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Functionalization of chitosan oligomers: from aliphatic epoxide to cardanol grafted oligomers for oil in water emulsions

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KEYWORDS: Oligochitosan, amphiphilic structures, oil in water emulsions

ABSTRACT

Hydrophobically modified chitooligosaccharides (COS) were tested for suitability as an emulsifier in cationic bituminous emulsions. COS of polymerization degree (DP) 5, 10, 15 and 20 were obtained by nitrous acid deamination. A complete study on depolymerization and precise product and side product characterization was led upstream. Chemical modification of COS was performed to reach amphiphilic structures using three fatty epoxide with growing chain length (Butyl (C4), octadecyl (C9) and hexadecyl glycidyl ether (C16)). The grafting efficiency according to reaction conditions were established. Different substitution degrees (DS) were obtained by modulating the ratio of fatty epoxy/NH₂. It was shown that after a certain DS, the oligomers thus formed were not water-soluble anymore. At the end, cardanol glycidyl ether was grafted on DP 5, 10 and 15 COS, cardanol being a biobased compound extracted from cashew nut shell, this reaction led to a potentially fully biobased structure. Water-soluble candidates with higher DS were used as surfactant to emulsify motor oil as a simulation of bitumen. Cardanol-chitosan based surfactant led to direct oil in water emulsion (60/40 w/w) formed of particles of 15 μm average that were stable at least for 24 h.

INTRODUCTION

Bitumen emulsions are composed of an oily phase composed of bitumen (40 to 80% (w/w)) and an aqueous phase (20 to 60% (w/w)) that contains a surfactant (between 0.1 and 2.5% (w/w))^{1 2}. The bitumen is sheared through a colloid mill and bitumen droplets of 1 to 20 μm are dispersed in the aqueous phase. Nowadays, bitumen emulsions are mainly cationic, meaning that they are stabilized by a cationic surfactant like fatty amines, fatty amidoamines, fatty imidazolines and fatty quaternary ammonium salts which are often toxic³.

Chitosan is a positively charged polysaccharide with a potential use in several areas, including applications in cosmetics, biotechnology and medicine^{4 5}. It is a natural nontoxic source of potentially cationic free amines for which emulsifying properties have already been investigated⁶. In some cases, chitosan can be used for Pickering emulsions^{7 8 9}. Interestingly, it was shown that crude chitosan might be used to partially substitute industrial surfactants (up to 60% replacement) for bitumen emulsions¹⁰. Those chitosan surfactant are high molecular weight polysaccharides that induce high viscosity when concentrated which make them difficult to modify chemically to reach efficient amphiphilic structures. When reducing the molecular weight of this hydrophilic polymer, chitooligosaccharides (COS) can be obtained. COS are easier to functionalized¹¹ to reach amphiphilic structure able to self-assemble. Generally, COS are modified with fatty acids and used to form micelles for drug delivery^{12 13} but they could find application in other area such as direct oil in water emulsion.

Several ways to obtain chitosan oligomers are reported in literature^{14 15 16}. Among them, the action of nitrous acid^{17 18} was shown to be applicable and reproducible on any kind of crude chitosan leading to low molecular weights oligomers¹⁹. Though, it leads to formation of 2,5-ahhydromannose (AMF) chain ends which are electrophilic and undergo intramolecular imines formation. This side reaction was underlined in a previous paper¹⁹. In this work, it was shown that imines represent 23% of the amines for DP10 COS. As

1
2
3 described before ²⁰, it is possible either to reduce or to react the aldehyde chain-end to
4
5 avoid this side reaction. Nevertheless, this reaction was never investigated directly on AMF
6
7 and no quantification or deep characterization were provided. In this work, we first focus
8
9 on the oligomer synthesis and try to deeper describe both reactions that can avoid imine
10
11 formation. The previous work was completed providing a model study on AMF, NMR
12
13 characterizations and quantifying the remaining aldehyde groups.
14
15

16
17 There is a growing interest in functionalization of COS in order to broaden their
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19 applications ^{13 21 22}. Thus, COS are easily functionalizable due to the presence of free
20
21 primary amines on the sugar units. Thanks to their shorter hydrophilic chain, their
22
23 functionalization with fatty moieties, one of the most common reaction described, can lead
24
25 to amphiphilic structures ^{23 24}. Nevertheless, the grafting of fatty epoxide with different chain
26
27 length studied in this work was never described before. Cardanol is a natural fatty oil
28
29 coming from cashew nutshell. COS modified with epoxidized cardanol seems to be a novel
30
31 opportunity to synthesize fully biobased amphiphilic moieties from waste recovery.
32
33

34
35 Hydrophobically modified COS appear to be a potential new biobased emulsifier for
36
37 bitumen emulsions. The objective of the present study was to reach amphiphilic COS and
38
39 evaluate their potential use in bitumen emulsion stabilization through a model study
40
41 involving motor oil.
42
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44

45 MATERIAL AND METHODS

46 47 **Material**

48
49 Chitosan DA around 15% CH30 (Mw 30 kg/mol) was purchased from Glentham
50
51 Science and used as received; butyl glycidyl ether (C4), octyl/decyl glycidyl ether (C9),
52
53 hexadecyl glycidyl ether (C16), triethylamine (Et₃N), hydroxylamine hydrochloride
54
55 (NH₂OH), sodium borohydride (NaBH₄), acetic acid (AcOH), sodium acetate (NaAc),
56
57 sodium nitrite (NaNO₂), lithium bromide (LiBr), sodium azide (NaN₃), trimethylsilyl-3-
58
59 propionic-2,2,3,3-D4 acid sodium salt, sodium hydroxide (NaOH), hydrochloric acid (HCl),
60

1
2
3 and acetone (>98%) were purchased from Sigma and used without purification; Methanol
4 (MeOH) and HPLC water (>99%) was purchased from VWR, engine oil Cirkan C was
5 purchased from Total.
6
7
8

9 10 **AMF and AMF oxime synthesis**

11
12 The AMF molecule was synthesized according to the previous protocol ¹⁹. Briefly,
13
14 1 g of glucosamine was dissolved in deionized water (20 mL) for 4h. One eq of NaNO₂
15
16 were added in 10 mL of distilled water to the solution. The reaction was carried out for 3 h.
17
18 AMF was then reduced to alcohol by adding NaBH₄ (1 eq) or transformed into an oxime by
19
20 adding NH₂OH (1 eq). Samples were taken after 30 min, 1 h, 2 h, 3 h and analyzed by UV.
21
22 At the end of the reaction, the products were freeze-dried and characterized by ¹H NMR
23
24 and SEC.
25

26
27 ¹H NMR of AMF (D₂O, 400 MHz, 298 K, pH 5.5): δ (ppm) 8.50 (s, 1H, H1), 5.10 (d,
28
29 5.0 Hz, 1H, H1' gemdiol), 4.20 (t, 6.0 Hz, 1H, H2), 4.10 (t, 6.0 Hz, 1H, H5), 4.00 (m, 1H,
30
31 H5), 3.90 (m, 1H, H3), 3.80 (m, 1H, H4 and 2H, H6);
32

33
34 ¹H NMR of AMF + NaBH₄ (D₂O, 400 MHz, 298 K, pH 5.5): δ (ppm) 8.50 (s, 1H, H1),
35
36 4.20 (t 6.0 Hz, 1H, H2), 4.00 (m, 1H, H5), 3.85 (m, 1H, H3), 3.80 (m, 1H, H5), 3.70-3.80
37
38 (m, 2H, H1' (after reduction), 1H H4 and 2H, H6)
39

