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Bio-inspired electrospun scaffolds as materials for biomedical applications

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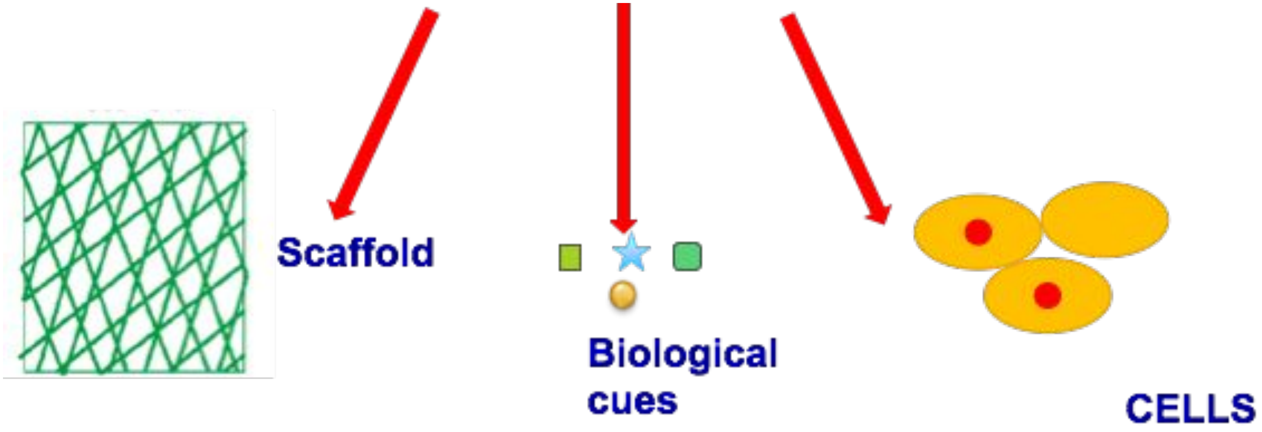
Laboratory of Bioinspired Materials: One of the Main Italian centers for biomaterials

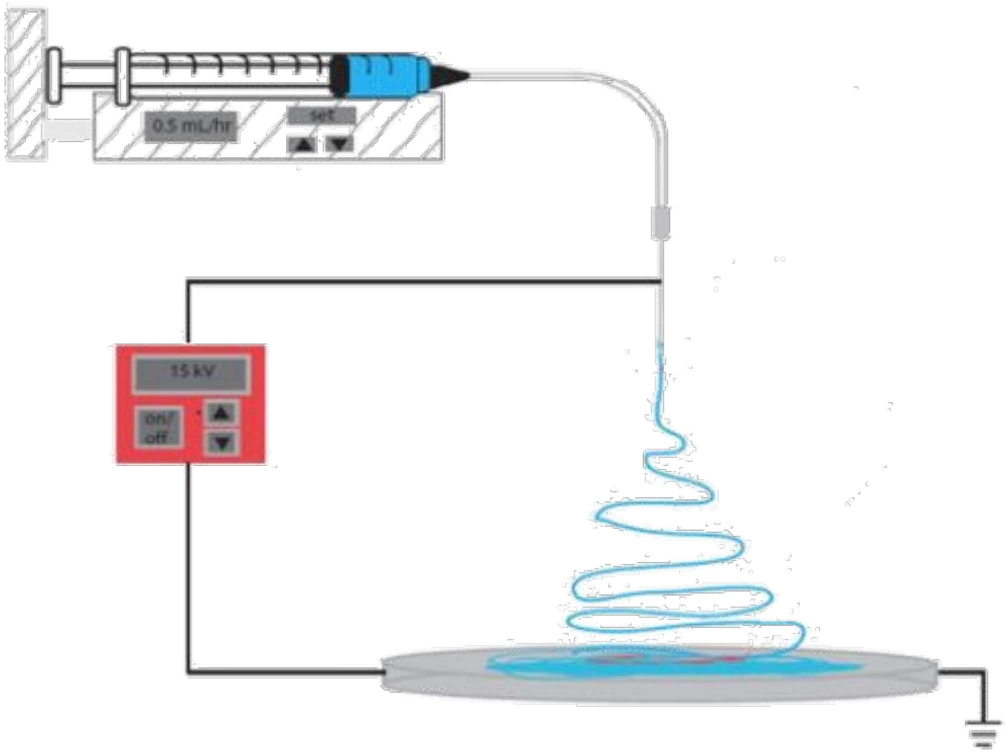
Figure 7. Distribution of the main research Italian centers for biomaterial production and testing.

Tissue engineering has emerged as an alternative to grafting approaches by designing and producing scaffolds that aim to mimic the architecture and the function of the extracellular matrix

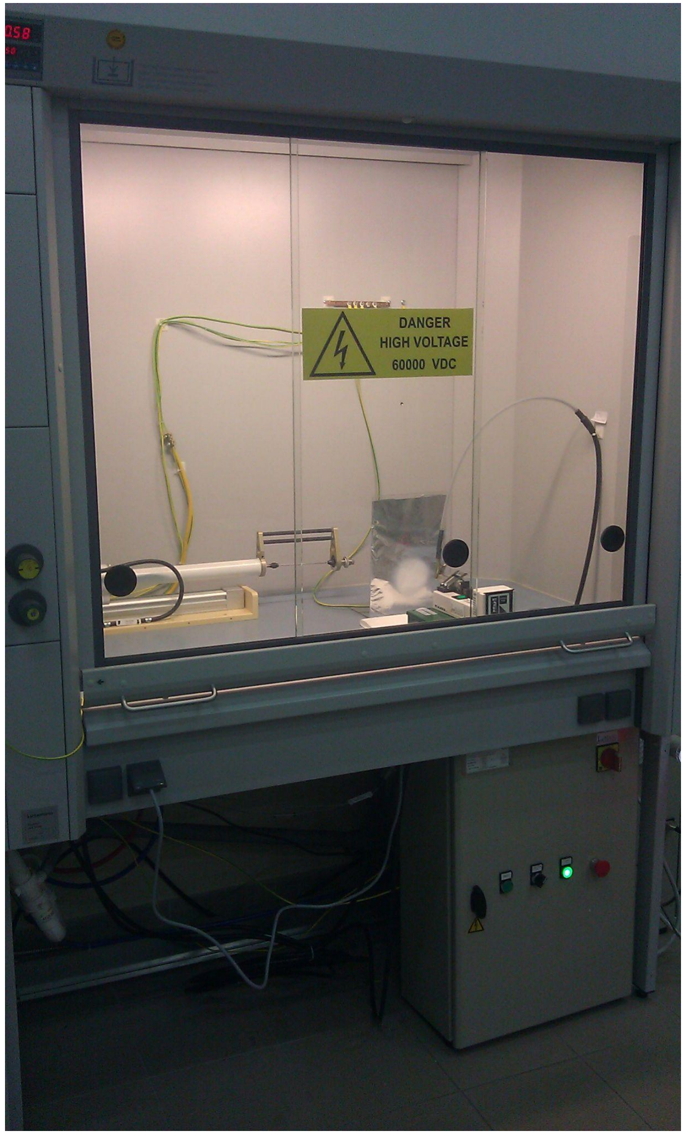
Biomimetic approach

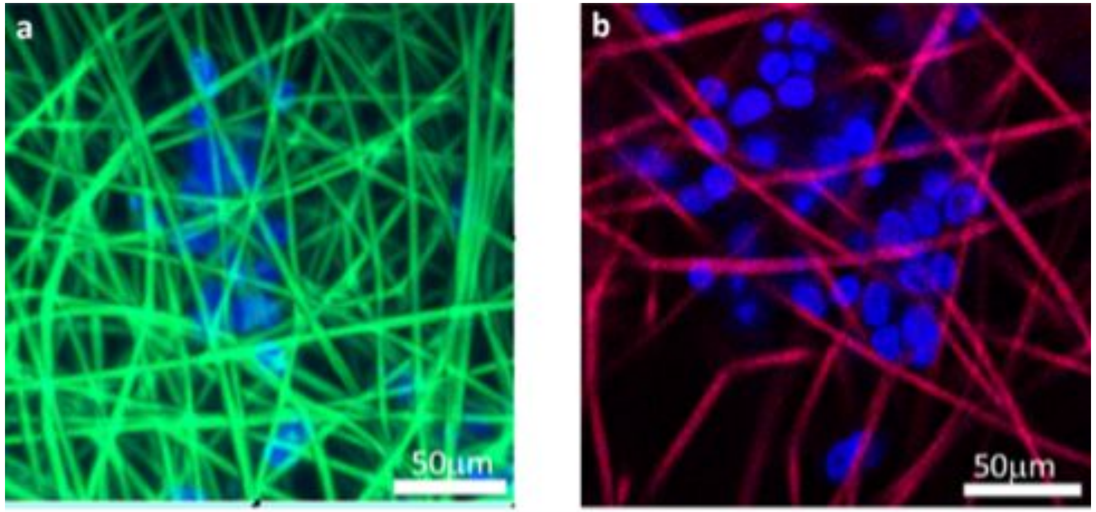


Electrospinning (Technique 1)



Electrospinning





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Chemistry
A European
Journal

