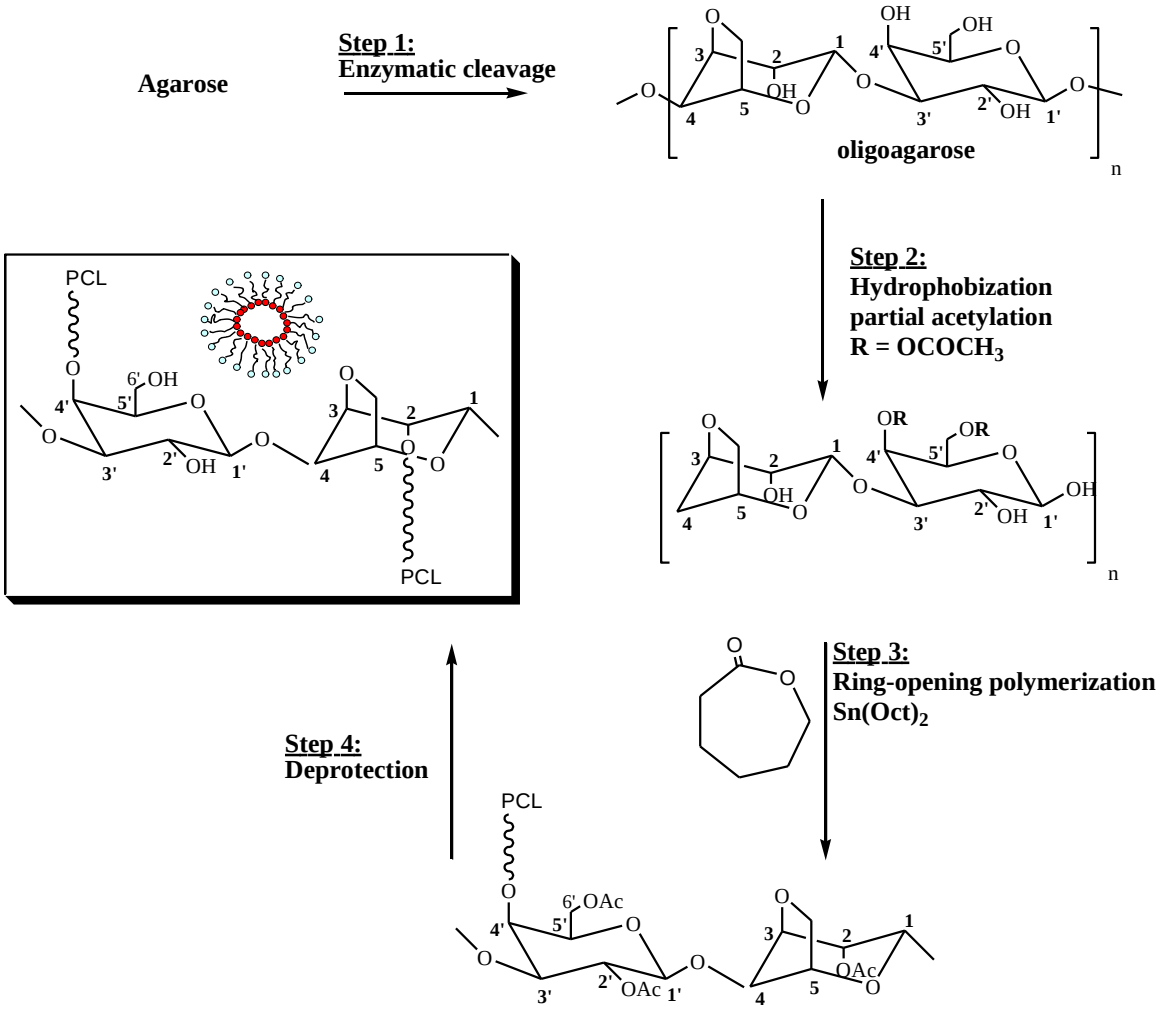
Electronic Supplementary Information

1. Materials

All solvents used were of HPLC/Analar grade. Phosphate buffer tablets were purchased from Sigma. ε-Caprolactone (99%) and tin(II) octanoate (96%) were used as received from Alfa Aesar. Oligoagarose was prepared by the group of William Helbert (author) at Roscoff Station (France) and sent to us under research cooperation agreement. It was used as received.

2. Synthesis and characterization of oligoagarose-*g*-polycaprolactone

Scheme 1 shows the method devised for the synthesis of oligoagarose-*g*-polycaprolactone.



Scheme 1

2.1 Hydrolysis of agar

Recombinant β-agarase B from *Zobellia galactanivorans* was purified. Agarose (0.5 % w/v in

water, Eurogentec) was molten in a boiling water bath and then cool down to 40°C to prevent the polysaccharide from gelling. 1L of this solution, referred to as melted agarose, was incubated with 500µL of enzymes. The amount of reducing sugars released was assayed using the ferricyanide method. The solution was filtered through a 30 kDa membrane in order to remove undigested fraction. Oligoagarose (Figure 1) samples were lyophilised and stored at room temperature.

