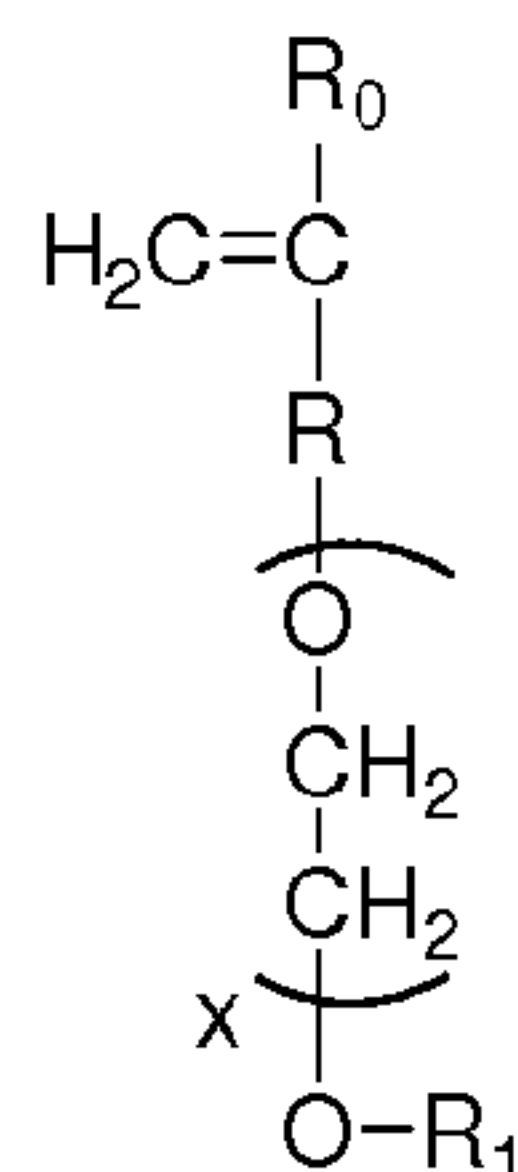
The aforementioned fabric hueing agents can be used in combination (any mixture of fabric hueing agents can be used).

Polymer: Suitable polymers include carboxylate polymers, polyethylene glycol polymers, polyester soil release polymers such as terephthalate polymers, amine polymers, cellulosic polymers, dye transfer inhibition polymers, dye lock polymers such as a condensation oligomer produced by condensation of imidazole and epichlorhydrin, optionally in ratio of 1:4:1, hexamethylenediamine derivative polymers, and any combination thereof.

Carboxylate polymer: Suitable carboxylate polymers include maleate/acrylate random copolymer or polyacrylate homopolymer. The carboxylate polymer may be a polyacrylate homopolymer having a molecular weight of from 4,000 Da to 9,000 Da, or from 6,000 Da to 9,000 Da. Other suitable carboxylate polymers are co-polymers of maleic acid and acrylic acid, and may have a molecular weight in the range of from 4,000 Da to 90,000 Da.

Other suitable carboxylate polymers are co-polymers comprising: (i) from 50 to less than 98 wt% structural units derived from one or more monomers comprising carboxyl groups; (ii) from 1 to less than 49 wt% structural units derived from one or more monomers comprising sulfonate moieties; and (iii) from 1 to 49 wt% structural units derived from one or more types of monomers selected from ether bond-containing monomers represented by formulas (I) and (II):

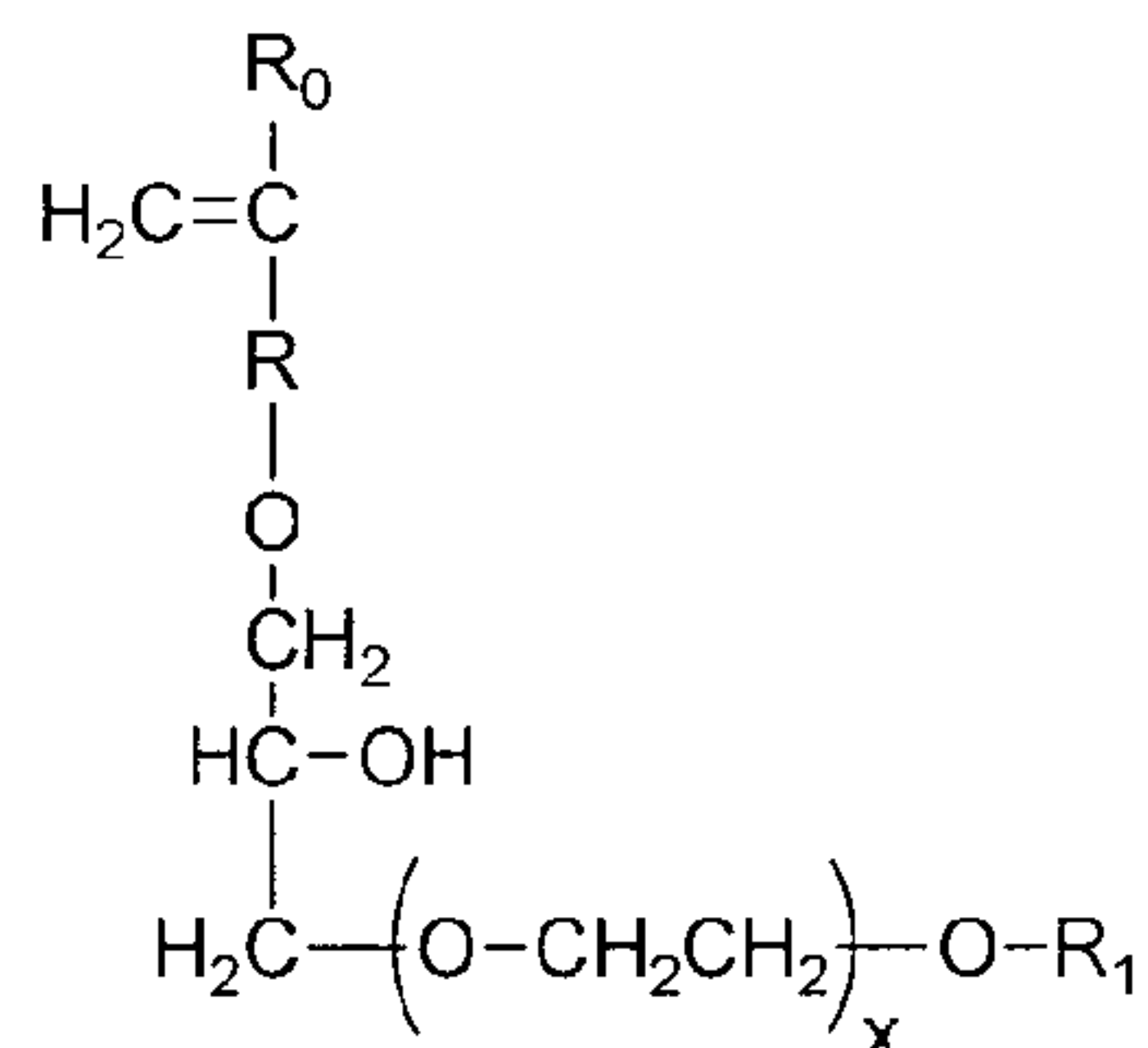
formula (I):



wherein in formula (I), R_0 represents a hydrogen atom or CH_3 group, R represents a CH_2 group, CH_2CH_2 group or single bond, X represents a number 0-5 provided X represents a number 1-5 when R is a single bond, and R_1 is a hydrogen atom or C_1 to C_{20} organic group;

formula (II)