40
41 ¹H NMR of AMF-oxime (D₂O, 400 MHz, 298 K, pH 5.5): δ (ppm) 8.20 (s, 1H, H1),
42
43 7.60 (d, 8Hz, 1H H1 (E)-oxime), 7.00 (d, 7.0 Hz, 1H H1 (Z)-oxime), 5.10 (d, 5Hz, 1H,
44
45 H1' gemdiol), 4.35 (t 4.0 Hz, 1H, H2''), 4.25 (m, 1H, H2), 4.10 (m, 1H, H5), 4.00 (m, 1H,
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47 H3), 3.70 (m, 1H, H4 and 2H, H6)
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49 50 **COS and COS oxime synthesis**

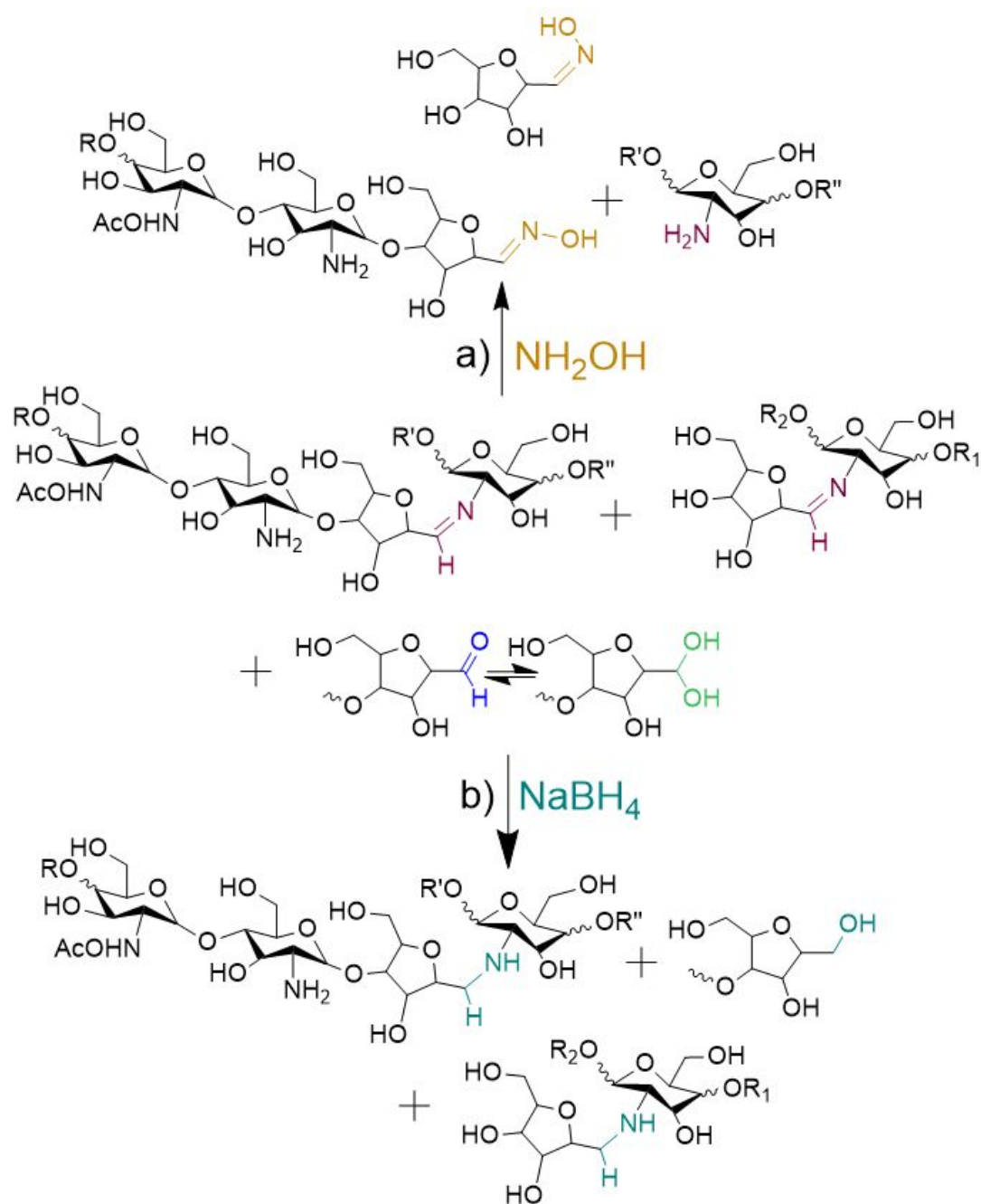
51
52 The method was adapted to chitosan as described before ²⁵. Briefly, 1 g of chitosan
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54 was dissolved in a solution of acetic acid (350 μL in 90 mL of distilled water) overnight. The
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56 next day, 0.4 eq (to obtain a DP10) and 0.8 eq (to obtain a DP5) of NaNO₂ were added in
57
58 10 mL of distilled water to the solution. The reaction was carried out for 3 h at 50 ° C. At
59
60 the end of the reaction, the pH was raised to 8 by adding a concentrated solution of NaOH

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3 and 0.4 eq (0.8 eq respectively) of NaBH₄ in the case of reduction or of NH₂OH in the case
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5 of formation of oximes were added to the solution. Samples were taken after 30 min, 1 h,
6
7 2 h, 3 h and analyzed by UV. At the end of the reaction, the oligomers were obtained by
8
9 freeze-drying and characterized by ¹H NMR, 2D COSY NMR, FTIR and SEC (Scheme 1).
10

11
12 ¹H NMR of DP10 (D₂O, 400 MHz, 298 K, pH 5.5): δ (ppm) 8.20-8.50 (s, H
13
14 aldehydes), 8.00- 7.10 (m, H imines), 5.01 (d, 5 Hz, 1H, H1' gemdiol), 4.50 (m, 1H, H2-
15
16 AMF), 4.32 (m, 1H, H3-AMF), 4.10 (m, 1H, H5-AMF), 3.20-4.00 (m, 1H H2 acetyl, 1H, H3;
17
18 1H, H4; 1H, H5; 2H, H6; 1H, H4-AMF and 2H, H6-AMF), 2.50 (m, 1H, H2), 2.00 (s, 3H,
19
20 HCH₃), 4.80 (s, HOD)
21

22
23 ¹H NMR of DP10+NaBH₄ (D₂O, 400 MHz, 298 K, pH 5.5): δ (ppm) 8.20-8.50 (s, H
24
25 aldehydes), 4.50 (m, 1H H2-AMF), 4.30 (m, 1H, H3-AMF), 4.11 (m, 1H, H5-AMF), 4.00 (m,
26
27 1H, H4 2H, H6) 3.20-4.00 (m, 1H H2 acetyl, 1H, H3; 1H, H4; 1H, H5; 2H, H6; 1H, H4-AMF
28
29 and 2H, H6-AMF), 2.50 (m, 1H, H2), 2.00 (s, 3H, HCH₃), 4.80 (s, HOD)
30

31
32 ¹H NMR of DP10-oxime (D₂O, 400 MHz, 298 K, pH 5.5): δ (ppm) 8.20-8.50 (s, H
33
34 aldehydes), 7.56 (d, 8Hz, 1H, Ho1 (E)), 7.00 (d, 7.0 Hz, 1H, Ho1 (Z)), 5.00 (d, 5.0 Hz, 1H
35
36 Ho2 (Z)), 4.60 (m, 1H, Ho5), 4.48 (t, 4.0 Hz, 1H, Ho2 (E)), 4.39 (m, 1H, H2-AMF), 4.22 (m,
37
38 1H, Ho3), 3.20- 4.00 (m, 1H H2 acetyl, 1H, H3; 1H, H4; 1H, H5; 2H, H6; 1H, Ho4 and 2H,
39
40 Ho6), 3.00 (m, 1H, H2), 2.00 (s, 3H, HCH₃), 4.80 (s, HOD)
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Scheme 1. Reaction of a) NH_2OH to obtained COS-oxime b) NaBH_4 for reduction of the aldehyde chain end of COS

Functionalization of chitosan oligomers

The depolymerization of high molecular weight chitosans was performed by nitrous deamination involving NaNO_2 as previously described¹⁷. Briefly, 1g (w/w) of chitosan was dissolved in 80 mL of AcOH (350 μ L in 90 mL of deionized water) solution overnight (pH 4.5). Once solubilized, a freshly prepared solution (10 mL) containing the desired equivalent ratio of $\text{NaNO}_2/\text{NH}_2$ (0.1, 0.2, 0.4, 0.8) was added to the solution. The solution was stirred at 50°C for 3h. After cooling down, NH_2OH (respectively with the amount of NaNO_2) was added and the solution was stirred for another 24h. The final product was freeze-dried for 24h and oligomers of DP 20, 15, 10, 5 were collected.