Chemistry
Europe
European Chemical
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Accepted Article

Title: Elastin-Hyaluronan Bioconjugate as Bioactive Component in Electrospun Scaffolds

Authors: Antonio Laezza, Antonietta Pepe, and Brigida Bochicchio

This manuscript has been accepted after peer review and appears as an Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

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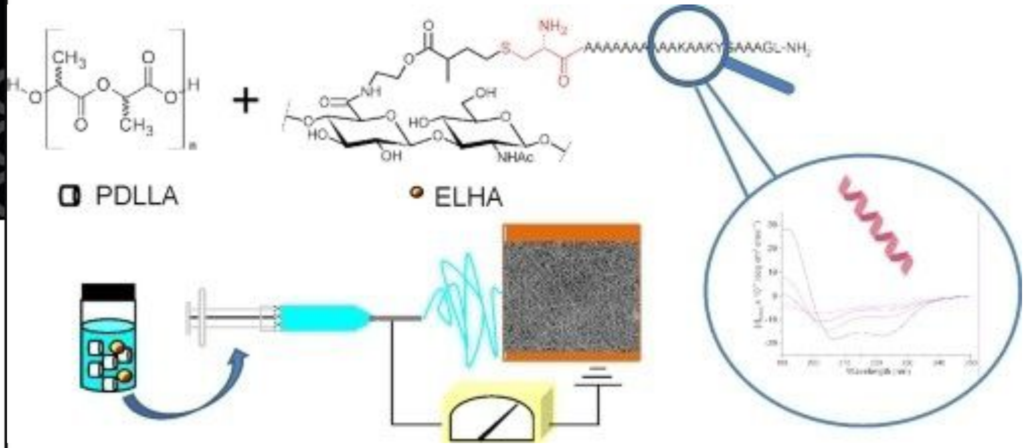
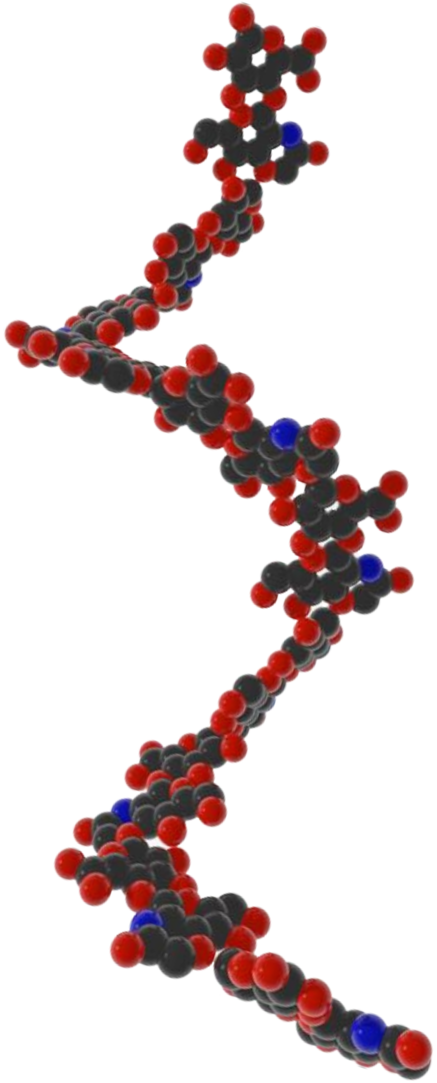


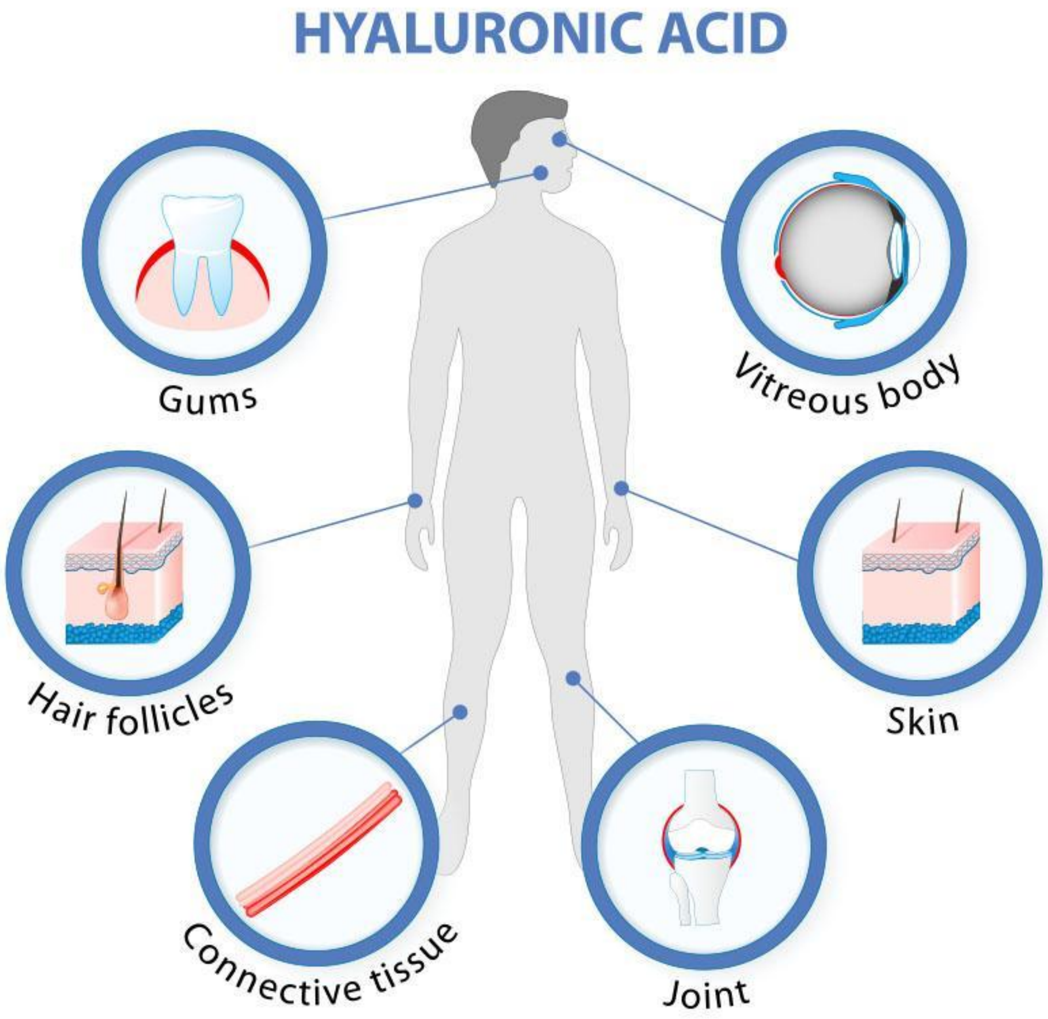
HYALURONIC ACID-MW

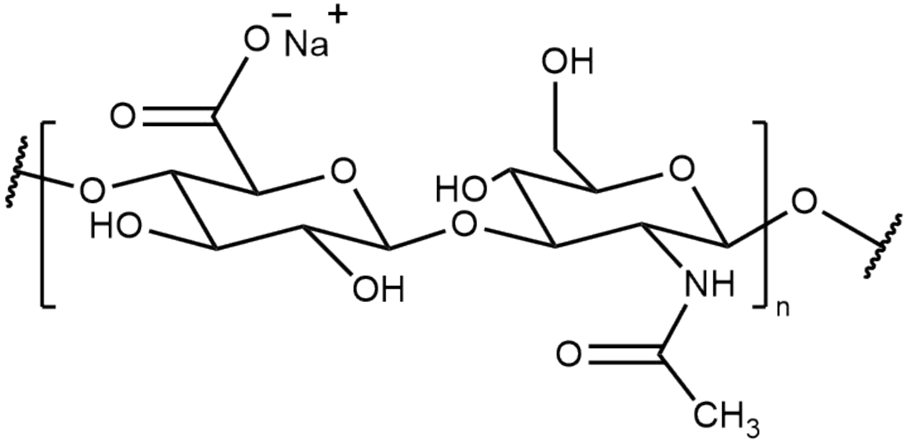


- O-HA, HA oligosaccaride;
- MMW-HA, HA (MW ~ 250-1000 kDa);
- HMW-HA, HA (MW ~ 1000-6000 kDa);
- vHMW-HA, HA (MW > 6000 kDa).