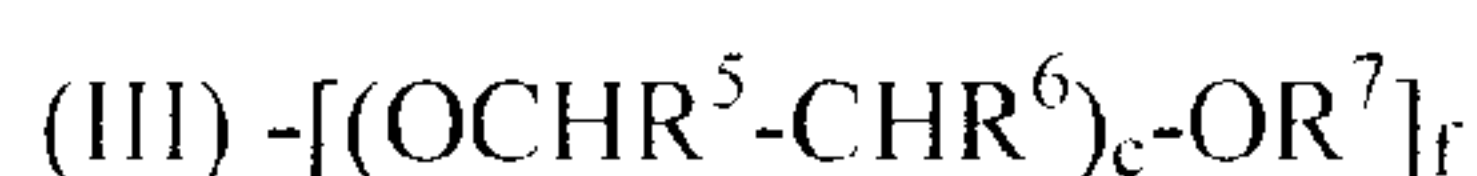
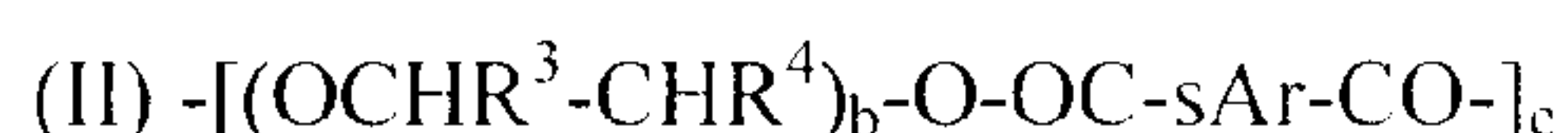
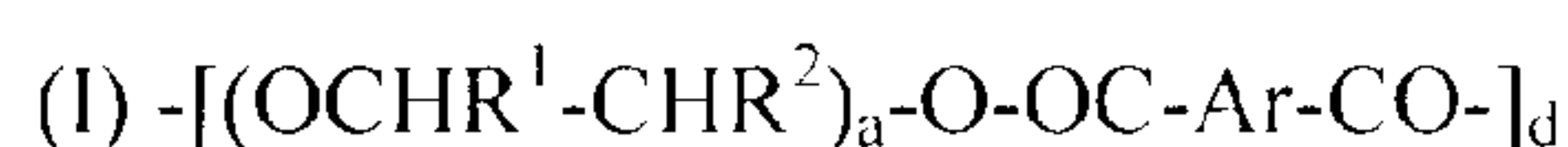
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in formula (II), R_0 represents a hydrogen atom or CH_3 group, R represents a CH_2 group, CH_2CH_2 group or single bond, X represents a number 0-5, and R_1 is a hydrogen atom or C_1 to C_{20} organic group.

10 **Polyethylene glycol polymer:** Suitable polyethylene glycol polymers include random graft co-polymers comprising: (i) hydrophilic backbone comprising polyethylene glycol; and (ii) hydrophobic side chain(s) selected from the group consisting of: C_4 - C_{25} alkyl group, polypropylene, polybutylene, vinyl ester of a saturated C_1 - C_6 mono-carboxylic acid, C_1 - C_6 alkyl ester of acrylic or methacrylic acid, and mixtures thereof. Suitable polyethylene glycol polymers
 15 have a polyethylene glycol backbone with random grafted polyvinyl acetate side chains. The average molecular weight of the polyethylene glycol backbone can be in the range of from 2,000 Da to 20,000 Da, or from 4,000 Da to 8,000 Da. The molecular weight ratio of the polyethylene glycol backbone to the polyvinyl acetate side chains can be in the range of from 1:1 to 1:5, or from 1:1.2 to 1:2. The average number of graft sites per ethylene oxide units can be less than 1,
 20 or less than 0.8, the average number of graft sites per ethylene oxide units can be in the range of from 0.5 to 0.9, or the average number of graft sites per ethylene oxide units can be in the range of from 0.1 to 0.5, or from 0.2 to 0.4. A suitable polyethylene glycol polymer is SokalanTM HP22.

25 **Polyester soil release polymers:** Suitable polyester soil release polymers have a structure as defined by one of the following structures (I), (II) or (III):



wherein:

a, b and c are from 1 to 200;

d, e and f are from 1 to 50;

Ar is a 1,4-substituted phenylene;

5 sAr is 1,3-substituted phenylene substituted in position 5 with SO₃Me;

Me is H, Na, Li, K, Mg/2, Ca/2, Al/3, ammonium, mono-, di-, tri-, or tetraalkylammonium wherein the alkyl groups are C₁-C₁₈ alkyl or C₂-C₁₀ hydroxyalkyl, or any mixture thereof;

R¹, R², R³, R⁴, R⁵ and R⁶ are independently selected from H or C₁-C₁₈ n- or iso-alkyl; and

10 R⁷ is a linear or branched C₁-C₁₈ alkyl, or a linear or branched C₂-C₃₀ alkenyl, or a cycloalkyl group with 5 to 9 carbon atoms, or a C₈-C₃₀ aryl group, or a C₆-C₃₀ arylalkyl group. Suitable polyester soil release polymers are terephthalate polymers having the structure of formula (I) or (II) above.

Suitable polyester soil release polymers include the Repel-o-texTM series of polymers such as Repel-o-tex SF2 (Rhodia) and/or the TexcareTM series of polymers such as Texcare SRA300
15 (Clariant).

Amine polymer: Suitable amine polymers include polyethylene imine polymers, such as alkoxyated polyalkyleneimines, optionally comprising a polyethylene and/or polypropylene oxide block.

Cellulosic polymer: The composition can comprise cellulosic polymers, such as polymers
20 selected from alkyl cellulose, alkyl alkoxyalkyl cellulose, carboxyalkyl cellulose, alkyl carboxyalkyl, and any combination thereof. Suitable cellulosic polymers are selected from carboxymethyl cellulose, methyl cellulose, methyl hydroxyethyl cellulose, methyl carboxymethyl cellulose, and mixtures thereof. The carboxymethyl cellulose can have a degree of carboxymethyl substitution from 0.5 to 0.9 and a molecular weight from 100,000 Da to
25 300,000 Da. Another suitable cellulosic polymer is hydrophobically modified carboxymethyl cellulose, such as FinnfixTM SH-1 (CP Kelco).

Other suitable cellulosic polymers may have a degree of substitution (DS) of from 0.01 to 0.99 and a degree of blockiness (DB) such that either DS+DB is of at least 1.00 or DB+2DS-DS² is at least 1.20. The substituted cellulosic polymer can have a degree of substitution (DS) of at
30 least 0.55. The substituted cellulosic polymer can have a degree of blockiness (DB) of at least 0.35. The substituted cellulosic polymer can have a DS + DB, of from 1.05 to 2.00. A suitable substituted cellulosic polymer is carboxymethylcellulose.

Another suitable cellulosic polymer is cationically modified hydroxyethyl cellulose.

Dye transfer inhibitor (DTI) polymer: The laundry detergent compositions may comprise DTI polymers. Suitable DTIs include polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof. The DTI polymers discussed above are well known
5 in the art and commercially available, for example PVP-K15 and K30 (Ashland), Sokalan HP165, HP50, HP53, HP59, HP56K, HP56, HP66 (BASF), Chromabond™ S-400, S403E and S-100 (Ashland), and Polyquart™ FDI (Cognis).

Builder: Suitable builders include zeolites, phosphates, citrates, and any combination thereof.

10 **Zeolite builder:** The composition may be substantially free of zeolite builder. Substantially free of zeolite builder typically means comprises from 0wt% to 10wt%, zeolite builder, or to 8wt%, or to 6wt%, or to 4wt%, or to 3wt%, or to 2wt%, or even to 1wt% zeolite builder. Substantially free of zeolite builder preferably means “no deliberately added” zeolite
15 builder. Typical zeolite builders include zeolite A, zeolite P, zeolite MAP, zeolite X and zeolite Y.

Phosphate builder: The composition may be substantially free of phosphate builder. Substantially free of phosphate builder typically means comprises from 0wt% to 10wt% phosphate builder, or to 8wt%, or to 6wt%, or to 4wt%, or to 3wt%, or to 2wt%, or even to 1wt% phosphate builder. Substantially free of zeolite builder preferably preferably means “no
20 deliberately added” phosphate builder. A typical phosphate builder is sodium tri-polyphosphate (STPP).

Citrate: A suitable citrate is sodium citrate. However, citric acid may also be incorporated into the composition, which can form citrate in the wash liquor.

25 **Buffer and alkalinity source:** Suitable buffers and alkalinity sources include carbonate salts and/or silicate salts and/or double salts such as burkeite.

Carbonate salt: A suitable carbonate salt is sodium carbonate and/or sodium bicarbonate. The carbonate salt may have a weight average mean particle size of from 100 to 500 micrometers. Alternatively, the carbonate salt may have a weight average mean particle size of from 10 to 25 micrometers.

30 **Silicate salt:** The silicate can be crystalline or amorphous. Suitable crystalline silicates include crystalline layered silicate, such as SKS-6. Other suitable silicates include 1.6R silicate and/or 2.0R silicate. A suitable silicate salt is sodium silicate. Another suitable silicate salt is sodium metasilicate.

Sulphate salt: A suitable sulphate salt is sodium sulphate. The sulphate salt may have a weight average mean particle size of from 100 to 500 micrometers, alternatively, the sulphate salt may have a weight average mean particle size of from 10 to 45 micrometers.