1g of DP20 oligomers were solubilized in 20 mL deionized water with 1 eq AcOH/ NH_2 . 1g of DP15 oligomers were solubilized in 20mL deionized water. 1g of DP10 and DP5 were solubilized in 10 mL deionized water and Et_3N was added to reach pH7. 20 mL of MeOH were added to each solution. To this oligomer solution, butylglycidyl ether (C4), octyl/decyl glycidyl ether (C9) or hexadecyl glycidyl ether (C16) in 10 mL MeOH was added with various amounts. The solution was stirred for 24h at 80°C. The products were precipitated in 400 mL of cold acetone, washed with acetone and centrifuged to be collected. Finally, the product was dried under vacuum for 6h to get rid of residual solvent.

The integrals provided are those expected for 100% functionalization.

^1H NMR of DP10 + C4 (D_2O , 400 MHz, 298 K, pH 5.5): δ (ppm) 8.20-8.50 (s, H aldehydes), 7.56 (d, 8Hz, 1H, Ho1 (E)), 7.00 (d, 7.0 Hz, 1H, Ho1 (Z)), 5.00 (d, 5.0 Hz, 1H Ho2 (Z)), 4.65 (m, 1H, Hb), 4.60 (m, 1H, Ho5), 4.48 (t, 4.0 Hz, 1H, Ho2 (E)), 4.39 (m, 1H, H2-AMF), 4.22 (m, 1H, Ho3), 3.20- 4.00 (m, 1H H2' acetyl, 1H, H3; 1H, H4; 1H, H5; 2H, H6; 2H Hg; 1H, Ho4 and 2H, Ho6), 2.95 (m, 2H, Hc), 2.75 (m, 2H, Ha), 2.55 (m, 1H, H2), 2.00 (s, 3H, HCH_3), 1.40 (t, 2H, Hf), 1.18 (m, 2H, He), 0.74 (t, 3H, Hd), 4.80 (s, HOD)

^1H NMR of DP10 + C9 (D_2O , 400 MHz, 298 K, pH 5.5): δ (ppm) 8.20-8.50 (s, H aldehydes), 7.56 (d, 8Hz, 1H, Ho1 (E)), 7.00 (d, 7.0 Hz, 1H, Ho1 (Z)), 5.00 (d, 5.0 Hz, 1H Ho2 (Z)), 4.65 (m, 1H, Hb), 4.60 (m, 1H, Ho5), 4.48 (t, 4.0 Hz, 1H, Ho2 (E)), 4.39 (m, 1H,

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2
3 H2-AMF), 4.22 (m, 1H, Ho3), 3.20- 4.00 (m, 1H H2' acetyl, 1H, H3; 1H, H4; 1H, H5; 2H,
4 H6; 2H Hg; 2H, Ha; 2H, Hc; 1H, Ho4 and 2H, Ho6), 2.55 (m, 1H, H2), 2.00 (s, 3H, HCH₃),
5 1.57 (m, 2H, Hf), 1.28 (m, 14H, He), 0.85 (m, 3H, Hd), 4.80 (s, HOD)
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9
10 ¹H NMR of DP10 + C16 (D₂O, 400 MHz, 298 K, pH 5.5): 8.20-8.50 (s, H aldehydes),
11 7.56 (d, 8Hz, 1H, Ho1 (E)), 7.00 (d, 7.0 Hz, 1H, Ho1 (Z)), 5.00 (d, 5.0 Hz, 1H Ho2 (Z)), 4.65
12 (m, 1H, Hb), 4.60 (m, 1H, Ho5), 4.48 (t, 4.0 Hz, 1H, Ho2 (E)), 4.39 (m, 1H, H2-AMF), 4.22
13 (m, 1H, Ho3), 3.20- 4.00 (m, 1H H2' acetyl, 1H, H3; 1H, H4; 1H, H5; 2H, H6; 2H Hg; 2H,
14 Ha; 2H, Hc; 1H, Ho4 and 2H, Ho6), 2.55 (m, 1H, H2), 2.00 (s, 3H, HCH₃), 1.57 (m, 4H, Hf),
15 1.27 (m, 14H, He), 0.87 (m, 3H, Hd), 4.80 (s, HOD)
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23 1g of oligomer DP5, DP10 or DP15 were solubilized in 20 mL of DMSO at 80°C.
24 Cardanol glycidyl ether in 10 mL of DMSO was added in different amount. The solution
25 was stirred for 24h at 80°C. The products were precipitated in 400 mL of cold acetone,
26 washed with acetone and centrifuged to be collected. Finally, the product was dried under
27 vacuum for 6h to get rid of residual solvent.
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34 ¹H NMR of DP10 + Cardanol glycidyl ether (D₂O, 400 MHz, 298 K, pH 5.5): 8.20-
35 8.50 (s, H aldehydes), 7.56 (d, 8Hz, 1H, Ho1 (E)), 7.00 (d, 7.0 Hz, 1H, Ho1 (Z)), 6.35-6.95
36 (m, 4H, H_{aromatic}) 5.1 (m, 6H, Hh), 5.00 (d, 5.0 Hz, 1H Ho2 (Z)), 4.60 (m, 1H, Ho5), 4.48 (t,
37 4.0 Hz, 1H, Ho2 (E)), 4.39 (m, 1H, H2-AMF), 4.22 (m, 1H, Ho3), 3.20-
38 4.00 (m, 1H H2' acetyl, 1H, H3; 1H, H4; 1H, H5; 2H, H6; 2H Hg; 2H, Ha; 2H, Hc; 1H, Ho4
39 and 2H, Ho6), 2.55 (m, 1H, H2), 2.00 (s, 3H, HCH₃), 1.87 (m, 6H Hi; 2H, Hg), 1.34 (m, 2H,
40 Hf), 1.15 (s, 10H, He), 0.69 (s, 3H, Hd), 4.80 (s, HOD)
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49 **Oil in water emulsions with modified COS**

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51 Motor oil was used to simulate bitumen. Motor oil having the same viscosity at 40°C
52 than bitumen at 150°C. Oil in water emulsions were produced as followed: COS cardanol
53 (0.2% (w/w) of the total emulsion) was dissolved in 0.2% HCl (w/w of the total emulsion))
54 aqueous solution (40% (w/w) of the total emulsion). Both oil and water were heated at
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3 40°C. Oil was added to the aqueous phase (60/40 (w/w)) and blended for 5 min with an
4
5 ultraturax at 9000 rpm.
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8 **Characterizations**