The hydroxyl content is calculated according to equation 1 taking into account that each repeat unit contain 4 OH groups and that a total of 6 OH groups are present on sugar units at chain ends.

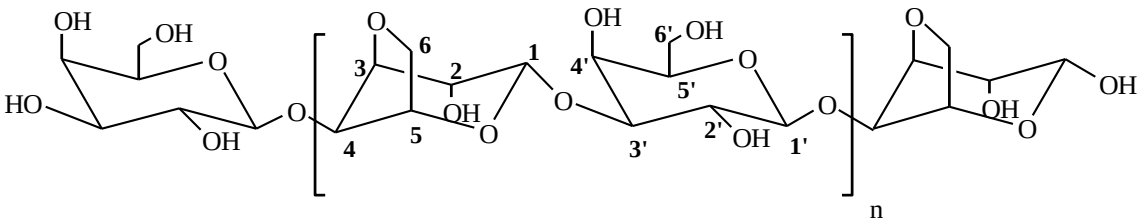


Figure 1. Oligoagarose repeat unit

Equation 1. Hydroxyl group content = (n x 4) + 6

Oligoagarose samples were characterized by HPAEC-PAD (Figure 2). The DP_n varied from 4 to 12 depending on samples. Their average hydroxyl group content per chain was calculated from the peak areas.

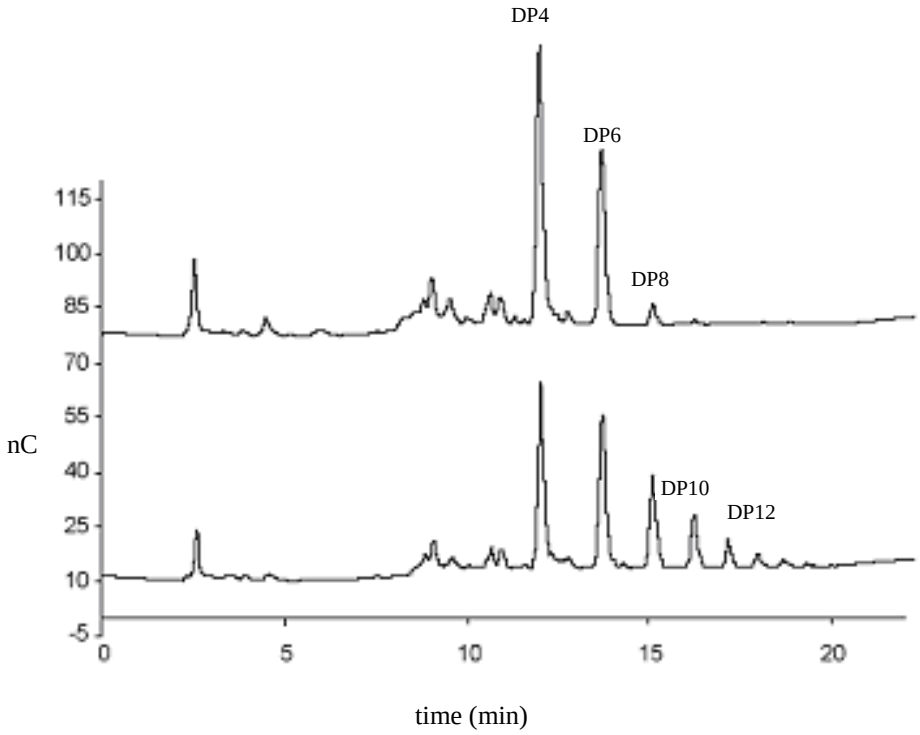
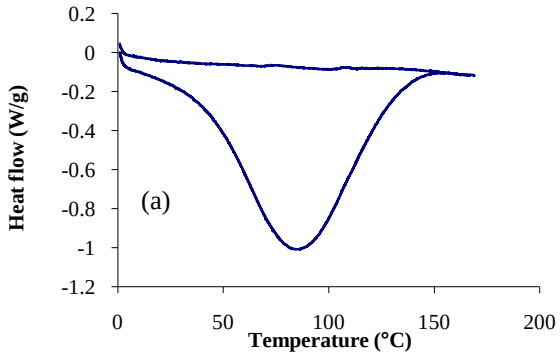


Figure 2. HPAEC-PAD profiles of two typical oligoagarose samples used.

DSC thermogram of oligoagarose showed a broad endotherm centered at 85°C (Figure 3a) while the graft copolymer with free hydroxyl groups exhibit a relatively sharper transition with three peaks in the range of 41.5-51.3°C (Figure 3b). DSC analysis of a physical mixture of oligoagarose and PCL_{10} gave a higher melting transition at 53.8-56.3°C (Figure 3c).