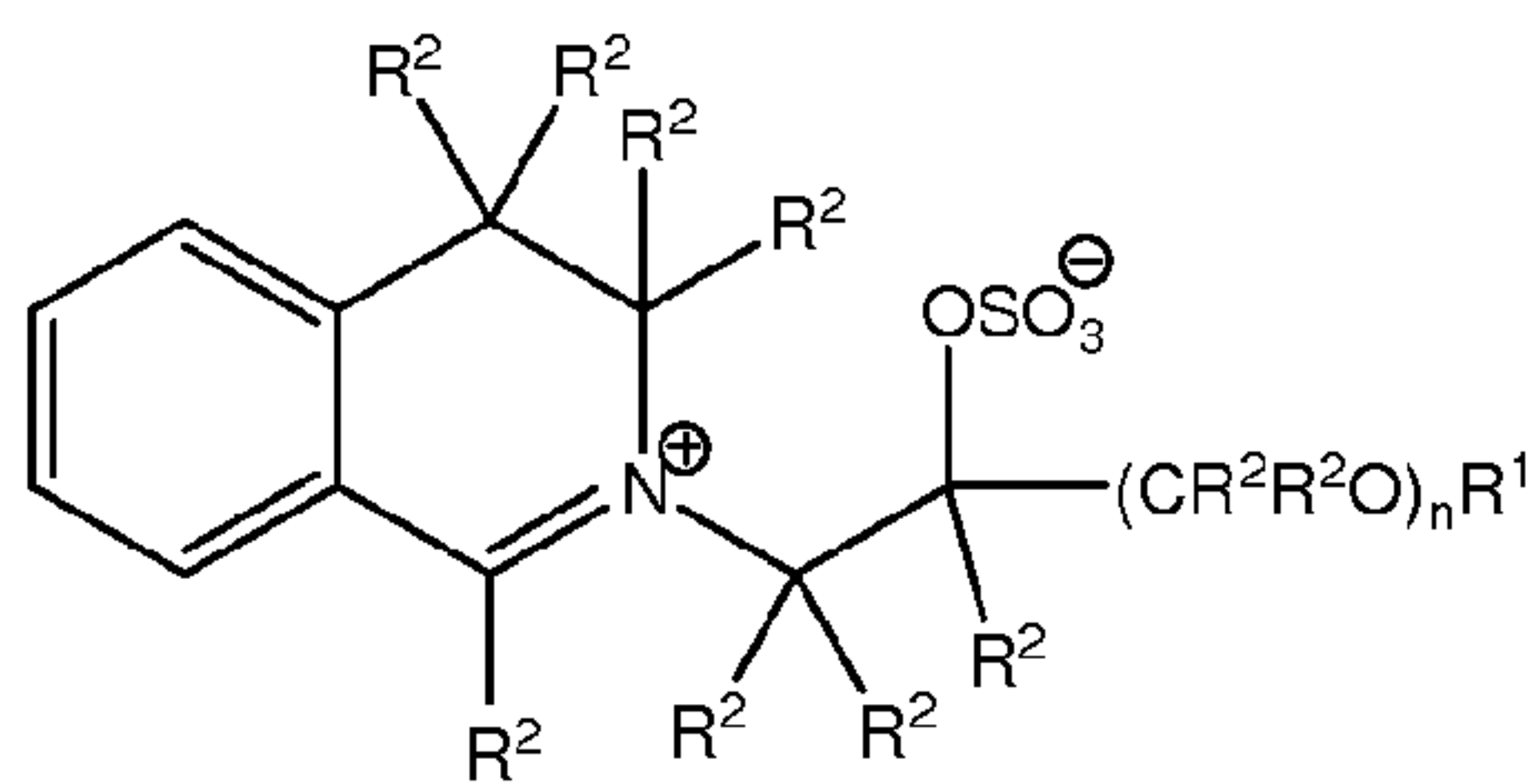
Bleach activator: Suitable bleach activators include: tetraacetythylenediamine (TAED); oxybenzene sulphonates such as nonanoyl oxybenzene sulphonate (NOBS), caprylamidononanoyl oxybenzene sulphonate (NACA-OBS), 3,5,5-trimethyl hexanoyloxybenzene sulphonate (Iso-NOBS), dodecyl oxybenzene sulphonate (LOBS), and any mixture thereof; caprolactams; pentaacetate glucose (PAG); nitrile quaternary ammonium; imide bleach activators, such as N-nonanoyl-N-methyl acetamide; and any mixture thereof.

Source of available oxygen: A suitable source of available oxygen (AvOx) is a source of hydrogen peroxide, such as percarbonate salts and/or perborate salts, such as sodium percarbonate. The source of peroxygen may be at least partially coated, or even completely coated, by a coating ingredient such as a carbonate salt, a sulphate salt, a silicate salt, borosilicate, or any mixture thereof, including mixed salts thereof. Suitable percarbonate salts can be prepared by a fluid bed process or by a crystallization process. Suitable perborate salts include sodium perborate mono-hydrate (PB1), sodium perborate tetra-hydrate (PB4), and anhydrous sodium perborate which is also known as fizzing sodium perborate. Other suitable sources of AvOx include persulphate, such as oxone. Another suitable source of AvOx is hydrogen peroxide.

Pre-formed peracid: A suitable pre-formed peracid is N,N-phthaloylamino peroxypropionic acid (PAP).

Bleach catalyst: Suitable bleach catalysts include oxaziridinium-based bleach catalysts, transition metal bleach catalysts and bleaching enzymes.

Oxaziridinium-based bleach catalyst: A suitable oxaziridinium-based bleach catalyst has the formula:



wherein: R^1 is selected from the group consisting of: H, a branched alkyl group containing from 3 to 24 carbons, and a linear alkyl group containing from 1 to 24 carbons; R^1 can be a branched alkyl group comprising from 6 to 18 carbons, or a linear alkyl group comprising from 5 to 18 carbons, R^1 can be selected from the group consisting of: 2-propylheptyl, 2-butyloctyl, 2-pentylnonyl, 2-hexyldecyl, n-hexyl, n-octyl, n-decyl, n-dodecyl, n-tetradecyl, n-hexadecyl, n-octadecyl, iso-nonyl, iso-decyl, iso-tridecyl and iso-pentadecyl; R^2 is independently selected from the group consisting of: H, a branched alkyl group comprising from 3 to 12 carbons, and a linear alkyl group comprising from 1 to 12 carbons; optionally R^2 is independently selected from H and methyl groups; and n is an integer from 0 to 1.

10 **Transition metal bleach catalyst:** The composition may include transition metal bleach catalyst, typically comprising copper, iron, titanium, ruthenium, tungsten, molybdenum, and/or manganese cations. Suitable transition metal bleach catalysts are manganese-based transition metal bleach catalysts.

15 **Reducing bleach:** The composition may comprise a reducing bleach. However, the composition may be substantially free of reducing bleach; substantially free means “no deliberately added”. Suitable reducing bleach include sodium sulphite and/or thiourea dioxide (IDO).

20 **Co-bleach particle:** The composition may comprise a co-bleach particle. Typically, the co-bleach particle comprises a bleach activator and a source of peroxide. It may be highly suitable for a large amount of bleach activator relative to the source of hydrogen peroxide to be present in the co-bleach particle. The weight ratio of bleach activator to source of hydrogen peroxide present in the co-bleach particle can be at least 0.3:1, or at least 0.6:1, or at least 0.7:1, or at least 0.8:1, or at least 0.9:1, or at least 1.0:1.0, or even at least 1.2:1 or higher.

25 The co-bleach particle can comprise: (i) bleach activator, such as TAED; and (ii) a source of hydrogen peroxide, such as sodium percarbonate. The bleach activator may at least partially, or even completely, enclose the source of hydrogen peroxide.

The co-bleach particle may comprise a binder. Suitable binders are carboxylate polymers such as polyacrylate polymers, and/or surfactants including non-ionic deterative surfactants and/or anionic deterative surfactants such as linear C_{11} - C_{13} alkyl benzene sulphonate.