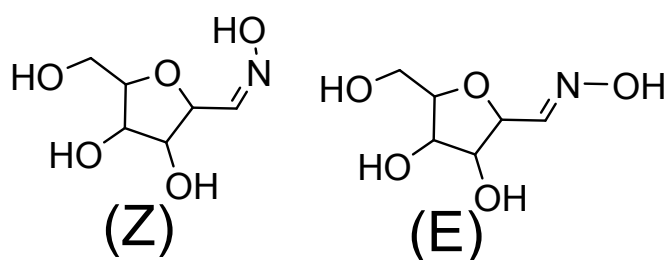
9
10 ¹H NMR analyses were performed using a Bruker Avance 400 MHz NMR
11 spectrometer at 298 K. NMR samples were prepared by dissolving 15 mg of product in 0.5
12 mL of D₂O with trimethylsilyl-3-propionic-2,2,3,3-d₄ acid sodium salt as internal reference
13 (δ 0.00 ppm). The chemical shifts were reported in part per million relative to tetra
14 methylsilane. Spin multiplicity is shown by s=singlet, d=doublet, t=triplet, q=quadruplet and
15 m=multiple. Substitution degrees (DS) of oligo chitosans were determined by ¹H NMR. IR
16 spectra were recorded on Nicolet 6700 Fourier Transform Infra-Red spectrometer. Molar
17 masses were recorded using size exclusion chromatography (SEC) with RI detection (PL
18 aquagel-OH 30 8 μm 100-60.000 g/mol, PL aquagel-OH 40 8 μm 10.000-200.000 g/mol,
19 TSK gel G Oligo PW 7 μm 7.8 mm ID ×30 cm 0-6000 g/mol) calibrated using PEO/PEG
20 standards. 10 mg/mL solutions of oligochitosans were prepared in acetate buffer (1,5%
21 AcOH, 0.8% NaAc, 1 ppm NaN₃, 5 ppm LiBr). The pH (correction for isotopic effect:
22 pD=pH+0.4) were measured by use of a Mettler Toledo pH electrode. UV analyses were
23 carried out using a Beckman DU 640 spectrophotometer. For this, 100 μL of reaction
24 medium were taken and diluted in 900 μL of distilled water and analyzed under UV between
25 200 and 400 nm. Size distribution of emulsions were measured by optical microscope on
26 a Leica microsystem equipped with a 50x objective. 200 droplets were measured using the
27 Leica software.
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48 **RESULTS AND DISCUSSION**

49 **Synthesis of chitosan oligomer-oximes**

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52 Regarding the reduction of the aldehyde chain end, reduction and reaction with
53 hydroxylamine was already described beforehand on 5 and 10 kg/mol COS²⁰.
54
55 Nonetheless, no information whereas provided on lower molecular weight COS (DP5 to
56 DP20). In addition, we thought it was important to complete this work by first doing a model
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3 study on AMF in order to better understand both reactions (Scheme 1) and quantify all the
4 species involved. Products were therefore characterized by ^1H NMR (Figure 2), UV and
5 size exclusion chromatography. When reduced with NaBH_4 , the percentage of residual
6 aldehyde was quantified by ^1H NMR by using the ratio between the aldehyde signal and
7 the signals corresponding to the five other protons present on AMF. It was also noted that
8 the signal corresponding to the gem-diol form no longer appears in the reduced version of
9 AMF (Figure 2 1) b)). Thus, 18% of aldehyde was not reduced using one equivalent of
10 NaBH_4 . The shift between aldehyde peaks is due to the pH differences, in particular due to
11 the presence of residual salts. Regarding the use of hydroxylamine, ^1H NMR spectroscopy
12 confirmed the formation of oximes with the appearance of signals at 7.5 and 7 ppm
13 (Figure 2 1) c)). The two signals correspond to the (E) and (Z) form of the oxime,
14 respectively (Figure 1). Signals of each isomer in water were attributed by analogy with
15 other oximes and oxime ethers described in the literature.^{26 27 28 29}



41 **Figure 1.** Z and E isomers of AMF-oxime

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43
44 The proton of the (E) isomer is the most de-shielded as described several times in
45 the literature^{26, 30} (Figure 2 1.c)). Form (E) is therefore the most abundant (75%). Moreover,
46 the coupling constants measured are in agreement with the literature since J_{trans} (8 Hz) >
47 J_{cis} (7Hz).
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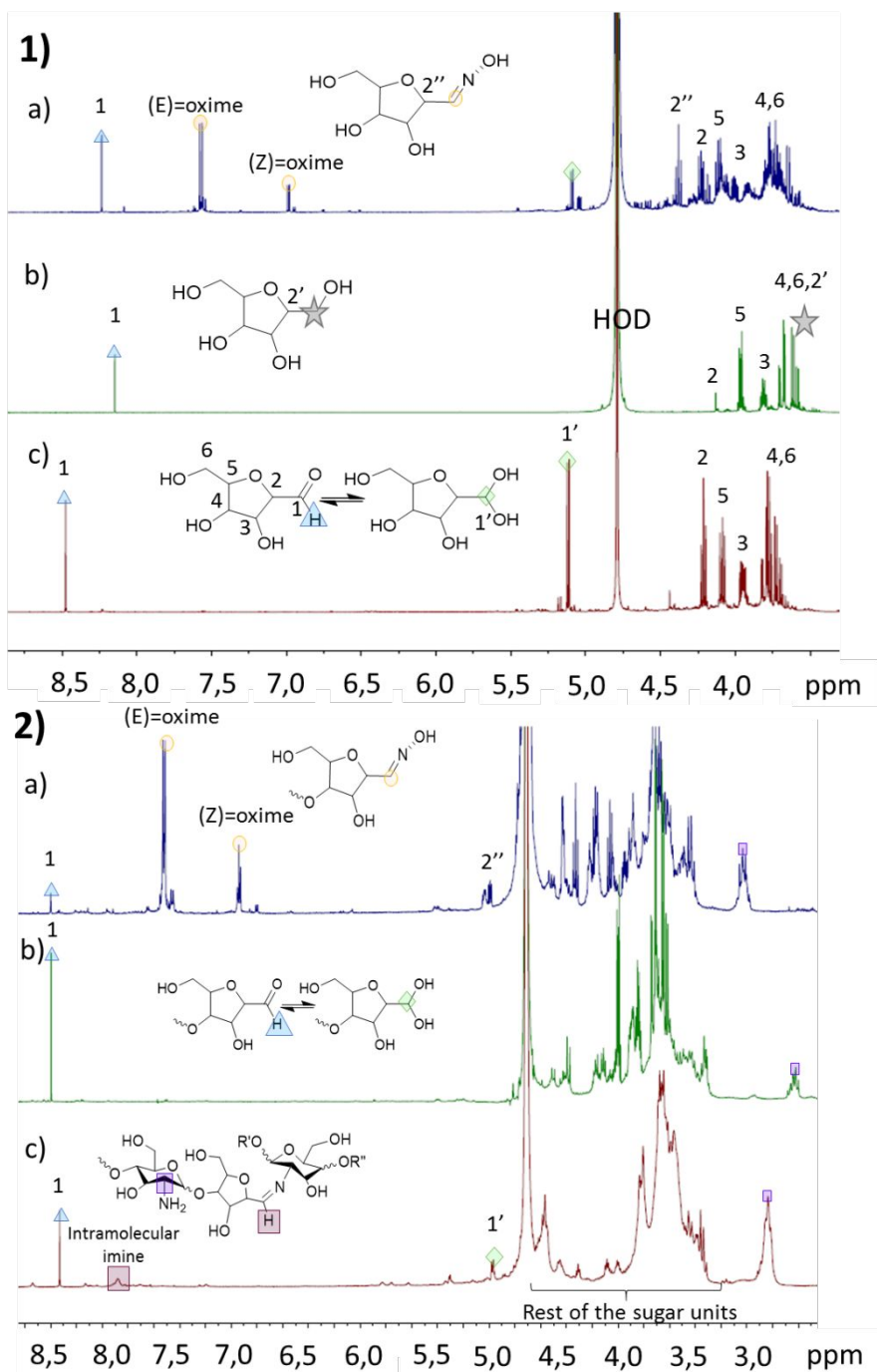


Figure 2. ^1H NMR spectra in D_2O of 1) AMF a) oxime formation b) NaBH_4 reduction c) crude oligomer and 2) COS DP5 with a) oxime formation b) NaBH_4 reduction c) crude oligomer

These two reactions were then transposed to DP5 and DP10 oligomers. First, the reactions were monitored by UV by observing the signal at 270 nm corresponding to both imines and aldehydes formed during the depolymerization. It was shown that the signal intensity decreased over time when NaBH_4 and NH_2OH were respectively added. The UV

spectra in Figure 3 represent the difference between the signals of the oligomers of DP5 and DP10 before and after 3h reactions with NaBH_4 or NH_2OH respectively. In both cases, a large decrease in absorbance is visible after 3 hours and no further evolution was observed after this time. Nevertheless, there is still a signal corresponding to the residual aldehydes. We noticed a more important decrease when using hydroxylamine.