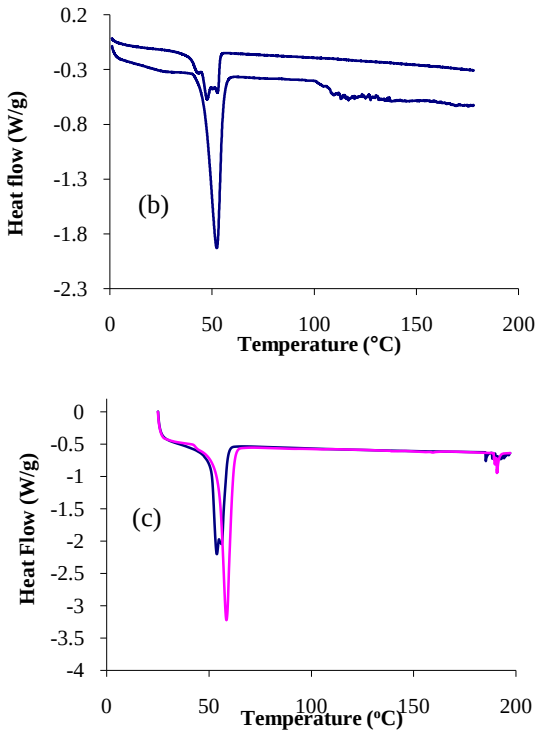


Figure 3. DSC thermogram of (a) oligoagarose (b) HO-OligoAga-*g*-PCL$_{10}$ (c) physical mixture of oligoagarose and PCL$_{10}$

The samples were further characterised by ^{1}H NMR (Figure 4) and the results compared with HPAEC-PAD profiles (Table 1). ^{13}C NMR (Table 2) was also used to characterise the oligoagarose samples.

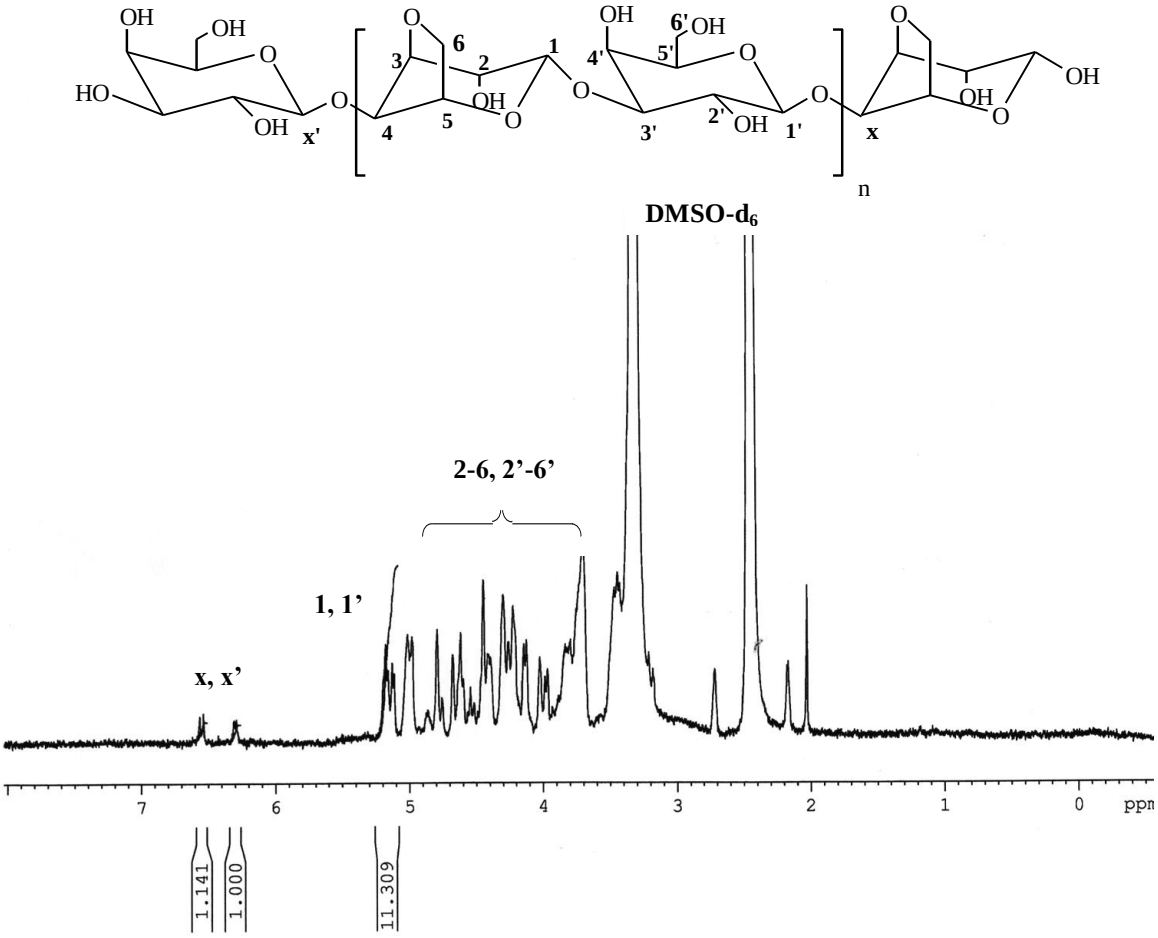


Figure 4. ^{1}H NMR spectrum of oligoagarose 3 in DMSO-d_6.