30 The co-bleach particle may comprise bleach catalyst, such as an oxaziridium-based bleach catalyst.

Chelant: Suitable chelants are selected from: diethylene triamine pentaacetate, diethylene triamine penta(methyl phosphonic acid), ethylene diamine- N' N' -disuccinic acid, ethylene

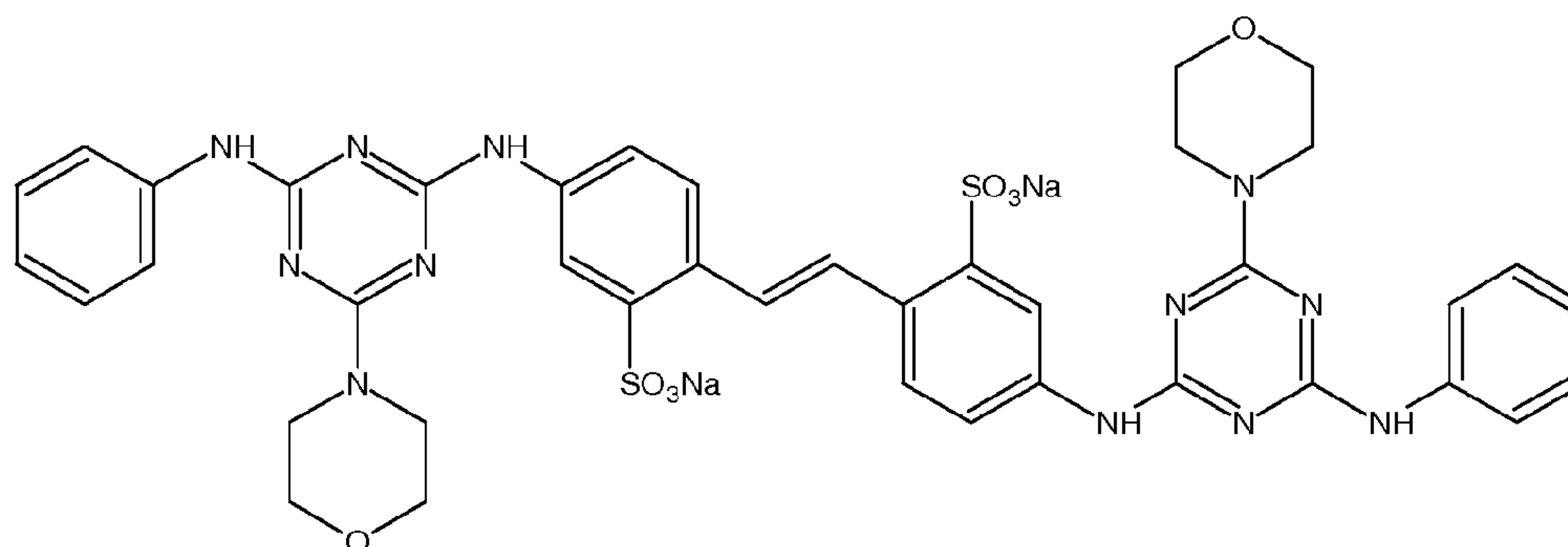
diamine tetraacetate, ethylene diamine tetra(methylene phosphonic acid), hydroxyethane di(methylene phosphonic acid), and any combination thereof. A suitable chelant is ethylene diamine-N'N'-disuccinic acid (EDDS) and/or hydroxyethane diphosphonic acid (HEDP). The laundry detergent composition may comprise ethylene diamine-N'N'-disuccinic acid or salt thereof. The ethylene diamine-N'N'-disuccinic acid may be in S,S enantiomeric form. The composition may comprise 4,5-dihydroxy-m-benzenedisulfonic acid disodium salt. Suitable chelants may also be calcium crystal growth inhibitors.

Calcium carbonate crystal growth inhibitor: The composition may comprise a calcium carbonate crystal growth inhibitor, such as one selected from the group consisting of: 1-hydroxyethanediphosphonic acid (HEDP) and salts thereof; N,N-dicarboxymethyl-2-aminopentane-1,5-dioic acid and salts thereof; 2-phosphonobutane-1,2,4-tricarboxylic acid and salts thereof; and any combination thereof.

Photobleach: Suitable photobleaches are zinc and/or aluminium sulphonated phthalocyanines.

Brightener: The laundry detergent composition may comprise fluorescent brightener. Preferred classes of fluorescent brightener are: Di-styryl biphenyl compounds, e.g. TinopalTM CBS-X, Di-amino stilbene di-sulfonic acid compounds, e.g. TinopalTM DMS pure Xtra and BlankophorTM HRH, and Pyrazoline compounds, e.g. BlankophorTM SN. Preferred fluorescers are: sodium 2-(4-styryl-3-sulfophenyl)-2H-naphthol[1,2-d]triazole, disodium 4,4'-bis{[(4-anilino-6-(N-methyl-N-2-hydroxyethyl)amino-1,3,5-triazin-2-yl)]amino}stilbene-2,2'-disulfonate, disodium 4,4'-bis{[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)]amino}stilbene-2,2'-disulfonate, and disodium 4,4'-bis(2-sulfostyryl)biphenyl.

A particularly preferred fluorescent brightener is C.I. Fluorescent Brightener 260 having the following structure. For solid detergent compositions, this brightener may be used in its beta or alpha crystalline forms, or a mixture of these forms.



Enzyme: Suitable enzymes include proteases, amylases, cellulases, lipases, xylogucanases, pectate lyases, mannanases, bleaching enzymes, cutinases, and mixtures thereof.

For the enzymes, accession numbers and IDs shown in parentheses refer to the entry numbers in the databases Genbank, EMBL and/or Swiss-Prot. For any mutations, standard 1-
5 letter amino acid codes are used with a * representing a deletion. Accession numbers prefixed with DSM refer to micro-organisms deposited at Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Brunswick (DSMZ).

Protease. The composition may comprise a protease. Suitable proteases include metalloproteases and/or serine proteases, including neutral or alkaline microbial serine proteases,
10 such as subtilisins (EC 3.4.21.62). Suitable proteases include those of animal, vegetable or microbial origin. In one aspect, such suitable protease may be of microbial origin. The suitable proteases include chemically or genetically modified mutants of the aforementioned suitable proteases. In one aspect, the suitable protease may be a serine protease, such as an alkaline microbial protease or/and a trypsin-type protease. Examples of suitable neutral or alkaline
15 proteases include:

(a) subtilisins (EC 3.4.21.62), including those derived from *Bacillus*, such as *Bacillus lentus*, *Bacillus alkalophilus* (P27963, ELYA_BACAO), *Bacillus subtilis*, *Bacillus amyloliquefaciens* (P00782, SUBT_BACAM), *Bacillus pumilus* (P07518) and *Bacillus gibsonii* (DSM14391).

20 (b) trypsin-type or chymotrypsin-type proteases, such as trypsin (e.g. of porcine or bovine origin), including the *Fusarium* protease and the chymotrypsin proteases derived from *Cellulomonas* (A2RQE2).

(c) metalloproteases, including those derived from *Bacillus amyloliquefaciens* (P06832, NPRES_BACAM).

25 Suitable proteases include those derived from *Bacillus gibsonii* or *Bacillus Lentus* such as subtilisin 309 (P29600) and/or DSM 5483 (P29599).

Suitable commercially available protease enzymes include: those sold under the trademarks Alcalase®, Savinase®, Primase®, Durazym®, Polarzyme®, Kannase®, Liqueanase®, Liqueanase Ultra®, Savinase Ultra®, Ovozyme®, Neutrase®, Everlase® and Esperase® by
30 Novozymes A/S (Denmark); those sold under the tradename Maxatase®, Maxacal®, Maxapem®, Properase®, Purafect®, Purafect Prime®, Purafect Ox®, FN3®, FN4®, Excellase® and Purafect OXP® by Genencor International; those sold under the trademark

Opticlean® and Optimase® by Solvay Enzymes; those available from Henkel/Kemira, namely BLAP (P29599 having the following mutations S99D + S101 R + S103A + V104I + G159S), and variants thereof including BLAP R (BLAP with S3T + V4I + V199M + V205I + L217D), BLAP X (BLAP with S3T + V4I + V205I) and BLAP F49 (BLAP with S3T + V4I + A194P + V199M + V205I + L217D) all from Henkel/Kemira; and KAP (Bacillus alkalophilus subtilisin with mutations A230V + S256G + S259N) from Kao.

Other suitable protease enzymes are fungal serine proteases. Suitable enzymes are variants or wild-types of the fungal serine proteases endogenous to *Trichoderma reesei* strain QM9414, *Malbranchea cinnamomea* strain ALK04122, *Fusarium graminearum* strain ALK01726, *Fusarium equiseti* strain CBS 119568 and *Fusarium acuminatum* strain CBS 124084. Examples of commercially available fungal serine proteases are Biotouch ROC and Biotouch Novia, both supplied by AB Enzymes, Darmstadt, Germany.