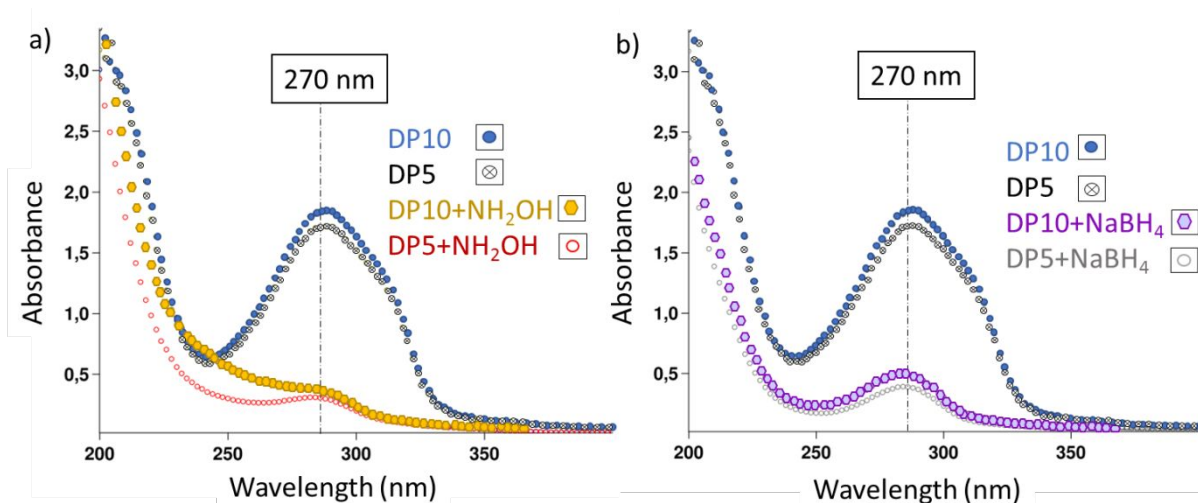


Figure 3. UV spectra of a) DP5 and DP10 before and after action of NH_2OH b) DP5 and DP10 before and after action of NaBH_4

Both reactions were confirmed by ^1H NMR (Figure 2 2)). When reduced with NaBH_4 , a disappearance of the signal corresponding to the gem-diol form (5 ppm) and a decrease in the aldehyde signal is visible from COSY. At the end, 80% of the aldehydes/gem-diols were reduced to alcohol. There are no more intramolecular imines in the medium since they were modified by the action of the reducing agent. The use of NH_2OH also avoided intramolecular imine formation. In general, heteroatoms directly linked to another heteroatom exhibit increased nucleophilicity compared to systems in which the heteroatom is replaced by a carbon. This observation can be explained by examining the most occupied molecular orbital (HOMO) of the nucleophile. Overall, the higher is the energy of a nucleophile's HOMO orbital, the higher is the reactivity. The presence of a heteroatom raises the energy of the HOMO orbital and therefore makes it more nucleophilic. This phenomenon is known as the α effect³¹. Therefore, hydroxylamine, with a nitrogen atom directly bonded

to an oxygen atom, is much more nucleophilic than the amines of COS. Thus, the reaction between AMF and NH_2OH is favored over the formation of intramolecular imines. The reaction is indeed based on the imines reversibility. Therefore, the addition of hydroxylamine resulted in the formation of oligomers with oximes at their chain end (Scheme 1). Similarly to AMF alone, the signals corresponding to the two isomers (Z) and (E) of the oxime (7.0 and 7.5 ppm respectively) are present. However, closely to AMF, the reaction was not complete and 10% of aldehyde and gem-diol remained unreacted. Finally, the imine signal between 7.1 and 8 ppm has completely disappeared, which implied the regeneration of 20% additional free amines and less steric hindrance in the oligomer chain. This phenomena implies better potential for further functionalization of COS. In both cases, the presence of residual aldehyde / gem-diol was proven, for the first time in the literature.

The molar masses of the oligomers were measured before and after the action of NaBH_4 or NH_2OH respectively (Table 1). As in the work of Bezrodnykh et al,²⁰ it has been proven that NaBH_4 reduced imines thus creating irreversible branched structures and increasing molar masses, by 30% for DP5 and 10% for DP10. On the contrary, the use of NH_2OH prevented the formation of imines. Besides, hydroxylamine being a small molecule, its addition to oligomers did not imply any variation in molar mass.

Oligomer	Mn crude (g/mol)	Mn with NaBH_4 (g/mol)	Mn oxime (g/mol)
DP5	1,000 ± 35	1,300 ± 30	1,000 ± 40
DP10	1,600 ± 55	1,750 ± 50	1,600 ± 60

Table 1. Molar masses in PEO equivalent according to the type of reaction and DP of the oligomer.

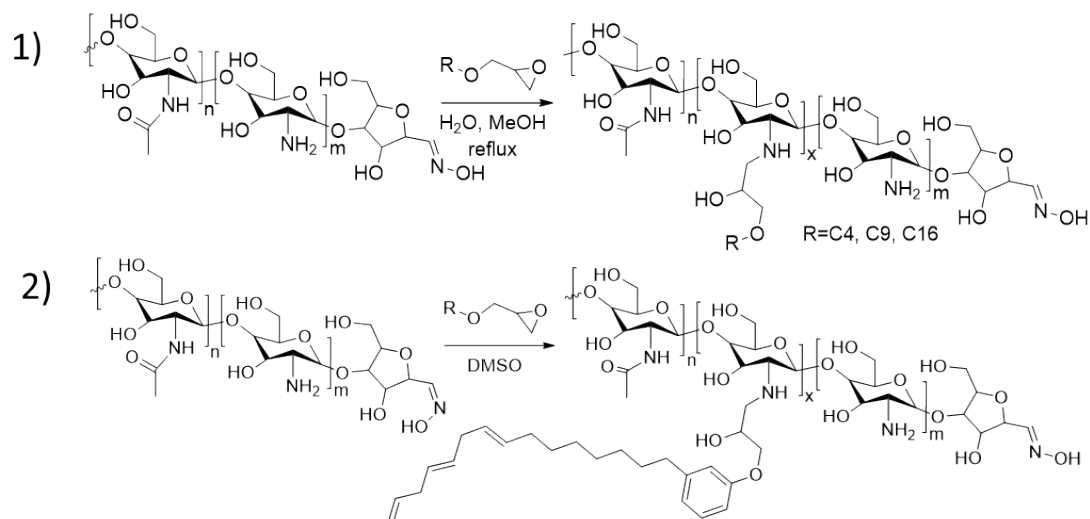
Two methods for preventing the formation of intramolecular imines were deeply investigated in this section. The first one was the reduction of aldehydes chain end by the action of NaBH_4 . This method was effective since 80% of the aldehydes were reduced. However, in this case, the intramolecular imines were also reduced, thus forming