Table 1. Experimental values of hydroxyl group content of oligoagarose chains

	Average hydroxyl content	
Sample	^{1}H NMR	HPAEC-PAD
1	36	36
2	24	28
3	28	27
4	30	30

Table 2. Assignment of diads of oligoagarose samples using ^{13}C NMR

Unit	C-1	C-2	C-3	C-4	C-5	C-6
Theoretical						
G	102.4	70.2	82.2	68.8	75.3	61.4
LA	98.3	69.9	80.1	77.4	75.7	69.4
Experimental						
G	101.5	70.0	80.5	68.0	75.0	60.5
LA	97.5	70.0	79.5	77.0	75.5	68.5

2.2 Synthesis of partially acetylated oligoagarose

Oligoagarose (0.46 g), acetic anhydride (4.8 ml) and pyridine (0.8 ml) were added in a glass tube at room temperature and stirred under nitrogen. After 3 h stirring, ice was added to the mixture to hydrolyse any unreacted acetic anhydride and the acetylated oligoagarose precipitated from cold methanol. It was obtained as a white residue and was dried under vacuum before characterization by NMR (Figures 5 and 6).

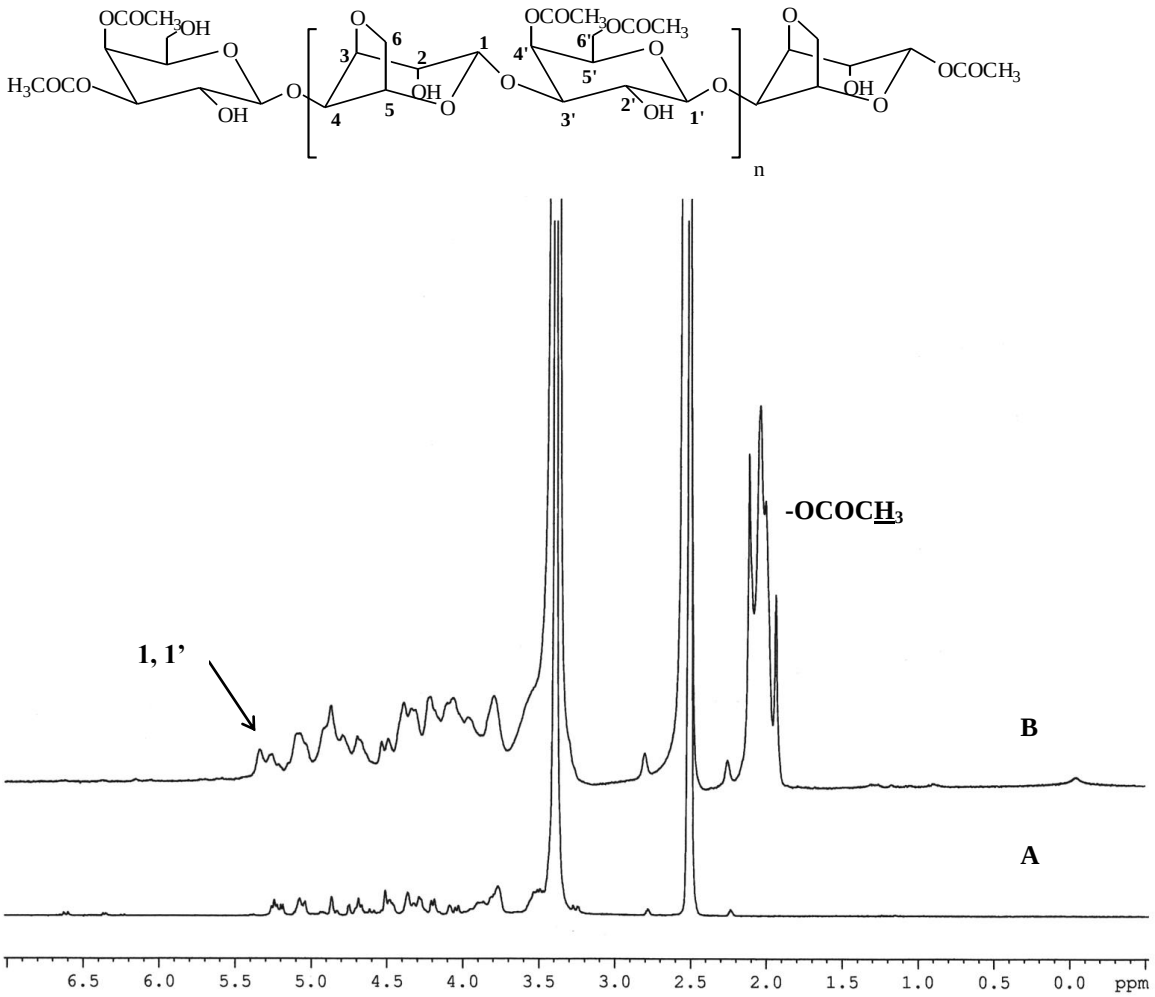


Figure 5. ^{1}H NMR spectra of (A) oligoagarose (B) AcO-oligoagarose derivative in DMSO-d_6