Amylase: Suitable amylases are alpha-amylases, including those of bacterial or fungal origin. Chemically or genetically modified mutants (variants) are included. A suitable alkaline alpha-amylase is derived from a strain of *Bacillus*, such as *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus stearothermophilus*, *Bacillus subtilis*, or other *Bacillus sp.*, such as *Bacillus sp.* NCIB 12289, NCIB 12512, NCIB 12513, sp 707, DSM 9375, DSM 12368, DSMZ no. 12649, KSM AP1378, KSM K36 or KSM K38. Suitable amylases include:

(a) alpha-amylase derived from *Bacillus licheniformis* (P06278, AMY_BACLI), and variants thereof, especially the variants with substitutions in one or more of the following positions: 15, 23, 105, 106, 124, 128, 133, 154, 156, 181, 188, 190, 197, 202, 208, 209, 243, 264, 304, 305, 391, 408, and 444.

(b) AA560 amylase (CBU30457, HD066534) and variants thereof, especially the variants with one or more substitutions in the following positions: 26, 30, 33, 82, 37, 106, 118, 128, 133, 149, 150, 160, 178, 182, 186, 193, 203, 214, 231, 256, 257, 258, 269, 270, 272, 283, 295, 296, 298, 299, 303, 304, 305, 311, 314, 315, 318, 319, 339, 345, 361, 378, 383, 419, 421, 437, 441, 444, 445, 446, 447, 450, 461, 471, 482, 484, optionally that also contain the deletions of D183* and G184*.

(c) variants exhibiting at least 90% identity with the wild-type enzyme from *Bacillus SP722* (CBU30453, HD066526), especially variants with deletions in the 183 and 184 positions.

Suitable commercially available alpha-amylases are Duramyl®, Liquezyme® Termamyl®, Termamyl Ultra®, Natalase®, Supramyl®, Stainzyme®, Stainzyme Plus®, Fungamyl® and BAN® (Novozymes A/S), Bioamylase® and variants thereof (Biocon India Ltd.), Kemzym®

AT 9000 (Biozym Ges. m.b.H, Austria), Rapidase® , Purastar®, Optimize HT Plus®, Enzysize®, Powerase® and Purastar Oxam®, Maxamyl® (Genencor International Inc.) and KAM® (KAO, Japan). Suitable amylases are Natalase®, Stainzyme® and Stainzyme Plus®.

Cellulase: The composition may comprise a cellulase. Suitable cellulases include those of bacterial or fungal origin. Chemically modified or protein engineered mutants are included. Suitable cellulases include cellulases from the genera *Bacillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, e.g., the fungal cellulases produced from *Humicola insolens*, *Myceliophthora thermophila* and *Fusarium oxysporum*.

Commercially available cellulases include Celluzyme®, and Carezyme® (Novozymes A/S), Clazinase®, and Puradax HA® (Genencor International Inc.), and KAC-500(B)® (Kao Corporation).

The cellulase can include microbial-derived endoglucanases exhibiting endo-beta-1,4-glucanase activity (E.C. 3.2.1.4), including a bacterial polypeptide endogenous to a member of the genus *Bacillus sp. AA349* and mixtures thereof. Suitable endoglucanases are sold under the trademarks Celluclean® and Whitezyme® (Novozymes A/S, Bagsvaerd, Denmark).

The composition may comprise a cleaning cellulase belonging to Glycosyl Hydrolase family 45 having a molecular weight of from 17kDa to 30 kDa, for example the endoglucanases sold under the trademark Biotouch® NCD, DCC and DCL (AB Enzymes, Darmstadt, Germany).

Suitable cellulases may also exhibit xyloglucanase activity, such as Whitezyme®.

Xyloglucanase: Suitable xyloglucanase enzymes may have enzymatic activity towards both xyloglucan and amorphous cellulose substrates. The enzyme may be a glycosyl hydrolase (GH) selected from GH families 5, 12, 44 or 74. The glycosyl hydrolase selected from GH family 44 is particularly suitable. Suitable glycosyl hydrolases from GH family 44 are the XYG1006 glycosyl hydrolase from *Paenibacillus polyxyma* (ATCC 832) and variants thereof.

Pectate lyase: Suitable pectate lyases are either wild-types or variants of *Bacillus*-derived pectate lyases (CAF05441, AAU25568) sold under the trademarks Pectawash®, Pectaway® and X-Pect® (from Novozymes A/S, Bagsvaerd, Denmark).

Mannanase: Suitable mannanases are sold under the trademarks Mannaway® (from Novozymes A/S, Bagsvaerd, Denmark), and Purabrite® (Genencor International Inc., Palo Alto, California).

Bleaching enzyme: Suitable bleach enzymes include oxidoreductases, for example oxidases such as glucose, choline or carbohydrate oxidases, oxygenases, catalases, peroxidases, like halo-, chloro-, bromo-, lignin-, glucose- or manganese-peroxidases, dioxygenases or

laccases (phenoloxidases, polyphenoloxidases). Suitable commercial products are sold under the Guardzyme® and Denilite® ranges from Novozymes. It may be advantageous for additional organic compounds, especially aromatic compounds, to be incorporated with the bleaching enzyme; these compounds interact with the bleaching enzyme to enhance the activity of the oxidoreductase (enhancer) or to facilitate the electron flow (mediator) between the oxidizing enzyme and the stain typically over strongly different redox potentials.

Other suitable bleaching enzymes include perhydrolases, which catalyse the formation of peracids from an ester substrate and peroxygen source. Suitable perhydrolases include variants of the *Mycobacterium smegmatis* perhydrolase, variants of so-called CE-7 perhydrolases, and variants of wild-type subtilisin Carlsberg possessing perhydrolase activity.

Identity. The relativity between two amino acid sequences is described by the parameter “identity”. For purposes of the present invention, the alignment of two amino acid sequences is determined by using the Needle™ program from the EMBOSS™ package version 2.8.0. The Needle program implements the global alignment algorithm described in Needleman, S. B. and Wunsch, C. D. (1970) *J. Mol. Biol.* 48, 443-453. The substitution matrix used is BLOSUM62™, gap opening penalty is 10, and gap extension penalty is 0.5.

Fabric-softener: Suitable fabric-softening agents include clay, silicone and/or quaternary ammonium compounds. Suitable clays include montmorillonite clay, hectorite clay and/or laponite clay. A suitable clay is montmorillonite clay. Suitable silicones include amino-silicones and/or polydimethylsiloxane (PDMS). A suitable fabric softener is a particle comprising clay and silicone, such as a particle comprising montmorillonite clay and PDMS.

Flocculant: Suitable flocculants include polyethylene oxide; for example having an average molecular weight of from 300,000 Da to 900,000 Da.

Suds suppressor: Suitable suds suppressors include silicone and/or fatty acid such as stearic acid.

Perfume: Suitable perfumes include perfume microcapsules, polymer assisted perfume delivery systems including Schiff base perfume/polymer complexes, starch-encapsulated perfume accords, perfume-loaded zeolites, blooming perfume accords, and any combination thereof. A suitable perfume microcapsule is melamine formaldehyde based, typically comprising perfume that is encapsulated by a shell comprising melamine formaldehyde. It may be highly suitable for such perfume microcapsules to comprise cationic and/or cationic precursor material in the shell, such as polyvinyl formamide (PVF) and/or cationically modified hydroxyethyl cellulose (catHEC).

Aesthetic: Suitable aesthetic particles include soap rings, lamellar aesthetic particles, gelatin beads, carbonate and/or sulphate salt speckles, coloured clay particles, and any combination thereof.

5

EXAMPLES

Example 1;

The improved cationic surfactant removal benefit of the method of the present invention was demonstrated in the following experiment.

10 A composition was prepared comprising alkyl ethoxylated sulphate anionic surfactant, a polydimethyl siloxane containing suds suppressor and sodium bicarbonate. This composition was labeled pre-treatment composition 1.

A second pre-treatment composition was prepared which was identical to pre-treatment composition 1, but which was also used in conjunction with a commercially available Lenor™ brand liquid softener, Pure Care Sensitive™.

15 A third pre-treatment composition was prepared which was identical to pre-treatment composition 1, but which also comprised a variant having at least 90% sequence identity to wild-type lipase from *Thermomyces lanuginosus* and having sequence substitutions T231R and N233R.

20 A fourth pre-treatment composition was prepared which was identical to pre-treatment composition 3, but which was also used in conjunction with a commercially available Lenor brand liquid softener.

A fifth pre-treatment composition was prepared which was identical to pre-treatment composition 1, but which also comprised a variant corresponding to Claim 5, part (u) of EP1290150B1.

25 A sixth pre-treatment composition was prepared which was identical to pre-treatment composition 5, but which was also used in conjunction with a commercially available Lenor brand liquid softener.

To summarise;

30

1. pre-treatment composition only
2. pre-treatment composition in conjunction with Lenor
3. pre-treatment composition comprising Lipex™
4. pre-treatment composition comprising Lipex in conjunction with Lenor

5. pre-treatment composition comprising *Humicola insolens* cutinase
6. pre-treatment composition comprising *Humicola insolens* cutinase in conjunction with Lenor.

