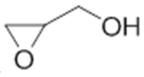
hydrogen. In this case, preferably at least two of R_1, R_2, R_3, R_4 and R_5 are hydrogen. More preferably, at least three of R_1, R_2, R_3, R_4 and R_5 are hydrogen. Even more preferably, four of R_1, R_2, R_3, R_4 and R_5 are hydrogen. Preferably, R_1 is hydrogen, and at least one of R_2 and R_3 is hydrogen. Alternatively R_2 and R_3 are hydrogen. Yet more preferably, R_1, R_2, and R_3 are hydrogen.

Most preferably, the grafting step (ii) involves reacting the substrate having one or more functional groups from which polymers may be grown with a plurality of compounds of formula 

, and/or its enantiomers.

In embodiments where the substrate is formed from nanofibres formed from different polymers, each different kind of polymer nanofibre may be grafted with a different polymer in the grafting step. That could, for instance, result from different functional groups being present on the different polymer nanofibres. Alternatively, the same polymer may be grafted to each of the different kinds of polymer nanofibre in the substrate.

Typical functional groups include hydroxyl, amino and carboxyl groups. In the case where the substrate is formed of one or more cellulose or cellulose acetate nanofibres, the functional groups are typically hydroxyl groups.

Typically, the substrate is treated between steps (i) and (ii) to introduce the one or more functional groups, or the substrate is treated between steps (i) and (ii) to deprotect or activate any functional groups on the substrate, or the substrate is treated between steps (i) and (ii) to increase the number/density of functional groups on the substrate. Preferably, the substrate is treated between steps (i) and (ii) to introduce the one or more functional groups, or to deprotect or activate any functional groups on the substrate. More preferably, the substrate is treated between steps (i) and (ii) to

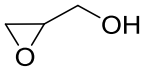
CLAIMS

1. A process for preparing a functionalised polymeric chromatography medium, which process comprises

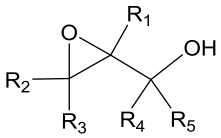
(i) providing a substrate formed of one or more polymer nanofibres,

(ii) grafting one or more neutral polymer chains from the substrate, and

(iii) contacting the grafted product with a functionalising reagent which functionalises the product of step (ii) with ligands selected from the group consisting of negatively charged moieties, positively charged moieties, proteins, peptides, antibodies or fragments thereof, dyes, histidine, groups containing a metal cation and hydrophobic groups,

wherein step (ii) comprises reacting a plurality of compounds of formula 

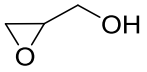
, and/or its enantiomers, and/or its derivatives of formula (I) and/or enantiomers and/or diastereomers thereof:



(I)

with one or more functional groups present on the nanofibre substrate, and growing one or more neutral polymer chains directly from the one or more functional group present on the nanofibre substrate,

wherein R_1, R_2, R_3, R_4 and R_5 may be the same or different, and are chosen from H, halo, C_1-C_4 alkyl, or C_1-C_4 alkoxy provided that at least one of R_1, R_2, R_3, R_4 or R_5 is not hydrogen.

2. The process according to claim 1, wherein step (ii) comprises reacting a plurality of compounds of formula 

, and/or its enantiomers, with one or more functional groups present on the nanofibre substrate.

3. The process according to claim 1 or claim 2, wherein the polymer nanofibres comprise a polymer selected from the group consisting of cellulose, cellulose acetate, polysulfones, polyamides, polyacrylic acid, polymethacrylic acid, polyacrylonitrile, polystyrene, polyethylene oxide, and mixtures thereof, preferably wherein the polymer is selected from the group consisting of cellulose, cellulose acetate, and a mixture therof.

4. The process according to any one of claims 1 to 3, wherein the amount of polymer introduced in step (ii) is between 500-60,000 μmol/g of grafted product.

5. The process according to any one of claims 1 to 4, wherein the step of contacting the grafted product with a functionalising reagent introduces one or more ligand groups into the grafted product which renders the functionalised product comprising the one or more ligand groups suitable for use as a chromatography medium.

6. The process according to claim 5, wherein the density of said ligand groups in the product of step (iii) is between 100-2,500μmol/g of chromatography medium.

7. The process according to any one of the preceding claims, wherein the pressure drop over the functionalised polymeric chromatography medium is less than 2MPa when a liquid phase is passed through a thickness of 0.05 to 10mm of the medium at a flow rate of between 1 to 640 membrane volumes per minute.

8. The process according to any one of the preceding claims, wherein the productivity of the functionalised polymeric chromatography medium is 50mg/mL/min to 75,000mg/mL/min.

9. The process according to any one of the preceding claims, wherein the productivity of the functionalised polymeric chromatography medium is greater than 200mg/ml/min.

10. The process according to any one of the preceding claims, wherein the grafting step (ii) has the effect of increasing the dynamic binding capacity (DBC) of the functionalised polymeric chromatography medium.

11. The process according to any one of claims 1 to 10, wherein the substrate is treated between steps (i) and (ii) to introduce the one or more functional groups, or the substrate is treated between steps (i) and (ii) to deprotect or activate any functional groups on the substrate, or the substrate is treated between steps (i) and (ii) to increase the number/density of functional groups on the substrate.

12. The process according to any one of claims 1 to 10, wherein the grafting step (ii) is carried out under conditions which additionally, in the same step, introduce the one or more functional groups, or deprotect or activate any functional groups on the substrate, or increase the number/density of functional groups on the substrate.