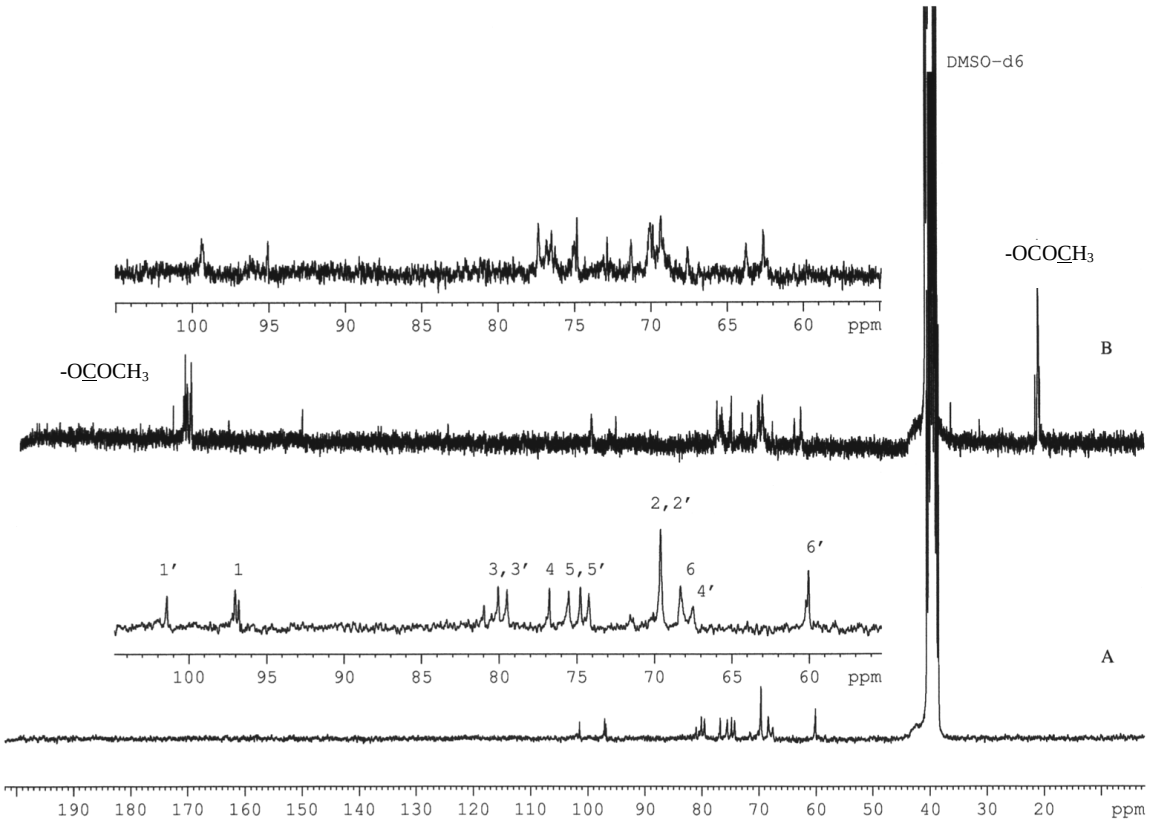


Figure 6. ^{13}C NMR spectra of (A) oligoagarose (B) partially AcO oligoagarose in DMSO-d_6.

2.3 Synthesis of graft copolymers: poly(O-acetyloligoagarose-g-caprolactone)

Acetylated oligoagarose (0.07 g) was measured in a glass tube. Tin(II) octanoate (17 mg) and toluene (0.5 ml) were added. The mixture was allowed to stir for 2 h at 40°C under nitrogen before the addition of ε-caprolactone (0.133 g). Polymerization was allowed to proceed at 100°C for 20 h. The crude product was then dissolved in chloroform followed by precipitation in cold methanol. The precipitate was isolated, dried under vacuum and characterized by NMR (Figure 7) and SEC (Figure 8).