5 Standard fabric swatches TF7436-M polycotton (25x20cm swatches) and Dacron™ 64 polyester (25 x20cm swatches) were obtained from Westlairs. Also obtained were standard Equest™ KC knitted cotton (25x20 cm) swatches. Two of each of these were added to a washing machine together with 455g of cotton tea towels as ballast.

The swatches were then washed in the 'short cotton cycle' (40°C) at 1600rpm together
10 with the relevant pre-treatment composition added to the drawer of the washing machine so that it would be added during the wash cycle and where applicable Lenor added to the drawer so that it would be added during the rinse cycle. The fabrics were then dried on a line. This was repeated so that all swatches had been washed four times together with the same pre-treatment composition and Lenor (where applicable), with drying between washes and a final tumble dry
15 after the last wash. The pre-treatment compositions were prepared such that the 13L wash liquor comprised 100ppm linear alkylbenzene sulphonate anionic surfactant present in the wash liquor. Sodium bicarbonate was added to the wash liquor at a concentration of 400ppm, and the suds suppressor (12.4% active) at a concentration of 46ppm. The lipid esterase was added to the wash liquor at a concentration of 1ppm (active enzyme protein). A volume of 70ml of the standard
20 Lenor liquid fabric softener was added to the drawer of the washing machine.

The lipid esterase concentration on polycotton and polyester fabrics for fabrics treated with pre-treatments 1-4 were tested using an enzyme linked immunosorbant assay (ELISA). A sample preparation buffer was first prepared by weighing 0.93g Trizma base, 4.96g sodium thiosulfate pentahydrate, 0.147g calcium chloride and 29.22g sodium chloride into a 1000ml
25 beaker. To this, 800ml deionised water was added and stirred to dissolve the ingredients. To this, 1g of bovine serum albumin (BSA) was added and the solution stirred. Hydrochloric acid was added to adjust the pH to 8 and then 0.1g sodium azide was added. 1ml of Tween 20 was then added. To this, the fabric swatch was added and agitated for 30 minutes. A volume of 25ml of this was solution was then taken and added to a centrifuge tube and placed in sample
30 rotator for at least 30 mins.

A volume of 100µl of this was placed in the well of microtitre plate, covered and allowed to incubate for 90 mins. A volume of 10µl of the appropriate detecting antibody (made using standard biochemical means) was added to 1ml of blocking buffer (2g of bovine serum albumin

dissolved in 100ml of wash buffer [wash buffer; 29.22g sodium chloride, 1.86g Trisma-base and 1g bovine serum albumin, dissolved in desionised water, pH adjusted to 8, 0.5ml TweenTM 20 added and the volume made up to 1000ml]) and mixed gently to produce a detecting antibody solution. The microtitre plate was washed with wash buffer, and 100µl of the detected antibody solution was added. To 11ml of blocking buffer, 10µl of a peroxide solution was added. The microtitre plate was washed with wash buffer and the peroxide in blocking buffer solution added. The plate was covered and allowed to stand for 60 mins at room temperature.

An OPD substrate solution was prepared by adding a 15mg tablet of OPD (commercially available from Sigma) to 30ml of a citrate/phosphate buffer (7.3g of citric acid monohydrate and 23.87g Na₂HPO₄.12H₂O dissolved in deionised water, pH adjusted to pH 5 and the volume made up to 1000ml) in a centrifuge tube wrapped in foil. The tube was capped and mixed gently. To the tube, 10µl of 30% hydrogen peroxide was added and the plate then washed with wash buffer. The plate was then washed with citrate/phosphate buffer and 100µl of OPD substrate solution added to the well. Following this, 150µl of 1M H₂SO₄ was added to the well to stop the reaction. The microtitre plate was read in a microtitre plate reader at 492 and 620nm (dual wavelength mode). The 620nm value was subtracted from the 492nm value. The final values obtained were then compared to a calibration curve prepared earlier. Those skilled in the art would know how to prepare a standard calibration curve. From the calibration curve the amount of enzyme present on the fabric was calculated. Results can be seen in Table1.

Table 1

Pre-treatment	Fabric	ng enzyme /g fabric
1	Polyester	18400
2	Polyester	17900
3	Polyester	1000
4	Polyester	370
1	Polycotton	4700
2	Polycotton	28000
3	Polycotton	1030
4	Polycotton	2170

The test fabrics were then agitated in 0.01% aqueous solution of bromophenol blue a tergotometer. Bromophenol blue is a dye that complexes with cationic surfactants. The fabrics were contacted with the bromophenol blue for 5 minutes.

The fabrics were then rinsed in the tergotometer. The conditions were 200rpm, for 5 minutes at 22°C. The rinse treatment solution also comprised 50ppm linear alkylbenzene sulphonate anionic surfactant.

Fabrics were then dried overnight on wire racks.

- 5 Following drying, the fabrics were imaged using Colour-Eye imaging apparatus. The Colour-eye imaging apparatus measures the optical b value of the fabrics. A more negative b value indicates that cationic surfactant residues are present. The results can be seen in Tables 2 and 3 below.

10 **Table 2: polycotton**

Treatment	b Value	Standard Error
1	-9.19	0.49
2	-17.05	0.38
3	-7.94	0.14
4	-7.82	0.19
5	-8.83	0.38
6	-9.00	0.74

(Standard error was calculated as $SE = SD/\sqrt{n}$ where SD = standard deviation and n = number of external replicates)

- 15 As can be seen from Table 2, polycotton treated with treatment 1, i.e. no enzyme or fabric softener gave a b value of -9.19. Treatment 2, which also included fabric softener gave a b value of -17.05, indicating that there is more cationic residue present. However, treatments 3 to 6 all gave similar b values. This indicates that when the fabric was pre-treated with lipid esterase prior to addition of cationic fabric softener (treatments 4 and 6), the amount of cationic residue
- 20 following washing was the same as when no cationic softener was added (treatments 3 and 5).

Table 3: Polyester and Cotton

Treatment	Polyester		Cotton	
	b Value	Standard Error	b Value	Standard Error
1	2.61	0.07	-14.91	0.17
2	0.57	1.12	-29.57	0.65
3	3.02	0.08	-14.66	0.17
4	2.70	0.42	-13.92	0.63

As can be seen from Table 3, the same effect is seen again. Fabrics treated with treatment 2 show a much more negative b value on both polyester and cotton than fabrics treated with treatment 1. This indicates that fabrics treated with treatment 2 had residual cationic material remaining on them. However, fabrics treated with treatment 4 showed a b value that was similar to fabrics treated with treatment 3. Therefore, fabrics treated with treatment 4 had lower residual cationic material than fabrics treated with treatment 2.

Examples 2-20;

The following examples are of laundry detergent compositions suitable for use in step (iii);

10 Examples 2-7

Granular laundry detergent compositions designed for hand washing or top-loading washing machines may be added to sufficient water to form a paste for direct contact with the surface to be treated, forming a concentrated cleaning composition.

	2 (wt %)	3 (wt %)	4 (wt %)	5 (wt %)	6 (wt %)	7 (wt %)
Linear alkylbenzenesulfonate	20	22	20	15	20	20
C ₁₂₋₁₄ Dimethylhydroxyethyl ammonium chloride	0.7	0.2	1	0.6	0.0	0
AE3S	0.9	1	0.9	0.0	0.5	0.9
AE7	0.0	0.0	0.0	1	0.0	3
Sodium tripolyphosphate	5	0.0	4	9	2	0.0
Zeolite A	0.0	1	0.0	1	4	1
1.6R Silicate (SiO ₂ :Na ₂ O at ratio 1.6:1)	7	5	2	3	3	5
Sodium carbonate	25	20	25	17	18	19
Polyacrylate MW 4500	1	0.6	1	1	1.5	1
Random graft copolymer ¹	0.1	0.2	0.0	0.0	0.0	0.0
Carboxymethyl cellulose	1	0.3	1	1	1	1
Stainzyme® (20 mg active/g)	0.1	0.2	0.1	0.2	0.1	0.1
Bacterial protease (Savinase®)	0.1	0.1	0.1	0.1		0.1

32.89 mg active/g)						
Natalase® (8.65 mg active /g)	0.1	0.0	0.1	0.0	0.1	0.1
Lipex® (18 mg active /g)	0.03	0.07	0.3	0.1	0.07	0.4
Biotouch® ROC (20mg active/g)	0.1	0.2	0.2	0.2	0.1	0.4
Fluorescent Brightener 1	0.06	0.0	0.06	0.18	0.06	0.06
Fluorescent Brightener 2	0.1	0.06	0.1	0.0	0.1	0.1
DTPA	0.6	0.8	0.6	0.25	0.6	0.6
MgSO ₄	1	1	1	0.5	1	1
Sodium Percarbonate	0.0	5.2	0.1	0.0	0.0	0.0
Sodium Perborate Monohydrate	4.4	0.0	3.85	2.09	0.78	3.63
NOBS	1.9	0.0	1.66	0.0	0.33	0.75
TAED	0.58	1.2	0.51	0.0	0.015	0.28
Sulphonated zinc phthalocyanine	0.0030	0.0	0.0012	0.0030	0.0021	0.0
S-ACMC	0.1	0.0	0.0	0.0	0.06	0.0
Direct Violet 9	0.0	0.0	0.0003	0.0005	0.0003	0.0
Acid Blue 29	0.0	0.0	0.0	0.0	0.0	0.0003
Sulfate/Moisture	Balance					

Examples 8-13

Granular laundry detergent compositions designed for front-loading automatic washing machines may be added to sufficient water to form a paste for direct contact with the surface to be treated, forming a concentrated cleaning composition.