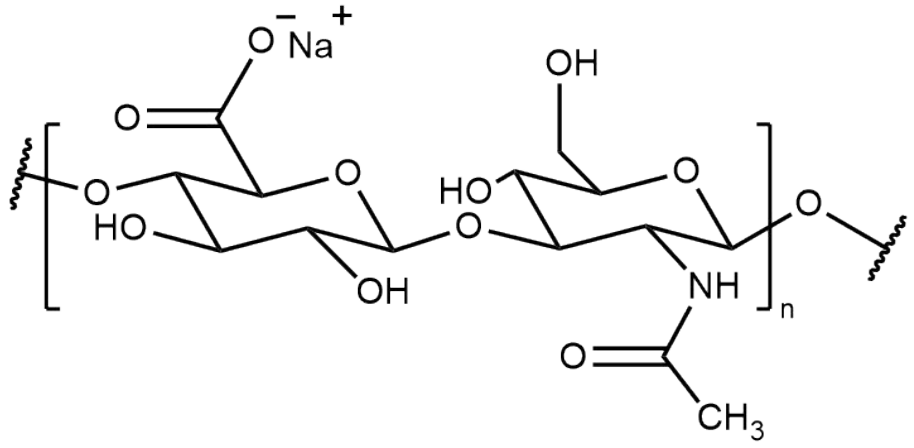
MMW-HA (186.0 kDa) by Altergon Italia

HYALURONIC ACID-MW

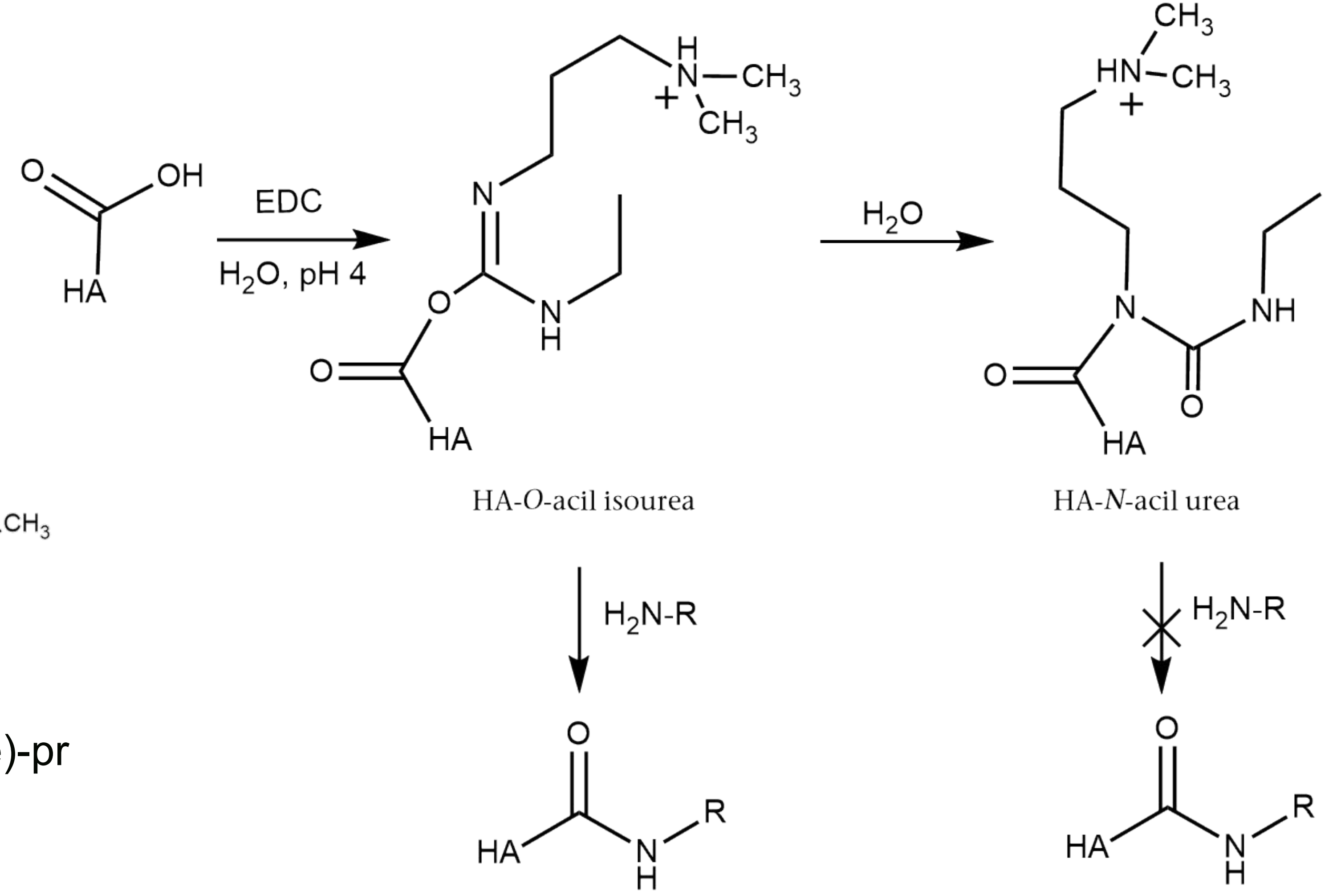


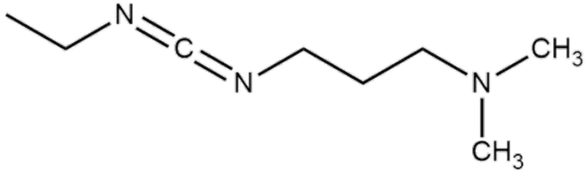


HYALURONIC ACID Chemical Modifications



HYALURONIC ACID Chemical Modifications

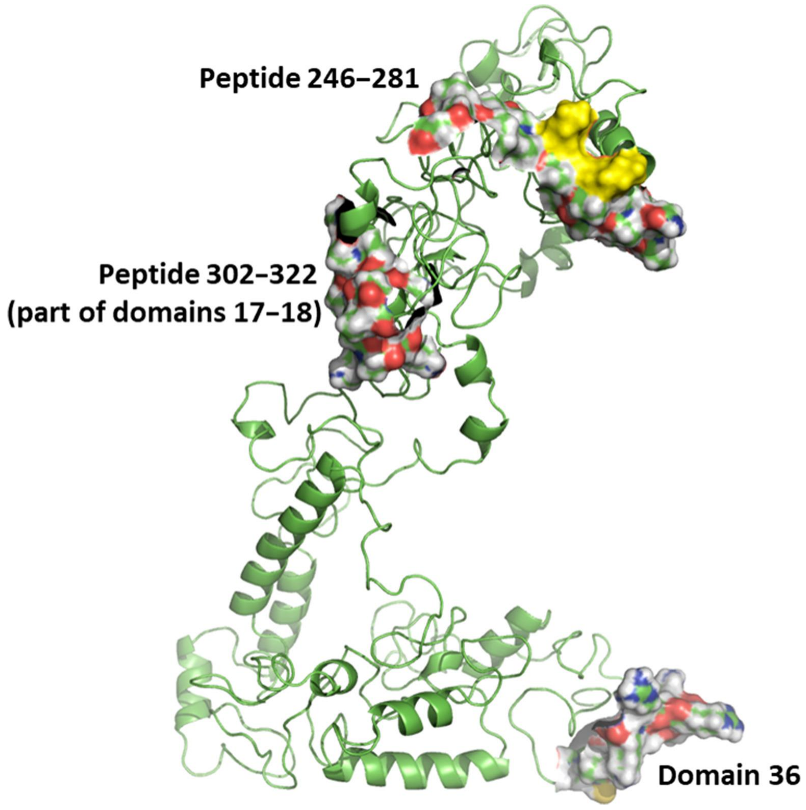




1-ethyl-3-[3-(dimetilamine)-propyl]-carboimmide

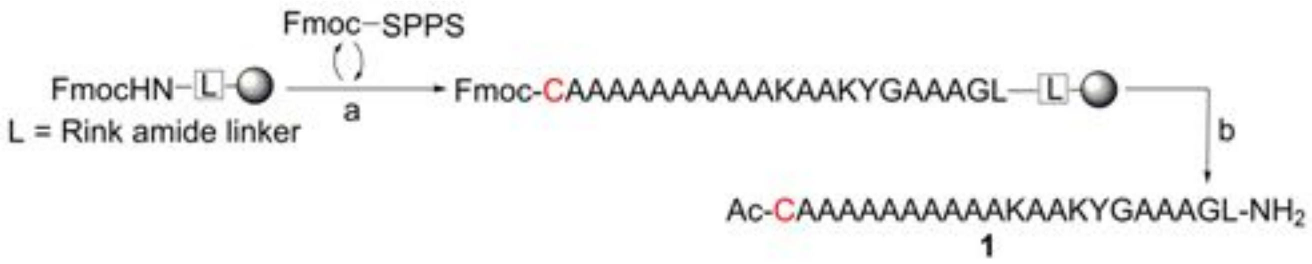
Elastin-Hyaluronan Bioconjugate: Solid Phase Peptide Synthesis (Technique 2)