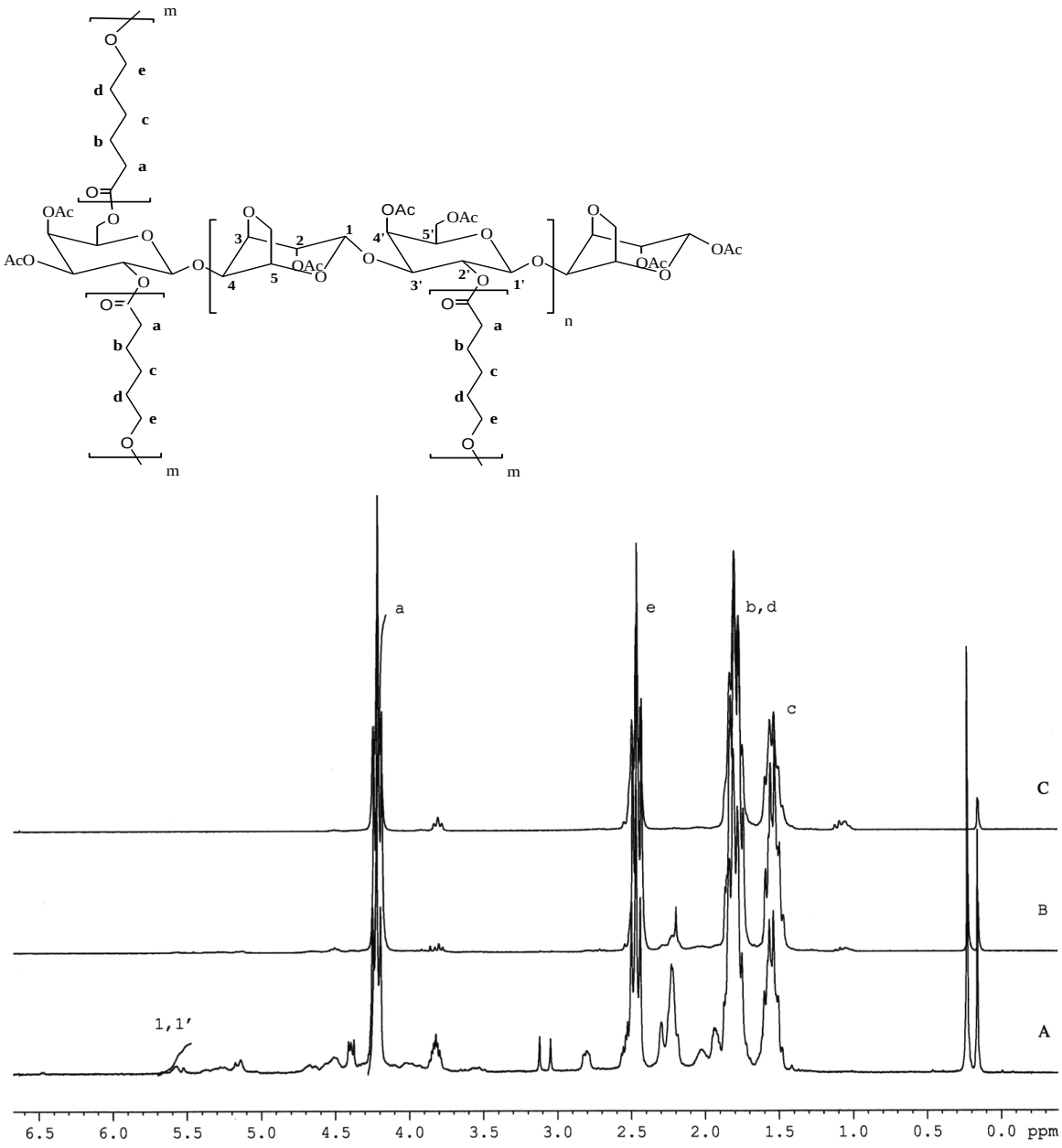


Figure 7. ^{1}H NMR spectra ($CDCl_3$) of poly(oligoagarose-*g*-caprolactone) using oligoagarose-OH as macroinitiator (A) 70% acetylated (B) 40% acetylated (c) 100% free OH

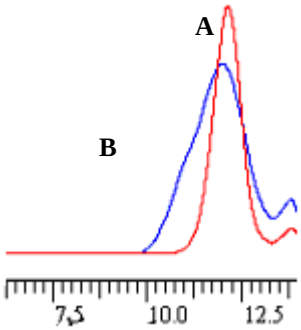


Figure 8. SEC chromatograms of (A) 70% AcO-oligoagarose, (B) 70% AcO-poly(oligoagarose-*g*-caprolactone) copolymer in THF as eluent.

2.4 Deprotection of acetyl groups of graft copolymers

Poly(O-acetyloligoagarose-*g*-caprolactone) was dissolved in mixed THF/CH_3OH solvent (v/v = 1/1) along with the addition of a catalytic amount of $NaOCH_3$ (pH = 8). After stirring at room temperature for 3 h, the deprotected graft copolymer was recovered by neutralization, precipitation, filtration and drying under vacuum. The resulting product was characterized by NMR (Figure 9).