5

	8 (wt%)	9 (wt%)	10 (wt%)	11 (wt%)	12 (wt%)	13 (wt%)
Linear alkylbenzenesulfonate	8	7.1	7	6.5	7.5	7.5
AE3S	0	4.8	0	5.2	4	4
C12-14 Alkylsulfate	1	0	1	0	0	0
AE7	2.2	0	3.2	0	0	0
C ₁₀₋₁₂ Dimethyl	0.75	0.94	0.98	0.98	0	0

hydroxyethylammonium chloride						
Crystalline layered silicate (δ - Na ₂ Si ₂ O ₅)	4.1	0	4.8	0	0	0
Zeolite A	5	0	5	0	2	2
Citric Acid	3	5	3	4	2.5	3
Sodium Carbonate	15	20	14	20	23	23
Silicate 2R (SiO ₂ :Na ₂ O at ratio 2:1)	0.08	0	0.11	0	0	0
Soil release agent	0.75	0.72	0.71	0.72	0	0
Acrylic Acid/Maleic Acid Copolymer	1.1	3.7	1.0	3.7	2.6	3.8
Carboxymethylcellulose	0.15	1.4	0.2	1.4	1	0.5
Bacterial protease (84 mg active/g)	0.2	0.2	0.3	0.15	0.12	0.13
Stainzyme® (20 mg active/g)	0.2	0.15	0.2	0.3	0.15	0.15
Lipex® (18.00 mg active/g)	0.05	0.15	0.1	0	0	0
Natalase® (8.65 mg active/g)	0.1	0.2	0	0	0.15	0.15
Celluclean™ (15.6 mg active/g)	0	0	0	0	0.1	0.1
Biotouch® ROC (20mg active/g)	0.2	0.1	0.2	0.2	0.2	0.2
TAED	3.6	4.0	3.6	4.0	2.2	1.4
Percarbonate	13	13.2	13	13.2	16	14
Na salt of Ethylenediamine-N,N'- disuccinic acid, (S,S) isomer (EDDS)	0.2	0.2	0.2	0.2	0.2	0.2
Hydroxyethane di phosphonate (HEDP)	0.2	0.2	0.2	0.2	0.2	0.2
MgSO ₄	0.42	0.42	0.42	0.42	0.4	0.4
Perfume	0.5	0.6	0.5	0.6	0.6	0.6
Suds suppressor agglomerate	0.05	0.1	0.05	0.1	0.06	0.05
Soap	0.45	0.45	0.45	0.45	0	0
Sulphonated zinc phthalocyanine (active)	0.0007	0.0012	0.0007	0	0	0

S-ACMC	0.01	0.01	0	0.01	0	0
Direct Violet 9 (active)	0	0	0.0001	0.0001	0	0
Sulfate/ Water & Miscellaneous	Balance					

Any of the above compositions is used to launder fabrics in the second step at a concentration of 7000 to 10000 ppm in water, 20-90 °C, and a 5:1 water:cloth ratio. The typical pH is about 10. The fabrics are then dried. In one aspect, the fabrics are actively dried using a dryer. In one aspect, the fabrics are actively dried using an iron. In another aspect, the fabrics are merely allowed to dry on a line wherein they are exposed to air and optionally sunlight.

Examples 14-19 Heavy Duty Liquid laundry detergent compositions

	14 (wt%)	15 (wt%)	16 (wt%)	17 (wt%)	18 (wt%)	19 (wt%)
AES C ₁₂₋₁₅ alkyl ethoxy (1.8) sulfate	11	10	4	6.32	0	0
AE3S	0	0	0	0	2.4	0
Linear alkyl benzene sulfonate	1.4	4	8	3.3	5	8
HSAS	3	5.1	3	0	0	0
Sodium formate	1.6	0.09	1.2	0.04	1.6	1.2
Sodium hydroxide	2.3	3.8	1.7	1.9	1.7	2.5
Monoethanolamine	1.4	1.49	1.0	0.7	0	0
Diethylene glycol	5.5	0.0	4.1	0.0	0	0
AE9	0.4	0.6	0.3	0.3	0	0
AE7	0	0	0	0	2.4	6
Chelant	0.15	0.15	0.11	0.07	0.5	0.11
Citric Acid	2.5	3.96	1.88	1.98	0.9	2.5
C ₁₂₋₁₄ dimethyl Amine Oxide	0.3	0.73	0.23	0.37	0	0
C ₁₂₋₁₈ Fatty Acid	0.8	1.9	0.6	0.99	1.2	0
4-formyl-phenylboronic acid	0	0	0	0	0.05	0.02

Borax	1.43	1.5	1.1	0.75	0	1.07
Ethanol	1.54	1.77	1.15	0.89	0	3
Ethoxylated (EO ₁₅) tetraethylene pentamine	0.3	0.33	0.23	0.17	0.0	0.0
Ethoxylated hexamethylene diamine	0.8	0.81	0.6	0.4	1	1
1,2-Propanediol	0.0	6.6	0.0	3.3	0.5	2
Bacterial protease (40.6 mg active/g)	0.8	0.6	0.7	0.9	0.7	0.6
Mannaway® (25 mg active/g)	0.07	0.05	0.045	0.06	0.04	0.045
Stainzyme® (15 mg active/g)	0.3	0.2	0.3	0.1	0.2	0.4
Natalase® (29 mg active/g)	0	0.2	0.1	0.15	0.07	0
Lipex® (18 mg active/g)	0.4	0.2	0.3	0.1	0.2	0
Biotouch® ROC (20mg active/g)	0.2	0.1	0.2	0.2	0.1	0.1
Liquitint® Violet CT (active)	0.006	0.002	0	0	0	0.002
S-ACMC	-	-	0.01	0.05	0.01	0.02
Water, perfume, dyes & other components	Balance					

Example 20

This composition may be enclosed in a polyvinyl alcohol pouch.

	19 (wt%)
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Alkylbenzene sulfonic acid	21.0
C ₁₄₋₁₅ alkyl 8-ethoxylate	18.0
C ₁₂₋₁₈ Fatty acid	15.0
Bacterial protease (40.6 mg active/g)	1.5
Natalase® (29 mg active/g)	0.2
Mannanase (Mannaway®, 11mg active/g)	0.1
Xyloglucanase (Whitezyme®, 20mg active/g)	0.2
Biotouch® ROC (20mg active/g)	0.2
Λ compound having the following general structure: bis((C ₂ H ₅ O)(C ₂ H ₄ O) _n)(CH ₃)-N ⁺ -C _x H _{2x} -N ⁺ -(CH ₃)-bis((C ₂ H ₅ O)(C ₂ H ₄ O) _n), wherein n = from 20 to 30, and x = from 3 to 8, or sulphated or sulphonated variants thereof	2.0
Ethoxylated Polyethylenimine ²	0.8
Hydroxyethane diphosphonate (HEDP)	0.8
Fluorescent Brightener 1	0.2
Solvents (1,2 propanediol, ethanol), stabilizers	15.0
Hydrogenated castor oil derivative structurant	0.1
Perfume	1.6
Core Shell Melamine-formaldehyde encapsulate of perfume	0.10
Ethoxylated thiophene Hueing Dye	0.004
Buffers (sodium hydroxide, Monoethanolamine)	To pH 8.2
Water* and minors (antifoam, aesthetics)	To 100%

* Based on total cleaning and/or treatment composition weight, a total of no more than 7% water

¹ Random graft copolymer is a polyvinyl acetate grafted polyethylene oxide copolymer having a polyethylene oxide backbone and multiple polyvinyl acetate side chains. The molecular weight of the polyethylene oxide backbone is about 6000 and the weight ratio of the polyethylene oxide to polyvinyl acetate is about 40 to 60 and no more than 1 grafting point per 50 ethylene oxide units.