Elastin-Hyaluronan Bioconjugate as Bioactive Component in Electrospun Scaffolds



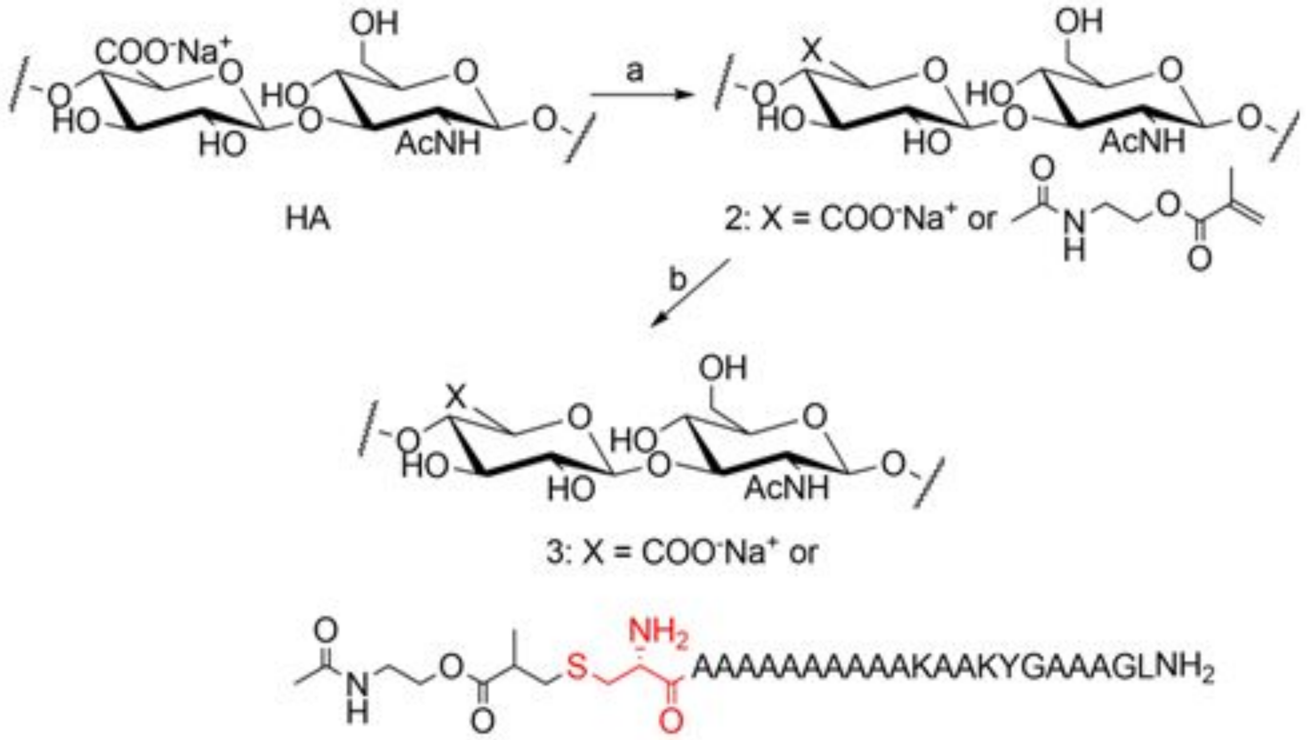
B. Bochicchio et al. The FEBS Journal, Volume: 288, Issue: 13, Pages: 4024-4038

Elastin-Hyaluronan Bioconjugate: Solid Phase Peptide Synthesis (Technique 2)



Scheme 1. Fmoc-SPPS synthesis of peptide 1. (a) i) 20% Piperidine, DMF, RT; ii) Fmoc-AA:HBTU:HOBT: DIPEA, 5:5:5:10, RT, DMF, temperature; iii) Ac_2O, DIPEA, DMF, RT; (b) i) 20% Piperidine, DMF, RT; ii) Ac_2O, DIPEA, DMF, RT; iii) 94% TFA, 2.5% EDT, 2.5% H2O, 1% TIS, RT, 1.5 h, yield 87%.

Elastin-Hyaluronan Bioconjugate: click chemistry



Scheme 2. Semi-synthesis of polysaccharide 3 . (a) 2-AEMA•HCl, EDC, s-NHS, H_2O, RT, pH = 6.8, overnight, yield 98%, **DS = 0.18**; (b) 1, TCEP•HCl, H2O, RT, pH = 8.9, 2d, yield = 67%, **DS = 1.0**

Elastin-Hyaluronan Bioconjugate Characterization: NMR (Technique 2)

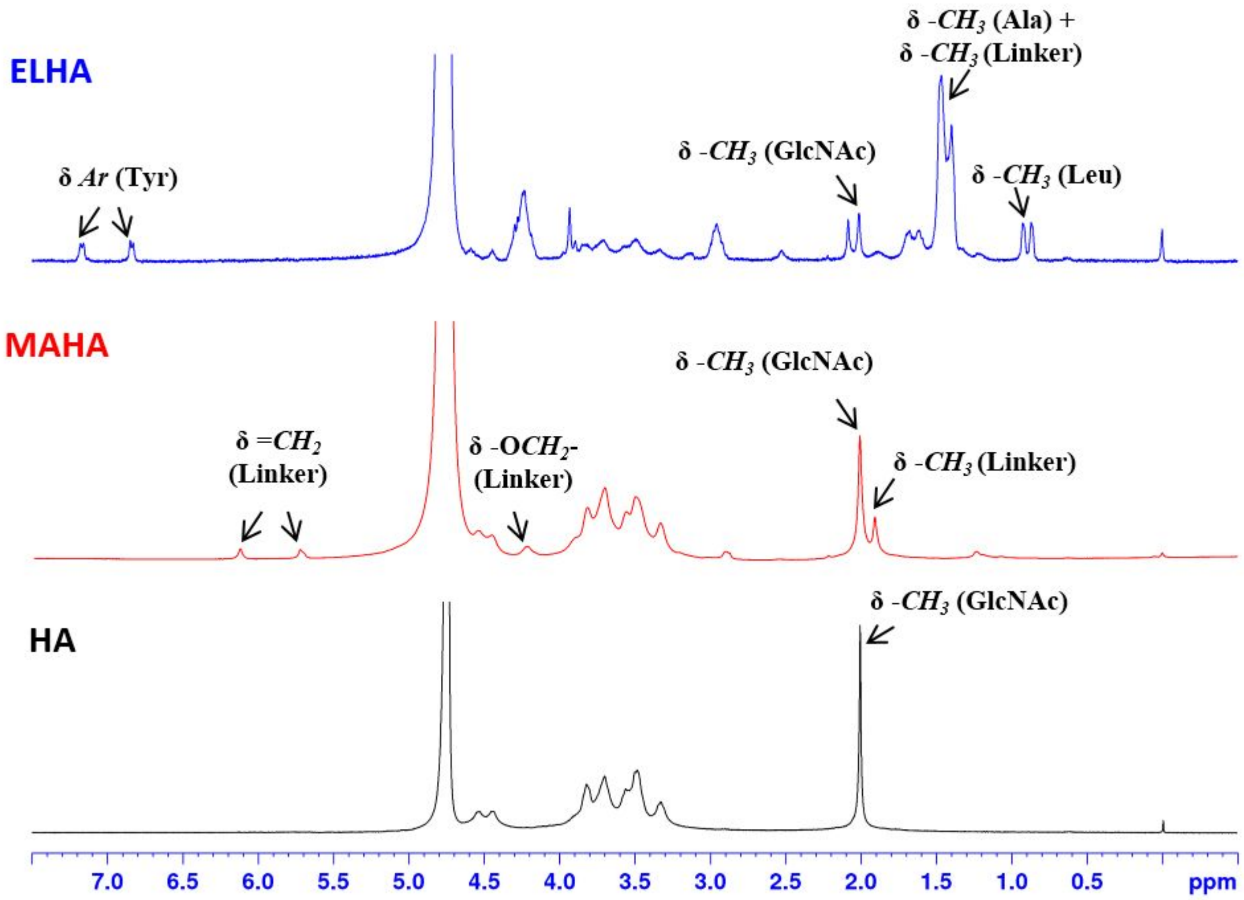


Figure 1. 1H-NMR spectra of HA (black), polysaccharide 2 (MAHA, red), and polysaccharide 3, (ELHA, blue) (400 MHz, D_2O, 298 K)

Elastin-Hyaluronan Bioconjugate charaterization: ATR-FTIR (Technique 3)