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3 irreversible branched structures confirmed by an increase in molar masses. The second
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5 method involved the use of NH_2OH , which reacted with the aldehydes to form oximes. The
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7 formation of two isomers (Z) and (E) was confirmed and shown by ^1H NMR, the (E) isomer
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9 being the most abundant. Thanks to 2D COSY NMR, signals were fully identified and
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11 attributed (SI 1). This second method allowed the regeneration of 20% of the amines
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13 previously involved in the formation of imines without increasing molar mass. Therefore it
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15 seemed that the method involving hydroxylamine is more suitable for the functionalization
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17 of oligomers.
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21 For the rest of the study, chitosan was depolymerized by the action of NaNO_2 and
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23 then hydroxylamine was added in order to obtain oxime-terminated oligomers that do not
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25 contain intramolecular imines and are therefore ready to be functionalized.
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27 **Functionalization of COS-oximes with aliphatic epoxide**

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29 Oxime ended DP5, DP10, DP15 and DP20 COS were functionalized with three
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31 different fatty epoxies (C4, C9 and C16) according to the reaction given in Scheme 2. At
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33 first, reactions were carried out at pH 5.5 which was the pH recorded when solubilizing
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35 oligomers in deionized water. For DP5 and DP10 reaction can be performed at higher pH
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37 as the pH range solubility is wider for smaller oligomers. This is why the reaction was led
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39 at pH 7 and it was shown that the DS obtained were higher than that at pH 5.5 (around
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41 20% higher). Indeed, at pH 5.5 primary amines are partially cationic whereas at pH 7, they
42
43 are more nucleophilic which makes them more suitable to epoxide ring opening. For DP15,
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45 the reaction was led at pH 5.5 and for DP20 acetic acid was added in order to solubilize
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47 the oligomers resulting in a pH 4.5.
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Scheme 2. Reaction of 1) fatty epoxides and 2) cardanol onto chitosan oligomers

The obtained products were characterized by ^1H NMR spectroscopy to confirm functionalization and calculate the DS (Figure 4). Signals were attributed thanks to 2D COSY NMR (SI 2-4). The grafting efficiency was confirmed for the three epoxy with the appearance of the signals corresponding to the alkyl chain. Fatty epoxy are not water soluble so the signals correspond to a graft oligomer. In addition, signals intensity corresponding to the proton in alpha of the free amine decreased meaning that free amines were consumed for ring opening. To calculate the DS of free amines, the signals d between 0.80 and 0.95 ppm corresponding to the terminal CH_3 of alkyl chains were compared to total intensity of the amine:

$$(1) \quad \text{DS} = \frac{\frac{1}{3}Ad}{\frac{1}{3}Ad + ANH2}$$

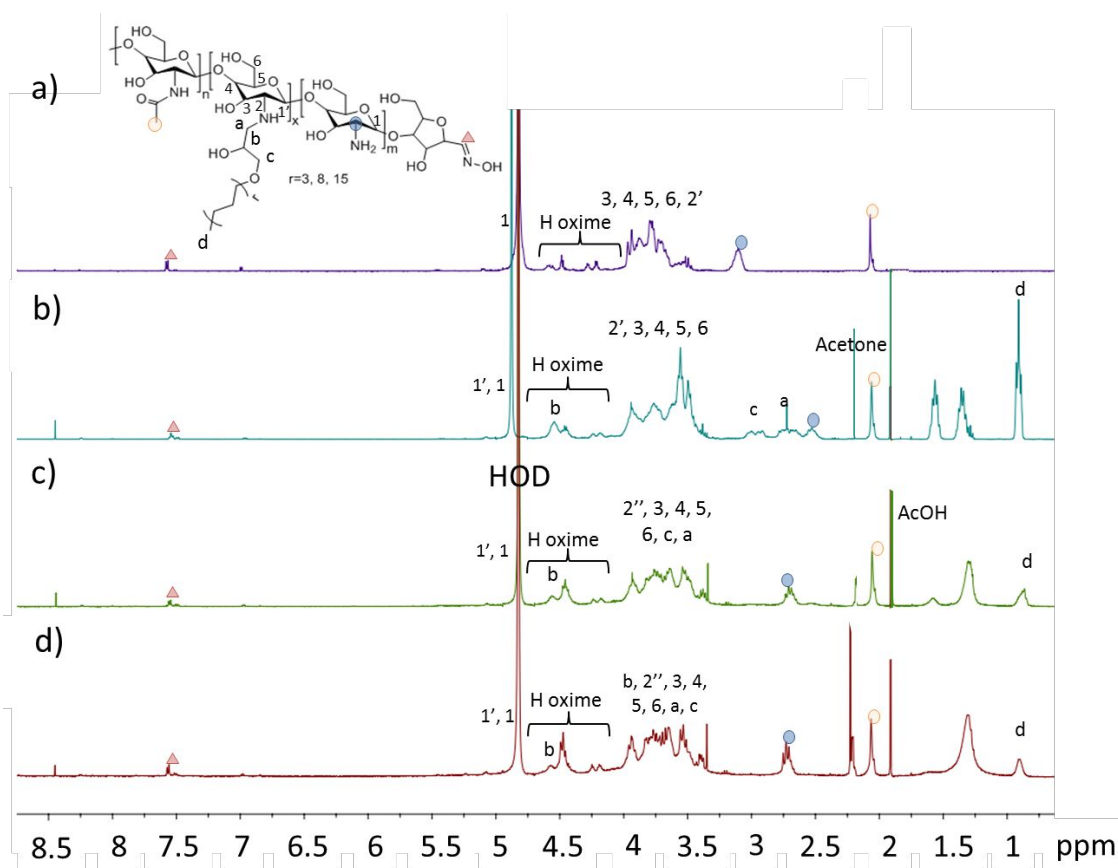


Figure 4. a) ^1H NMR spectrum of DP10 oligochitosan in D_2O b) ^1H NMR spectrum of DP10 oligochitosan modified with C4, DS 90% in D_2O c) ^1H NMR spectrum of DP10 oligochitosan modified with C9, DS 20% in D_2O d) ^1H NMR spectrum of DP10 oligochitosan modified with C16, DS 15% in D_2O

The epoxy/amine ratio was varied in order to reach the maximum DS. A wide DS range was thus obtained according to DPs and epoxides, by varying the equivalent number of fatty chains (Figure 5). Up to 100% DS were reached for C4 epoxy for DP5, 90% for DP10 and 80% for DP15. Those results were quite interesting and higher DS were obtained compared to similar grafting found in the literature. For example Illy et al.²¹ grafted allyl glycidyl ether (AGE, C3) on COS (>2,000 g/mol) for further thiol-ene reaction on the allyl double bond. The reaction was carried out in water at pH 8 as AGE is water-soluble and a DS of 33% was obtained with 2.2 eq of AGE. Mei et al.³² described the grafting of epichlorohydrin on very low molecular weight COS i.e. 550 g/mol, by epoxy amine reaction with 55% DS. Another classical reaction on chitosan is the grafting of the glycidyltrimethylammonium chloride³³.