13. The process according to claim 12, wherein the grafting step (ii) is effected in the presence of aqueous alkali, preferably NaOH or KOH, in water or water:ethanol, preferably in water.

14. The process according to any one of the preceding claims, wherein the one or more functional groups present on the nanofibre substrate comprise hydroxyl groups.

15. The process according to any one of the preceding claims, wherein in the functionalisation step (iii), the functionalising reagent is selected from the group consisting of glycidyltrimethylammonium chloride (GMAC), 1,4-butanesulfone, sodium chloroacetate, $NaIO_4$ followed by Protein A, divinylsulfone followed by Protein A, allyl glycidyl ether followed firstly by a halohydrin-forming reagent, preferably N-bromosuccinamide, and subsequently by Protein A, allyl glycidyl ether followed firstly by an epoxide-forming reagent and subsequently by Protein A, and combinations thereof.

16. The process according to any one of claims 10 to 15, wherein one or more of the polymer chains is branched.

17. The process according to claim 16, wherein

- the chromatography method is a cationic exchange method, and the reagent functionalises the chromatography medium with one or more charged groups comprising one or more carboxylate, sulphonate or phosphonate moieties;
- the chromatography method is an anionic exchange method, and the reagent functionalises the chromatography medium with one or more charged groups comprising one or more quaternary amino or diethylamine moieties;
- the chromatography method is an affinity capture chromatography method, and the reagent functionalises the chromatography medium with one or more proteins, peptides, antibodies or fragments thereof, dyes, histidine groups, or groups containing a metal

cation;

- the chromatography method is a hydrophobic interaction chromatography method, and the reagent functionalises the chromatography medium with one or more propyl, butyl, phenyl, or octyl groups; or
- the chromatography method is a mixed mode chromatography method, and the reagent functionalises the chromatography medium with one or more MEP, octylamine, N-benzyl methyl ethanolamine or N-benzoyl-homocysteine groups.

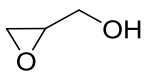
18. The process according to any one of the preceding claims, wherein in step (iii) the functionalising reagent functionalises the grafted product with one or more Protein A molecules.

19. The process according to any one of the preceding claims, wherein step (iii) involves multiple steps that together functionalise the product of step (ii) as a chromatography medium.

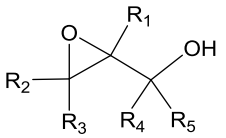
20. The process according to claim 19, wherein in step (iii):
(a) the grafted product is contacted with a functionalising reagent selected from the group selected from divinyl sulfone, allyl glycidyl ether, and combinations thereof;
(b) the product of step (a) is optionally treated with a halohydrin-forming reagent; and
(c) the product of step (b) is contacted with Protein A.

21. The process according to any one of the preceding claims, wherein the substrate is in the form of a membrane.

22. The process according to any one of the preceding claims, wherein the nanofibres have mean diameters from 10nm to 1000nm.

23. The process according to any one of the preceding claims, which process comprises
(i) providing a substrate formed of one or more cellulose nanofibres,
(ii) grafting one or more polyglycerol polymer chains directly from the substrate by reacting a plurality of compounds of formula 

, and/or its enantiomers, and/or its derivatives of formula (I) and/or enantiomers and/or diastereomers thereof:

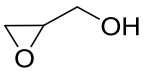


(I)

with one or more hydroxyl groups present on the substrate, and

(iii) contacting the grafted product with a reagent which functionalises the product of step (ii)with ligands selected from the group consisting of negatively charged moieties, positively charged moieties, proteins, peptides, antibodies or fragments thereof, dyes, histidine, groups containing a metal cation and hydrophobic groups,

wherein R_1, R_2, R_3, R_4 and R_5 may be the same or different, and are chosen from H, halo, C_1-C_4 alkyl, or C_1-C_4 alkoxy provided that at least one of R_1, R_2, R_3, R_4 or R_5 is not hydrogen.

24. The process according to claim 23, wherein step (ii) comprises grafting one or more polyglycerol polymer chains directly from the substrate by reacting a plurality of compounds of formula 

, and/or its enantiomers with one or more hydroxyl groups present on the substrate.

25. A functionalised chromatography mediumcomprising:

a substrate formed of one or more polymer nanofibers having one or more functional groups; and

one or more neutral polymer chains that have been directly grown from the one or more functional groups present on the nanofibre substrate and have then been functionalised with ligands selected from the group consisting of negatively charged moieties, positively charged moieties, proteins, peptides, antibodies or fragments thereof, dyes, histidine, groups containing a metal cation and hydrophobic groups, according to the process as claimed in any one of the preceding claims.

26. A process for preparing a chromatography cartridge, which process comprises carrying out the process of any one of claims 1 to 24 and incorporating the thus-obtained product into a cartridge.

27. A chromatography cartridge which (a) is obtainable by the process of claim 26, or (b) which comprises one or more functionalised chromatography media according to claim 25.

28. Use of a functionalised chromatography medium according to claim 25 or a chromatography cartridge according to claim 27 in chromatography.

29. A process for isolating one or more biological molecules from a mobile phase, which process comprises contacting one or more biological molecules in a mobile phase with a functionalised chromatography medium according to claim 25 or a chromatography cartridge according to claim 27.

30. The process according to claim 29 wherein the one or more biological molecules in a mobile phase is contacted with the functionalised chromatography medium for a period of time of one minute or less.

31. The process according to claim 29 or claim 30, wherein the one or more biological molecules are one or more monoclonal antibodies, or proteins engineered to exhibit a site with an affinity for Protein A binding, and the functionalised chromatography medium carries at least one Protein A ligand group.