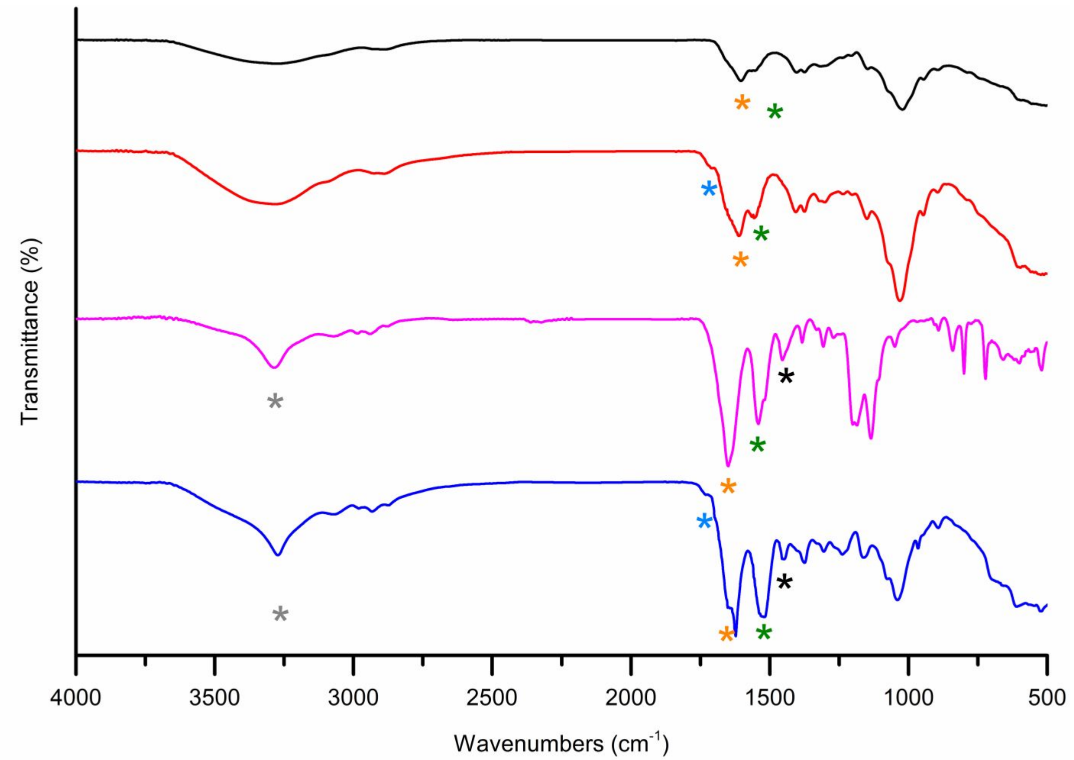
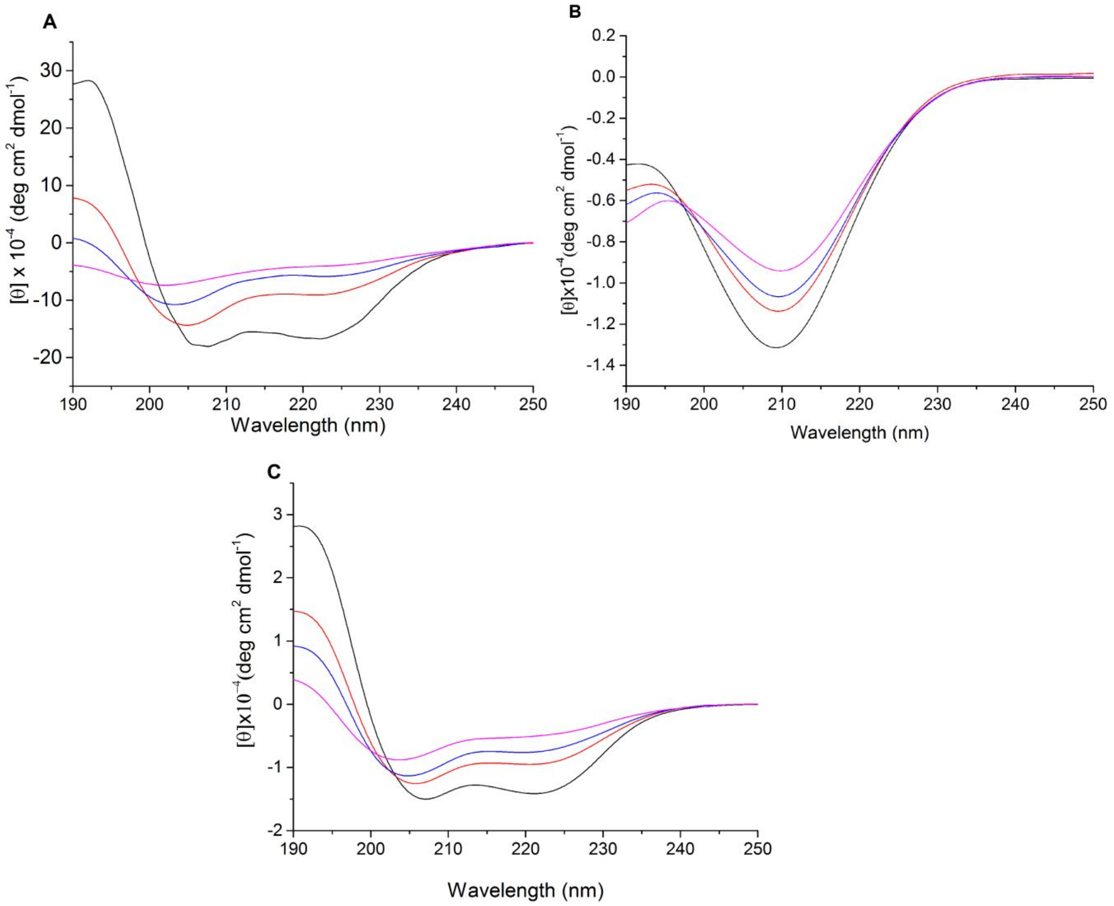


Figure 2. ATR spectra of HA (black), polysaccharide 2 (MAHA, red), peptide 1 (EL, purple), and polysaccharide 3, (ELHA, blue). N-H stretching vibration band (gray star), ester C=O stretching vibration (blue star), amide C=O stretching vibration (orange star), amide C-N stretching vibration (green star), C-H_3 bending vibration (black star).

Elastin-Hyaluronan Bioconjugate characterization: CD (Technique 4)



Temperature-dependent CD spectra in PBS solution of: A) peptide 1, B) HA, C) polysaccharide 3, (ELHA) recorded at 273 K (black), 298 K (red), 310 K (blue), 333 K (purple).

Elastin-Hyaluronan Bioconjugate: electrospun scaffolds (Scannin Electron Microscopy Technique 5)

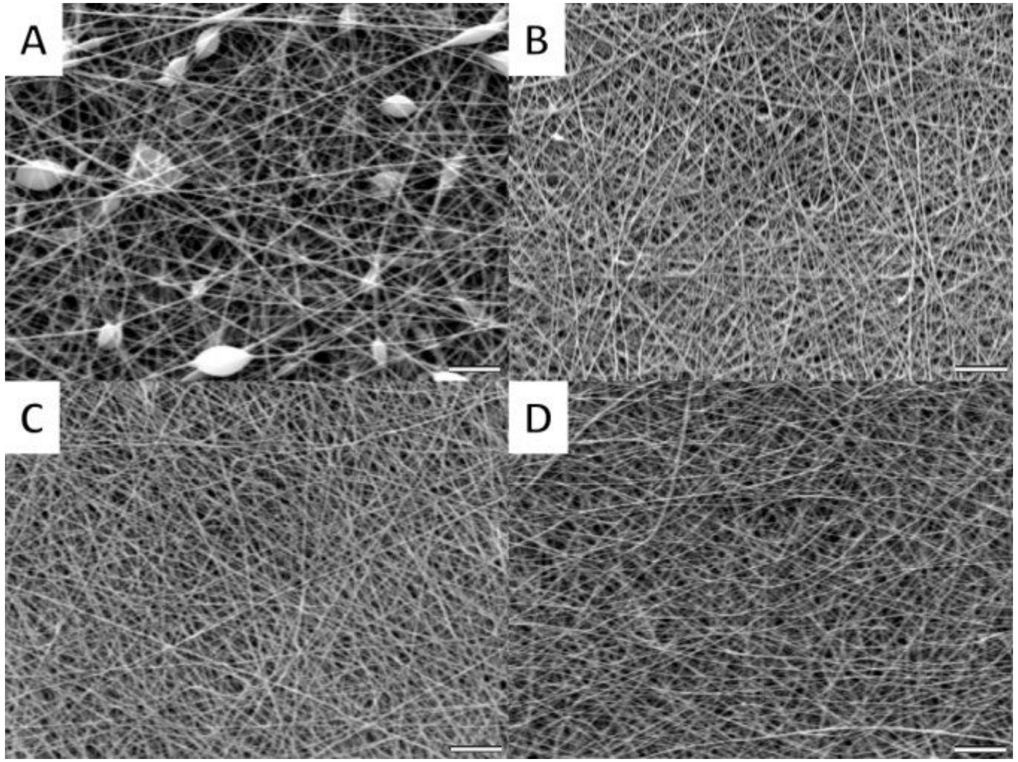


Figure 4. SEM images of electrospun scaffolds. P (A), HP (B), MHP (C), and EHP (D); (bar: 20 µm).