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Nevertheless, the previous work described the grafting of short epoxide and no example of longer chain such as C9 and C16 were found in the literature. It was shown that the longer are the fatty chains, the lower is the DS obtained, whatever the molar masses of the oligomers. For example, for a DP5, the maximum DS obtained with epoxy C4 is 100% while it is 25% for epoxy C16. Nevertheless, C16 epoxides were successfully grafted on the four different oligomers with relatively correct DS for fatty moieties. In comparison, grafting of fatty acids with such chain length can be found in the literature with a lot of working conditions such as temperature, time and solvent described ^{34 35 36}. DS obtained in this work are therefore included in the value range of DS obtained with fatty acids of C10 to C18. Similarly to fatty acid grafting ^{34 35 36}, it was shown that the higher is the DP, the lower is the DS. In addition, in some cases a limit of water solubility was reached. Indeed, the oligomers were functionalized and no longer soluble in water regardless of the pH, but only in DMSO, so it was impossible to determine their DS. For example, for a DP10 with 0.8 eq of C16, the products were no longer completely water-soluble. Thus, the maximum DS obtained is 15% with 0.5 eq of C16. In the case of DP15, the results were quite satisfactory for the C4 and C9 epoxides with maximum DS of 85 and 37% respectively. For C16 epoxide, the DS were lower which can be explained by lack of solubility for both oligomers and epoxy in the hydro-alcoholic mixture. In addition, the working pH being 5.5 due to the non-solubility of DP15 at higher pH, the amines of the oligomers were less reactive as some were under NH_3^+ form. Nevertheless, oligomers with 11% DS which represent more than one grafting per oligomer chain were obtained. Finally, with DP20, lower DS were obtained regardless to the size of the epoxide due to its greater steric hindrance and lower affinity with fatty chains. In addition, the amine were less nucleophilic due to the acidic environment.

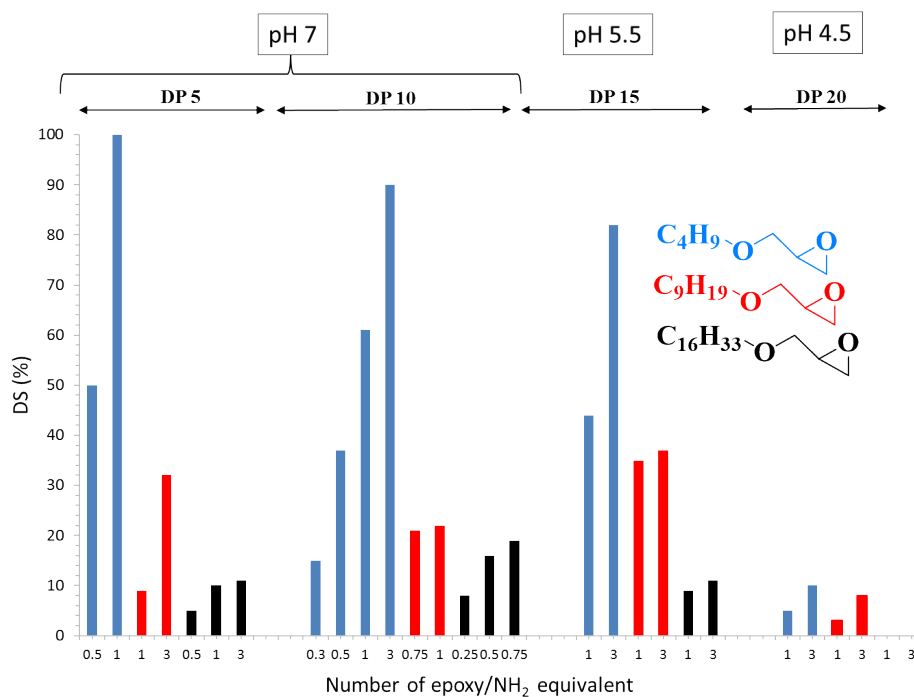


Figure 5. Evolution of DS according to number of equivalents of C₄, C₉ and C₁₆/NH₂ for DP₅, DP₁₀, DP₁₅ and DP₂₀

At the end of the reaction, the filtrate (mixture of acetone, water and methanol) was evaporated in order to analyze the non-precipitated residues. A soluble viscous mixture was obtained. The ¹H NMR analysis in D₂O shows the presence of unfunctionalized oligomers and residual free AMF. However, the product is not completely soluble in water. The ¹H NMR analysis in DMSO, in which the product is completely soluble, shows a mixture of oligomers and epoxides but it is not possible to differentiate functionalized oligomers from unreacted epoxides. Based on the previous results, it was assumed that both unreacted epoxides and oligomers with a DS above the water solubility limit are present in this fraction.

Through this study, it was shown that several parameters govern the chemical modification of COS with fatty epoxide, which are comparable to fatty acid addition previously described. Indeed, the longer is the fatty chain, the lower is the grafting efficiency. In addition, solubility limits were reached at relatively low DS with longer chains. Going further, DP₂₀ will be abandoned due to poor DS.

Functionalization of COS-oximes with cardanol

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3 Subsequently, the epoxide amine reaction was transposed to epoxidized cardanol
4 (Scheme 2 2)). Cardanol is a natural phenol, a commercial co-product of the non-edible oil
5 obtained from the cashew nut shell. Cardanol is interestingly composed of an aromatic ring
6 and an attached fatty carbon chain which can be saturated (2%), monounsaturated (34%),
7 bi-unsaturated (22%) and tri-unsaturated (41%). Epoxidized cardanol can be directly
8 obtained by epoxidation reaction of cardanol and is commercially available.
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16 Initially, the reaction onto oligomers of DP10 was carried out in an H₂O / MeOH
17 mixture in the presence of Et₃N (similar conditions to the aliphatic study). Epoxidized
18 cardanol is viscous and sparingly soluble in this mixture. Thus, after precipitation in
19 acetone, only crude oligomers were obtained. Unreacted cardanol which was not grafted
20 onto the COS was recovered in the filtrate. The reaction was therefore carried out in DMSO
21 where both cardanol and COS were soluble. COS grafting efficiency onto DP5, DP10 and
22 DP15 was confirmed by ¹H NMR (SI 5-6). The DS were calculated using the CH₃ 1 signal
23 which represents 59% of the cardanol chain.
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33 COS of DP5, DP10 and DP15 were grafted with cardanol with maximum DS of 12,
34 8 and 2% respectively. Literally, this represent 40%, 60% and 20% respectively of COS
35 oligomer chain bearing one cardanol chain, the rest of the polymer representing unmodified
36 COS. As for aliphatic epoxides, it was shown that the larger is the DP, the lower is the DS
37 (see SI 7). In addition, a plateau was reached very quickly even when increasing the
38 number of equivalents. Although cardanol is a much longer epoxy compound compared to
39 epoxides C4, C9 and C16, and the aromatic ring generates both hydrophobicity and steric
40 hindrance, maximum DS were comparable with C16 DS for DP5 and DP10, which were 12
41 and 21% respectively. Nonetheless the higher is the DP, the lower are the solubility and
42 sweliability in DMSO. Thus, DP15 showed poor solubility in DMSO, which explains DS
43 maximum of 2%. Besides, the longer is the oligomeric chain, the higher is the steric
44 hindrance. Finally, as shown beforehand, the maximum DS was limited by the solubility of
45 the final product.
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Oil in water emulsion

To evaluate the suitability of the newly synthesized surfactant in bitumen emulsion, a model engine oil was used. DP15, DP10 and DP5 grafted with C4, C9, C16 and cardanol with maximum DS were used to emulsify the motor oil. 60/40 (w/w) oil in water emulsions were made using 0.2% (w/w) of total emulsion of amphiphilic COS, pH was adjusted to 2 by addition of HCl (0.2% (w/w) of total emulsion) to obtain cationic surfactants.