² Polyethyleneimine (MW = 600) with 20 ethoxylate groups per -NH.

* Remark: all enzyme levels expressed as % enzyme raw material

Raw Materials and Notes For Composition Examples 2-20

- 5 Linear alkylbenzenesulfonate having an average aliphatic carbon chain length C_{11} - C_{12} supplied by Stepan, Northfield, Illinois, USA
- C_{12-14} Dimethylhydroxyethyl ammonium chloride, supplied by Clariant GmbH, Sulzbach, Germany
- AE3S is C_{12-15} alkyl ethoxy (3) sulfate supplied by Stepan, Northfield, Illinois, USA
- 10 AE7 is C_{12-15} alcohol ethoxylate, with an average degree of ethoxylation of 7, supplied by Huntsman, Salt Lake City, Utah, USA
- AE9 is C_{12-13} alcohol ethoxylate, with an average degree of ethoxylation of 9, supplied by Huntsman, Salt Lake City, Utah, USA
- HSAS is a mid-branched primary alkyl sulfate with carbon chain length of about 16-17
- Sodium tripolyphosphate is supplied by Rhodia, Paris, France
- 15 Zeolite A is supplied by Industrial Zeolite (UK) Ltd, Grays, Essex, UK
- 1.6R Silicate is supplied by Koma, Nestemica, Czech Republic
- Sodium Carbonate is supplied by Solvay, Houston, Texas, USA
- Polyacrylate MW 4500 is supplied by BASF, Ludwigshafen, Germany
- Carboxymethyl cellulose is Finnfix® V supplied by CP Kelco, Arnhem, Netherlands
- 20 Suitable chelants are, for example, diethylenetetraamine pentaacetic acid (DTPA) supplied by Dow Chemical, Midland, Michigan, USA or Hydroxyethane di phosphonate (HEDP) supplied by Solutia, St Louis, Missouri, USA Bagsvaerd, Denmark
- Savinase®, Natalase®, Stainzyme®, Lipex®, Celluclean™, Mannaway® and Whitezyme® are all products of Novozymes, Bagsvaerd, Denmark.
- 25 Biotouch® ROC is a product of AB Enzymes, Darmstadt, Germany.
- Bacterial protease (examples 8-13) described in US 6,312,936 B1 supplied by Genencor International, Palo Alto, California, USA
- Bacterial protease (examples 14-20) described in US 4,760,025 is supplied by Genencor International, Palo Alto, California, USA
- 30 Fluorescent Brightener 1 is Tinopal® AMS, Fluorescent Brightener 2 is Tinopal® CBS-X, Sulphonated zinc phthalocyanine and Direct Violet 9 is Pergasol® Violet BN-Z all supplied by Ciba Specialty Chemicals, Basel, Switzerland
- Sodium percarbonate supplied by Solvay, Houston, Texas, USA
- Sodium perborate is supplied by Degussa, Hanau, Germany
- 35 NOBS is sodium nonanoyloxybenzenesulfonate, supplied by Future Fuels, Batesville, Arkansas, USA
- TAED is tetraacetythylenediamine, supplied under the Peractive® brand name by Clariant GmbH, Sulzbach, Germany

S-ACMC is carboxymethylcellulose conjugated with C.I. Reactive Blue 19, sold by Megazyme, Wicklow, Ireland under the product name AZO-CM-CELLULOSE, product code S-ACMC.

Soil release agent is Repel-o-tex® PF, supplied by Rhodia, Paris, France

5 Acrylic Acid/Maleic Acid Copolymer is molecular weight 70,000 and acrylate:maleate ratio 70:30, supplied by BASF, Ludwigshafen, Germany

Na salt of Ethylenediamine-N,N'-disuccinic acid, (S,S) isomer (EDDS) is supplied by Octel, Ellesmere Port, UK

10 Hydroxyethane di phosphonate (HEDP) is supplied by Dow Chemical, Midland, Michigan, USA

Suds suppressor agglomerate is supplied by Dow Corning, Midland, Michigan, USA

HSAS is mid-branched alkyl sulfate as disclosed in US 6,020,303 and US 6,060,443

C₁₂₋₁₄ dimethyl Amine Oxide is supplied by Procter & Gamble Chemicals, Cincinnati, Ohio, USA

15 Liquitint® Violet CT is supplied by Milliken, Spartanburg, South Carolina, USA.

The dimensions and values disclosed herein are not to be understood as being strictly limited to the exact numerical values recited. Instead, unless otherwise specified, each such dimension is intended to mean both the recited value and a functionally equivalent range surrounding that value. For example, a dimension disclosed as “40 mm” is intended to mean

20 “about 40 mm.”

CLAIMS

1. A method of laundering a fabric, comprising the steps of:
 - i) contacting a fabric with a lipid esterase;
 - ii) contacting the fabric from step (i) with a cationically charged fabric softening active, wherein the cationically charged fabric softening active is a substrate for the lipid esterase; and
 - iii) contacting the fabric from step (ii) with a laundry detergent composition, wherein the laundry detergent composition comprises an anionic deterative surfactant and at least one acceptable laundry detergent ingredient, wherein the anionic deterative surfactant is present at the ratio of anionic surfactant to fabric on a weight to weight basis of from 1:150 to 1:500.
2. The method according to claim 1, wherein the lipid esterase comprises at least a first and a second lipid esterase.
3. The method according to claim 1 or 2, wherein the fabric in step (iii) is contacted with the laundry detergent composition in a wash cycle of an automatic washing machine and wherein the wash cycle is at least 30 seconds.
4. The method according to claim 1, 2 or 3, wherein the fabric in step (iii) is contacted with the laundry detergent composition in a wash cycle of an automatic washing machine and wherein the wash cycle is run at a temperature of between 5°C and 50°C.
5. The method according to any one of claims 1 to 4, wherein the lipid esterase is selected from E.C. class 3.1.
6. The method according to claim 5, wherein the lipid esterase is selected from class E.C. 3.1.1.3.
7. The method according to any one of claims 1 to 6, wherein the lipid esterase comprises a variant having at least 90% sequence identity to wild-type lipase from *Thermomyces lanuginosus* and having sequence substitutions T231R and N233R, or a variant having

substitutions G8D, N15D, S48E, A88H, N91H, A130V, and R189V compared to a wild-type *Humicola insolens* cutinase strain DSM 1800, or a combination thereof.

8. The method according to any one of claims 1 to 7, wherein in step (i) the fabric is contacted with the lipid esterase, the lipid esterase being present at a concentration of between 30 and 55,000 ng enzyme/g fabric.
9. The method according to any one of claims 1 to 8, wherein the cationic charged fabric softening active comprises a cationic surfactant.
10. The method according to claim 9, wherein the cationic surfactant comprises a quaternary ammonium compound.
11. The method according to any one of claims 1 to 10, wherein the anionic detergent surfactant comprises an anionic surfactant.
12. The method according to claim 11, wherein the anionic surfactant is selected from linear alkyl benzene sulfonate, alkoxyated anionic surfactant, and a combination thereof.
13. The method according to any one of claims 1 to 12, wherein the laundry detergent composition of step (iii) further comprises a hueing agent, a polymer or a combination thereof.
14. The method according to any one of claims 1 to 13, wherein the laundry detergent composition of step (iii) further comprises from 0wt% to 10wt% zeolite builder on an anhydrous basis, from 0wt% to 10wt% phosphate builder, or a combination thereof.
15. The method according to claim 3, wherein the fabric in step (iii) is pre-treated with the laundry detergent composition prior to being subjected to the wash cycle of an automatic washing machine.
16. The method according to claim 5, wherein the E.C. class 3.1 is 3.1.1.1, 3.1.1.3, 3.1.1.74 or a combination thereof.

17. The method according to claim 3, wherein the wash cycle is at least 3 minutes.
18. The method according to claim 3, wherein the wash cycle is at least 6 minutes.
19. The method according to claim 3, wherein the wash cycle is no more than 30 minutes.
20. The method according to claim 3, wherein the wash cycle is no more than 45 minutes.
21. The method according to claim 3, wherein the wash cycle is no more than 60 minutes.
22. Use of a lipid esterase of E.C. class 3.1 deposited on a fabric to improve the removal of a fabric softening active from the fabric in a subsequent wash.