Elastin-Hyaluronan Bioconjugate: MTS cells assay and absence of citotoxicity

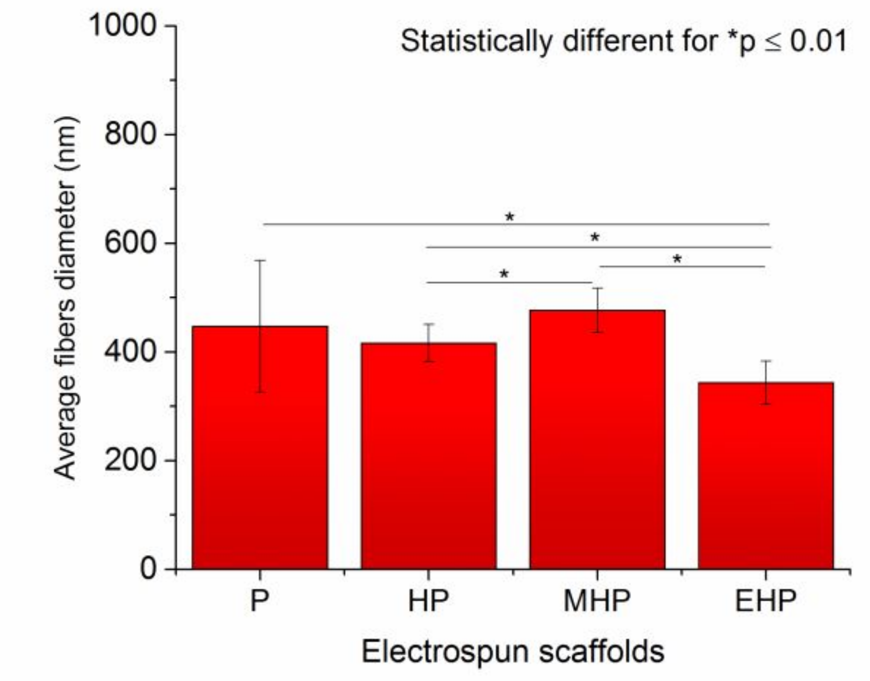
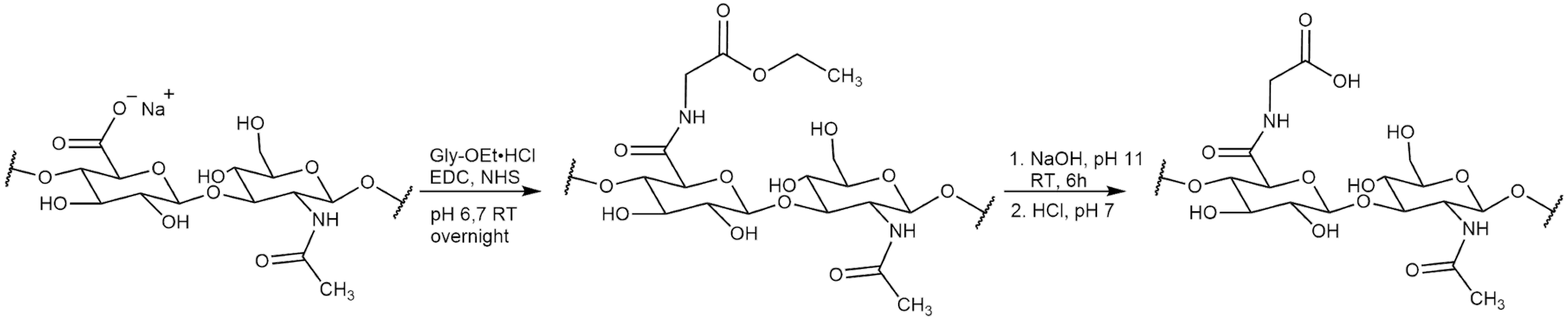


Figure 5. Histogram of average fibers diameter for P, HP, MHP, and EHP electrospun scaffolds

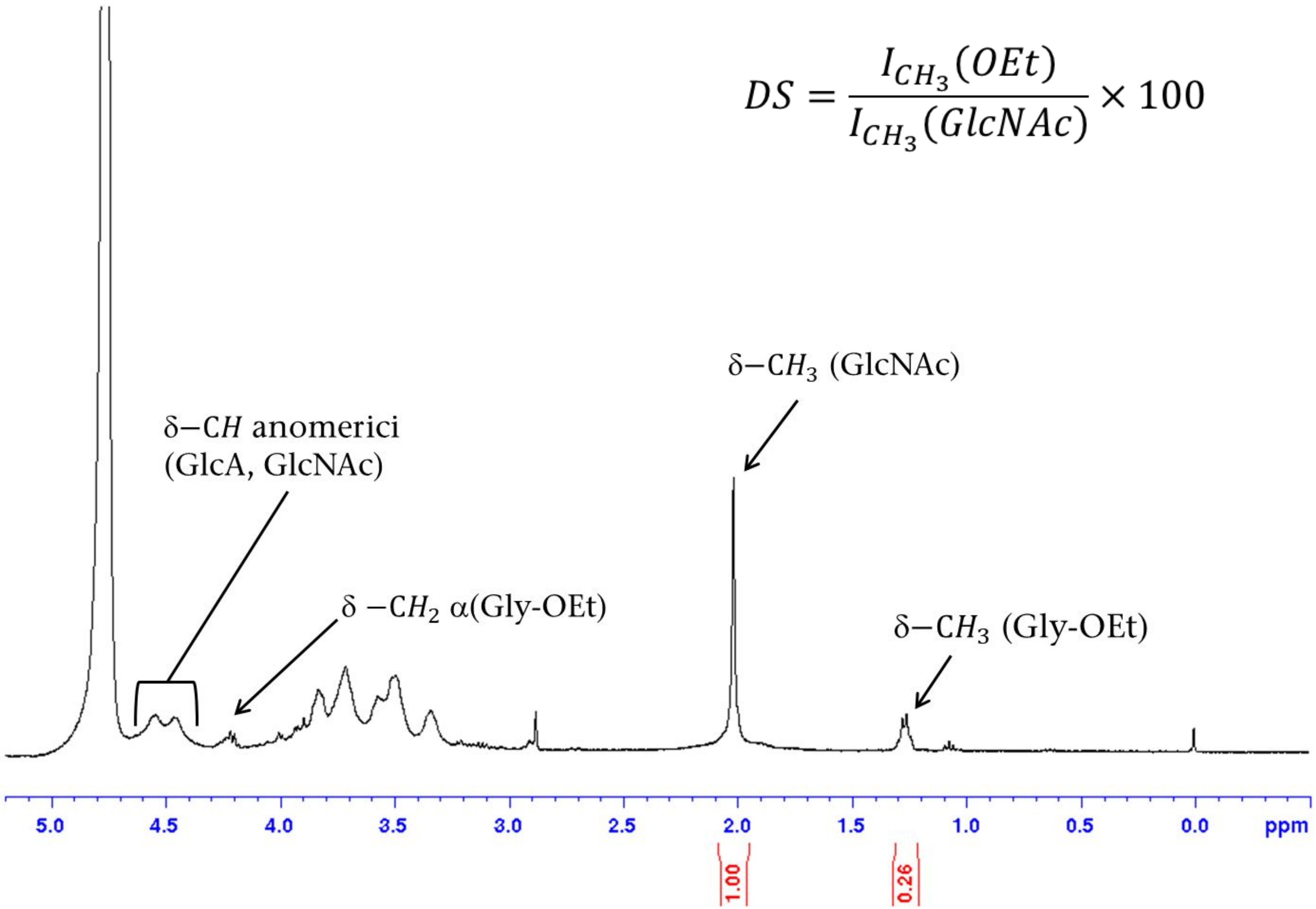
HYALURONIC ACID and alpha-aminoacids: (3 years Laurea)

HYALURONIC ACID and glycine: bioconjugation

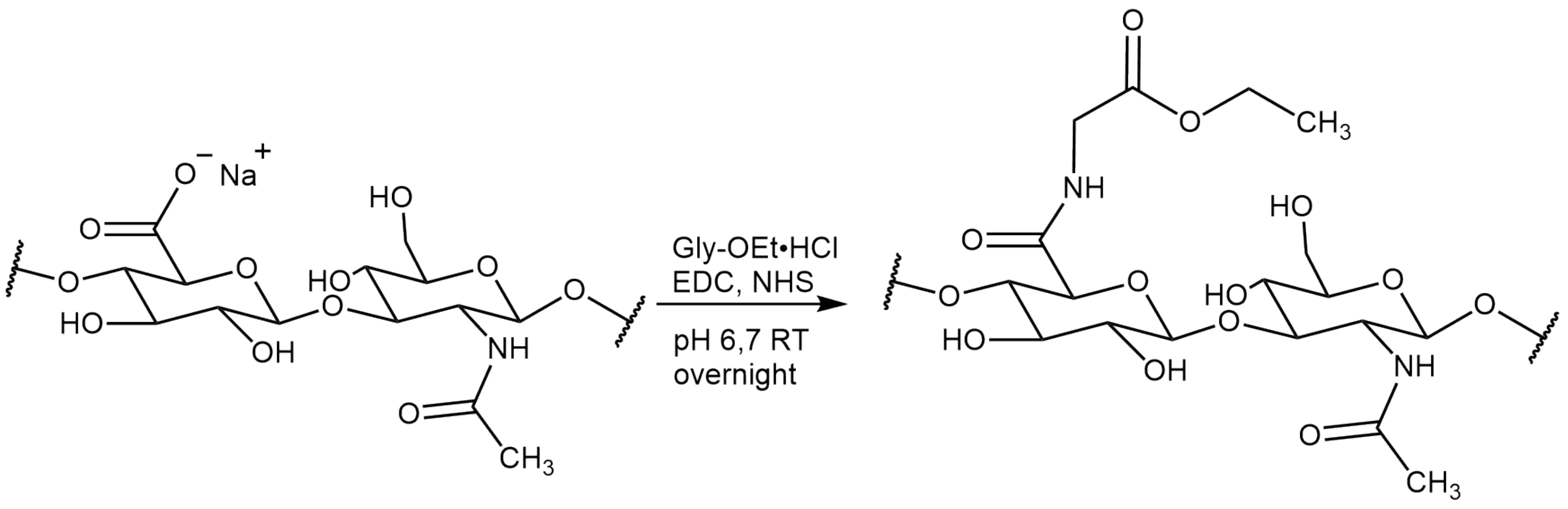


Glycine-Hyaluronan Bioconjugate Characterization: NMR (Technique 2)

$$DS = \frac{I_{CH_3}(OEt)}{I_{CH_3}(GlcNAc)} \times 100$$



BIOCONJUGATION OF HA WITH Gly-OEt



HA (1 eq)