	C4			C9			C16			Cardanol		
	Emulsion type	Size (μm)	Stability (h)	Emulsion type	Size (μm)	Stability (h)	Emulsion type	Size (μm)	Stability (h)	Emulsion type	Size (μm)	Stability (h)
DP5	No	N/A	N/A	Direct	5-20	<1	Direct	N/A	<0.1	Direct	N/A	<0.1
DP10	No	N/A	N/A	Direct	5-20	24	Direct	5-20	12	Direct	5-20	24-48
DP15	No	N/A	N/A	Direct	N/A	<0.01	No	N/A	N/A	No	N/A	N/A

Table 2. Type, size of the droplets and stability of emulsions made with DP5 DP10 and DP15 functionalized with C4, C9, C16 and cardanol with maximum DS

At the end, direct emulsions were obtained for both DP10 and DP5 functionalized with C9, C16 and cardanol and DP15 functionalized with C9. Indeed, the obtained emulsions were able to disperse in water. Oligomer functionalized with C4 epoxide could not emulsify oil. This confirmed that the C4 chain was too short to provide sufficient amphiphilic properties to COS despite maximum DS of 90 and 100% reached for DP5 and DP10 respectively. DP15 surfactant were not able to give stable emulsions. Indeed, direct emulsion was only obtained with COS modified with C9 but destabilized immediately. In the same way, emulsions obtained with DP5 were not stable, less than 5 min for Cardanol and C16. This might be explained by the number of active species in the mixture. Indeed, DP5 cardanol were grafted with 12% DS which represent only 40% of oligomers bearing one cardanol chain. It means that 60% of the product is not suitable for stabilization of oil

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3 droplets in water. DP5 modified with C9 gave direct emulsions but they were only stable
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5 1 h, which is not enough for the application.
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8 In the case of DP10, direct oil in water emulsions were obtained for C9, C16 and
9 cardanol. In addition, emulsions were diluted and observed under a microscope to
10 determine the size of the droplets (example in Figure 7 b) with DP10 cardanol). At the end,
11 the size distribution was quite narrow for the three samples and droplets averages of 15 μm
12 were obtained in accordance to expectation ². DP10 C16 was stable less than 5 min despite
13 15% DS and DP10 C9 was stable for 1 h. The most stable emulsions appeared to be those
14 stabilized with DP10-cardanol presented in Figure 7 with destabilization starting between
15 24h and 48 h. In this case, a DS 8% represent 60% of oligomers grafted with one cardanol
16 chain ((Figure 7 a)) and SI 8 for color code). Those results showed that the efficiency of
17 the surfactant depends on several factors: the size of the hydrophilic chain, the size of the
18 hydrophobic chain and the DS.
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31 Those were quite promising preliminary results. Indeed, it was the first time that
32 COS modified with fatty epoxide and particularly with cardanol, potentially 100% biobased
33 and biodegradable ³⁷, was successfully synthesized and used as a surfactant for
34 concentrated oil in water emulsion.
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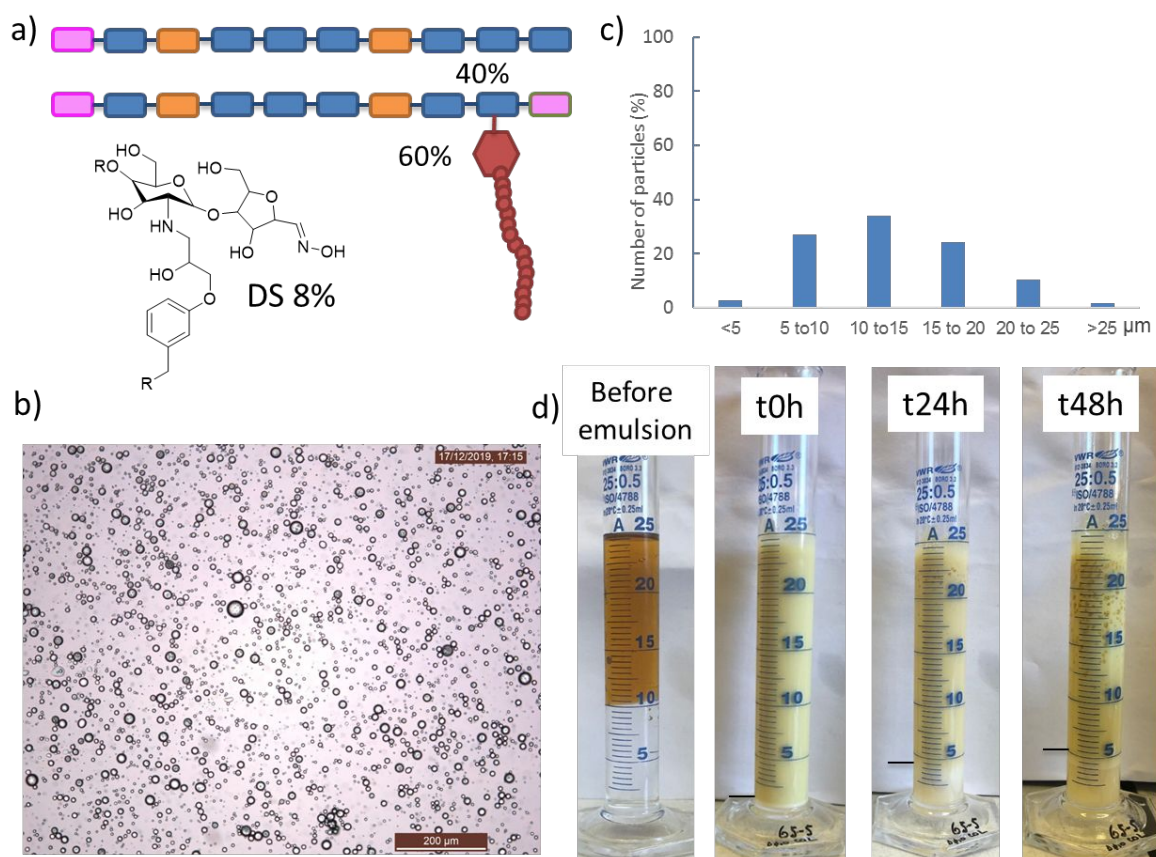


Figure 6. a) Simplified depiction of DP10-cardanol b) DP10-cardanol oil in water (60/40 /w/w) emulsion under microscope at $t=0.5$ h c) Size distribution of droplets obtained by microscopic measurement from DP10-cardanol oil in water (60/40 /w/w) emulsion d) Pictures of DP10-cardanol emulsion after 0, 24 and 48 h

CONCLUSION

In this study, new potential chitosan based surfactant for bitumen emulsions were introduced. At first, the controlled depolymerization of chitosan was investigated to reach the cationic head of the future surfactant suitable for further functionalization. Thus, the functionalization of COS-oxime by different fatty epoxide including epoxidized cardanol, a reaction that as far as we know was never described in the literature before was investigated. Oligomer of DP5, DP10 and DP15 grafted with cardanol were obtained with maximum DS of 12, 8 and 2% respectively. It was shown that direct oil in water (60/40) emulsions stable for 24-48 h, with 15 μm medium size droplets can be obtained with DP10-Cardanol acting as a surfactant. In order to improve the stability of emulsions, the amount

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3 of surfactant could be increased to reach higher quantity of active species. Studies on pH
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5 influence might also be led.
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8 This work showed a way to obtain potentially biobased and biodegradable COS
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10 amphiphilic structures that have been of great interest these last decades. Those products
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12 have proved their potential for cationic surfactant substitution in bitumen emulsions
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14 applications.
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20 ASSOCIATED CONTENT

21 **Supporting Information**

22
23 ¹H NMR spectrum of COS-cardanol, table of DS for COS cardanol and color code for figure 7 were
24 provided in supporting information.
25

26 AUTHOR INFORMATION

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35 **Author Contributions**

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38 All authors have given approval to the final version of the manuscript. The
39 experiment were performed by C.C. C.C. and G.D. wrote the manuscript with in-depth
40 editing from C.N., S.C., and contributions from all authors.
41
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47
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49 **Notes**

50
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52 The authors declare no competing financial interest.
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56 ABBREVIATIONS

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3 AGE: allyl glycidyl ether; AMF: 2,5-anhydromannofuranose; COS: chitooligosaccharides; DP:
4 polymerization degree; DS: substitution degree; FTIR:Fourier transform infrared
5 spectroscopy ; SEC:size-exclusion chromatography;
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