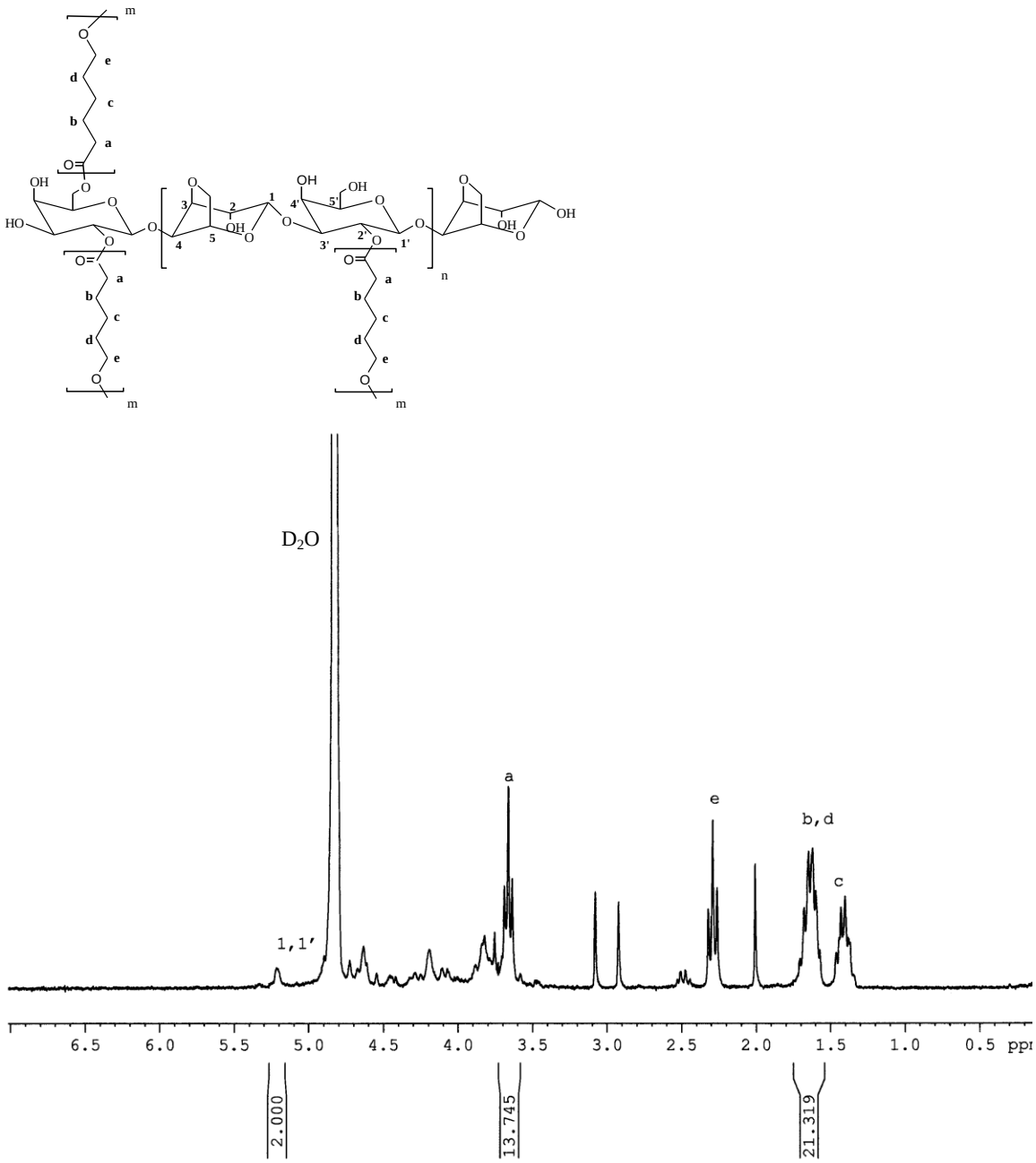


Figure 9. ^{1}H NMR spectrum of deprotected poly(oligoagarose-*g*-caprolactone) recorded in D_2O.

3. Drug loading using acetone volatilization method

The graft copolymer (10 mg) and ketoprofen (4 mg) was transferred into a clean beaker. Acetone (2 mL) was added and the mixture was stirred. Distilled deionised water (10 mL) was added drop wise to the stirring mixture. The mixture was allowed to stir for 3½ hours. The mixture was then filtered by means of 0.4 µm microporous filter into dialysis tubing. The filtered mixture was

dialysed against distilled deionised water for a period of 24 hours. The dialysed solution was then analysed by UV spectroscopy (Figure 10).

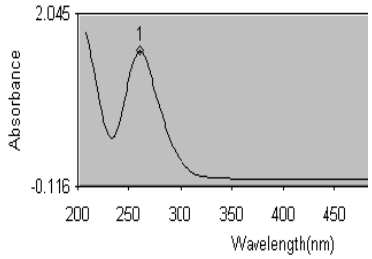


Figure 10. UV spectrum of a ketoprofen loaded HO-OligoAgo-g-PCL$_{10}$ using 4 mg of ketoprofen and 10 mg of copolymer.