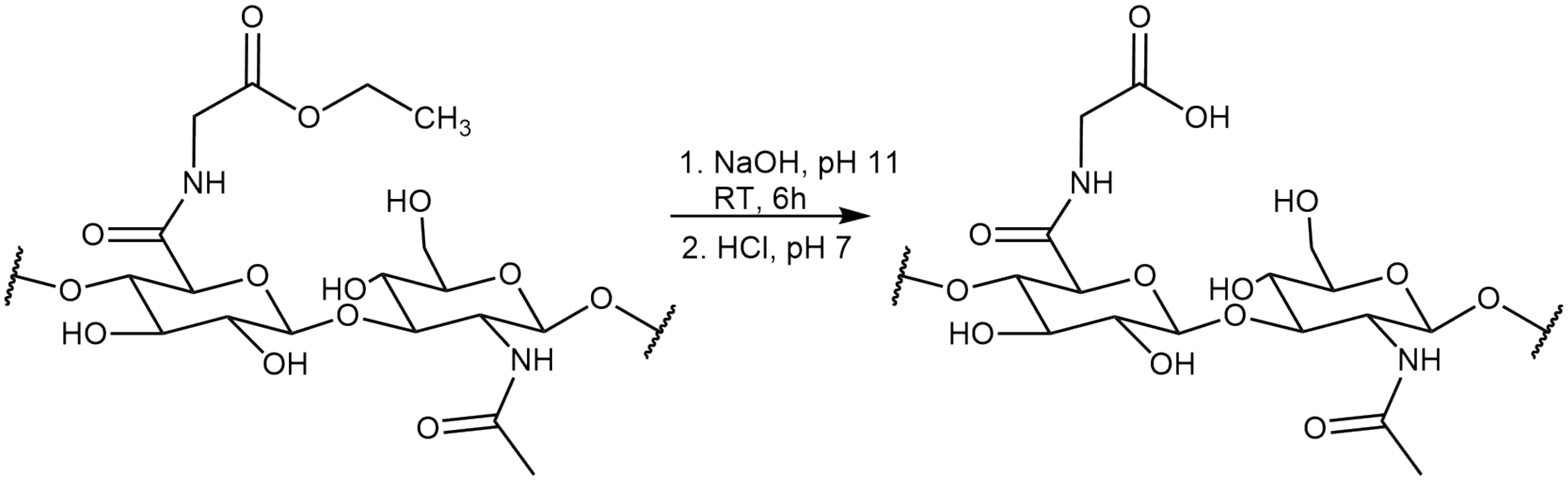
EDC (2 eq)

NHS (1 eq)

Gly-OEt·HCl (2 eq)