4. Characterization

Oligo-agarose samples were analysed by High Performance Anion Exchange Chromatography with pulsed amperometric detection (HPAEC-PAD), using a Dionex chromatograph DX 500 equipped with a 50 µl injection loop, a CarboPac PA100 column (4 mm× 250 mm) and an electrochemical detector with a gold electrode. Analyses were performed according to a method adapted from Weinberger *et al.*[16] as follows. Elution was carried out at 1 ml.min^{-1} with a 30 min linear gradient of 0–300 mM sodium acetate in 150 mM NaOH. The column was calibrated with neoagarotetraose and neoagarohexaose standards (Dextra Laboratory).

NMR analysis was carried out in DMSO-d_6 or D_2O or $CDCl_3$ at room temperature on a FT Bruker Spectrometer 250 MHz. Size Exclusion Chromatography was performed using a Polymer Standards Systems (PSS) apparatus with a refractive index detector. Calibration was done using polystyrene standards and, THF and water were used as eluent.

CMC was determined using DLS according to method described by Nagy et al[1] (Figure 11).

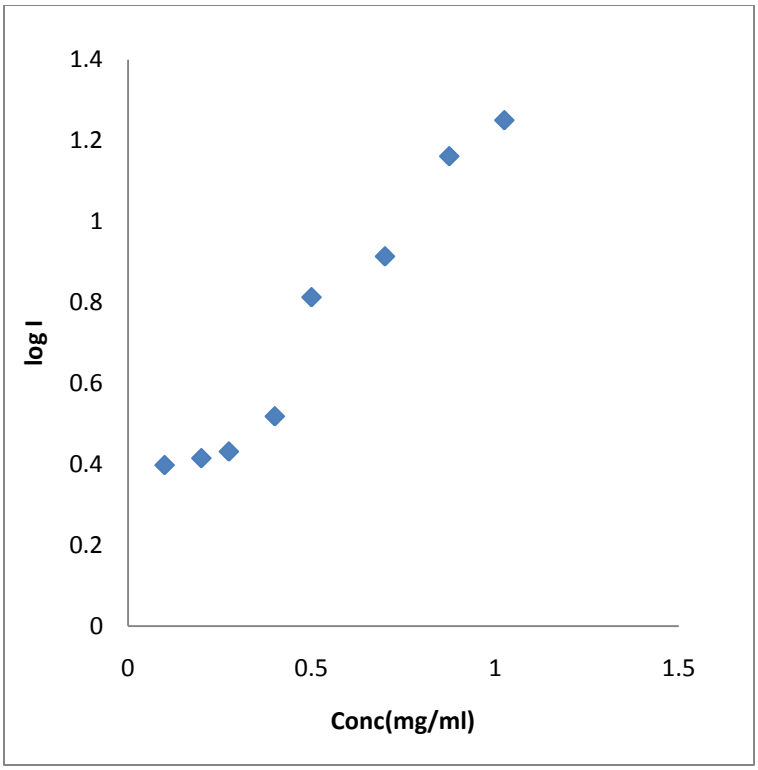


Figure 11. Determination of cmc using DLS.

SEM was recorded on a Leo 1430VP Scanning Electron Microscope. A thin layer of polymer was spread on a metal mount. The samples were then coated for 90s in a 20 mV argon atmosphere on S150A sputter coater.

Melting temperatures (T_m) and enthalpies of fusion (ΔH_m) were measured with a TA Instrument Q100 Differential Scanning Calorimeter. The DSC cell was purged with nitrogen gas flow of 50 $mlmin^{-1}$. Experiments were performed in aluminium hermetic pans using a heating and cooling rate of 10 $^oC\ min^{-1}$. The melting temperature was taken at the maximum of the melting peak from the second heating curve

Morphologies were analyzed by HAR-027-JOEL-2100 TEM (CNS, Harvard University) operating at an accelerating voltage of 200KV. Samples were dissolved in $CHCl_3$ or $CHCl_3$/THF. 5ul of sample was placed on Cu grid coated with carbon film and allowed to dry at room temperature overnight.

5. TEM statistical calculation

5.1 Oligoaga-g-PCL$_{10}$

Measurements in nm
15. Entries Total

Mean = 11.821 nm
Dev(rms)= 5.355 nm
12.6
11.7
11.2
10.6
10.8
20.1
8.70
28.4
11.7
11.0
7.55
6.78
8.62
8.36
9.21

5.2 Oligoaga-g-PCL$_{15}$

Measurements in nm
49. Entries Total
Mean = 8.073 nm
Dev(rms)= 1.444 nm
9.66
9.24
8.84
7.97
8.51
8.80
7.89
6.65
7.97
8.81
9.30
8.27
8.52
7.51
7.92
7.33
5.93
9.05
10.1
10.4
9.98
8.70
8.40
8.01

5.62
7.13
9.97
6.96
7.05
8.48
7.24
7.22
9.91
5.52
6.97
10.1
6.63
8.74
4.82
4.52
10.2
6.42
7.65
8.34
9.36
9.22
9.98
7.23
6.53

References

1. M. Nagy, L Szöllösi, S. Kéki, R. Faust and M Zsuga, J. Macromol. Sci., Part A: Pure and Appl. Chem., 2009, **46**, 331.