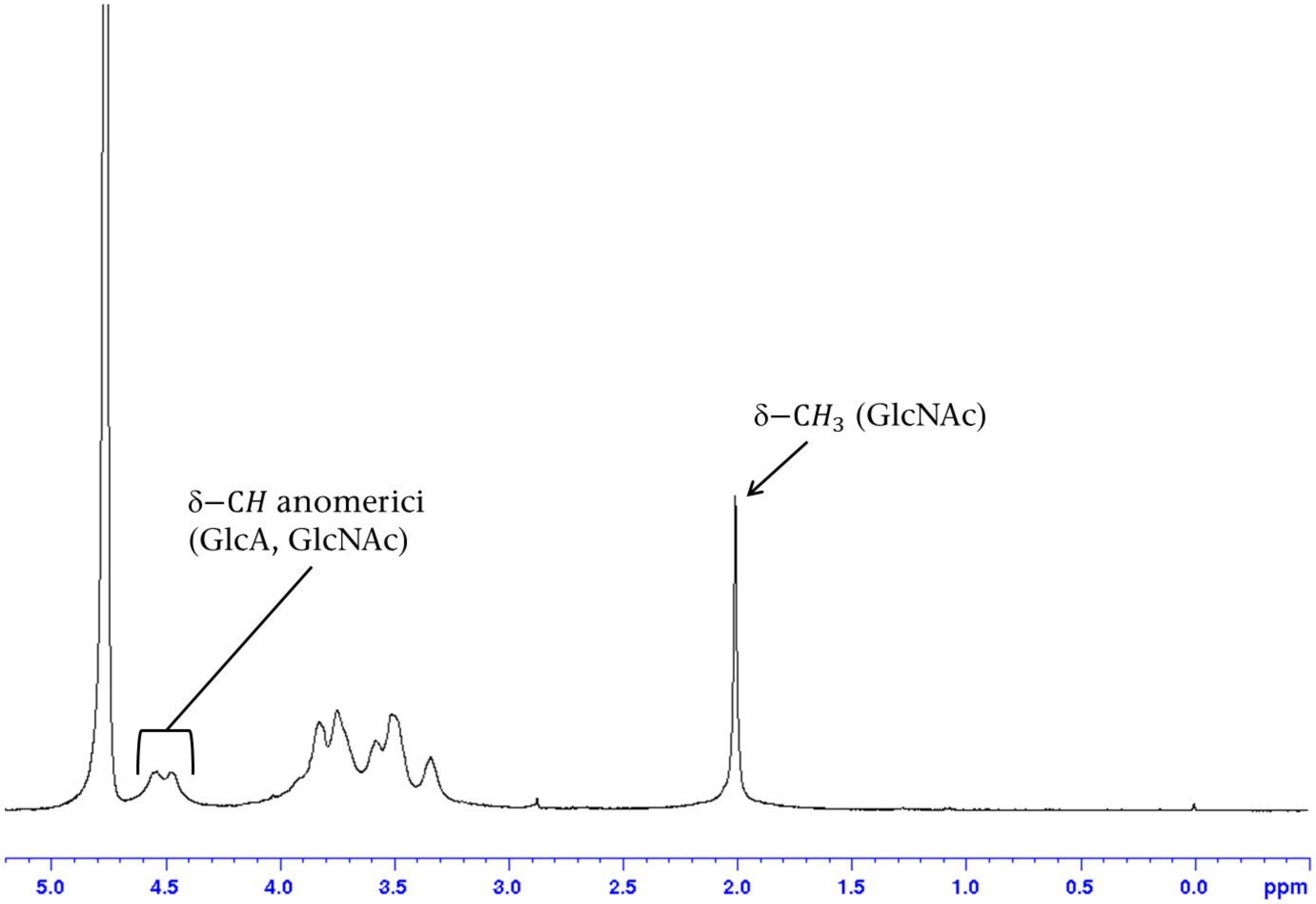
DS = 26,3 % 94,6%

HYDROLYSIS OF Gly-OEt

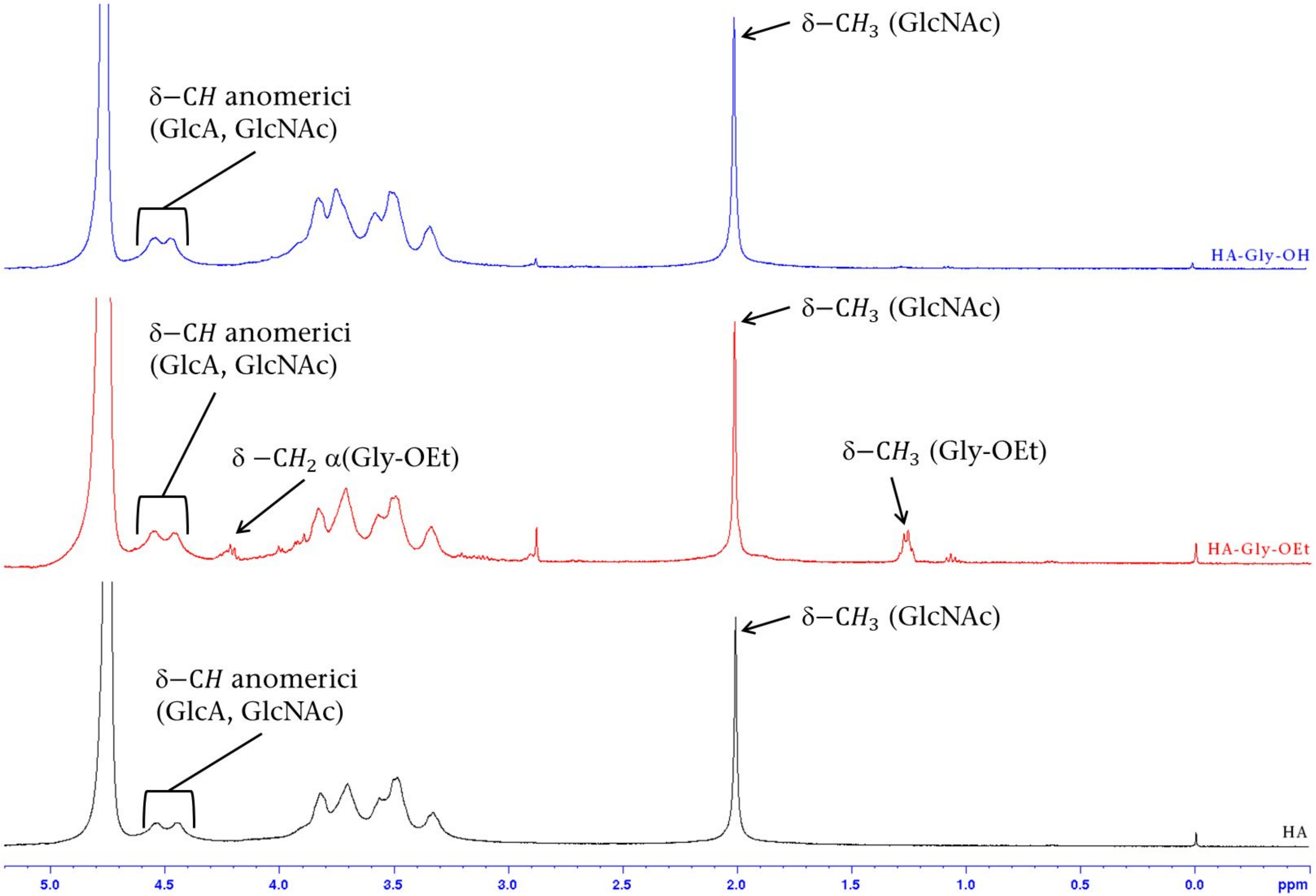


DS = 100 % 53,6%

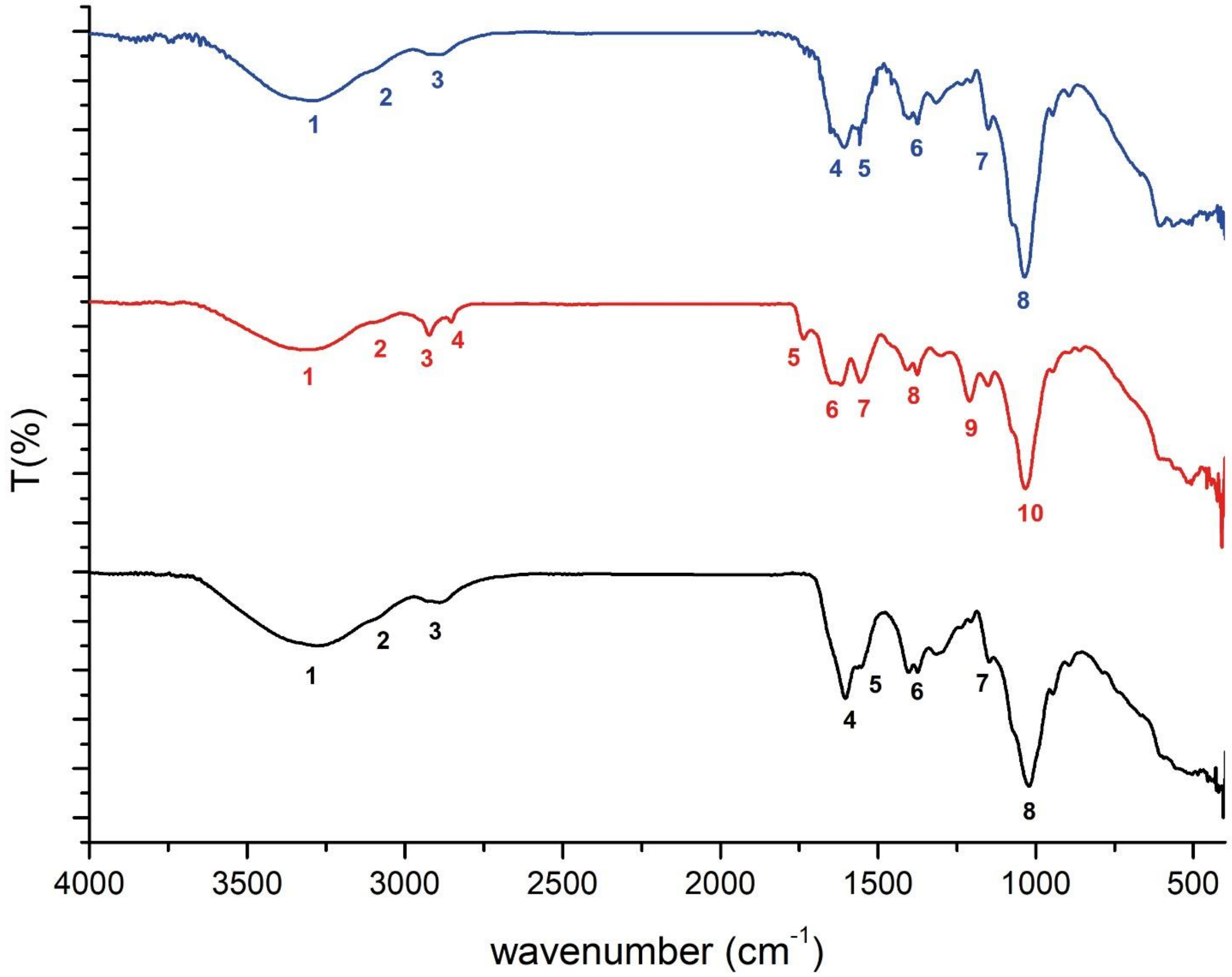
^{1}H-NMR


δ−CH₃ (GlcNAc)
δ−CH anomerici
(GlcA, GlcNAc)
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm

^{1}H-NMR

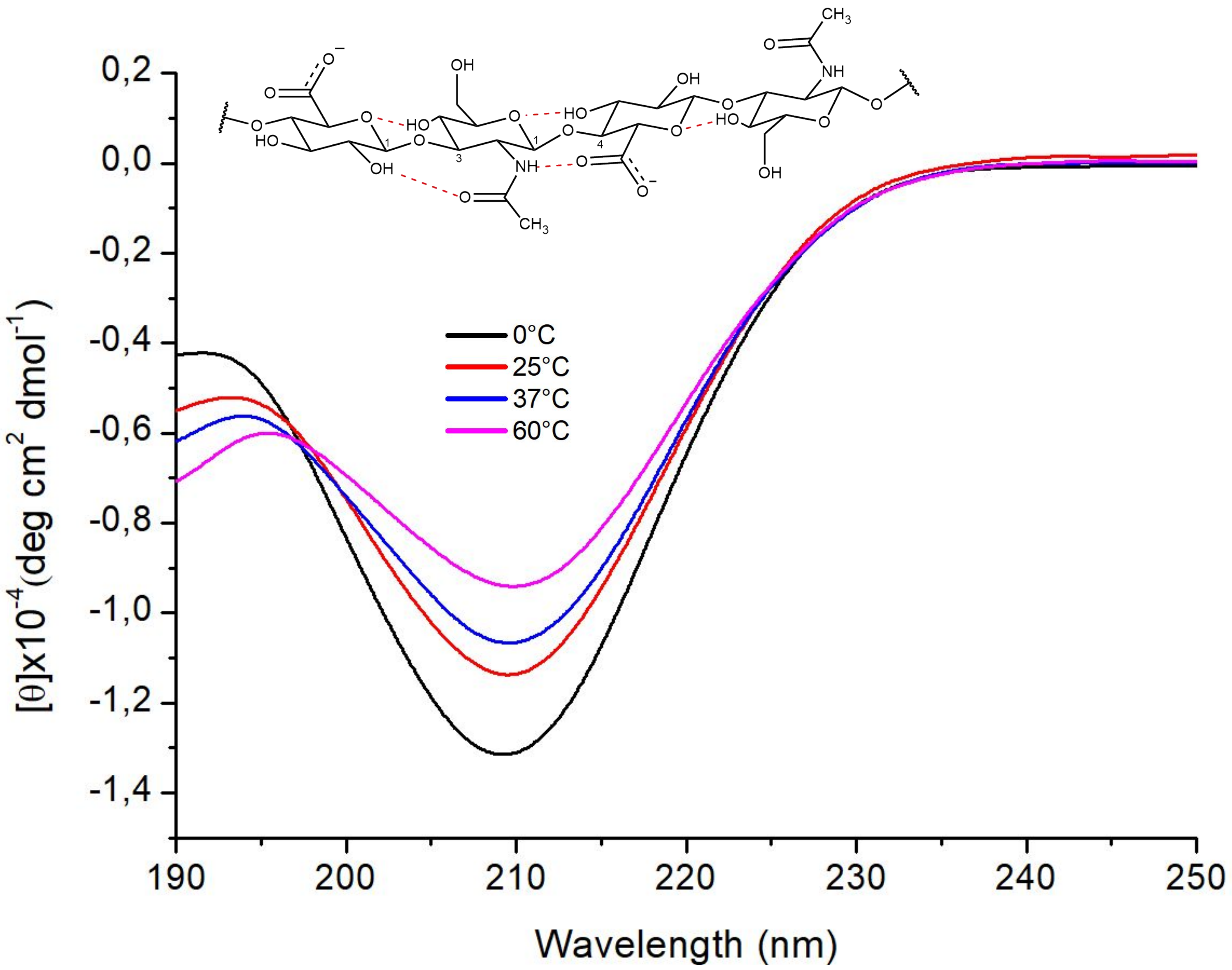

δ−CH₃ (GlcNAc)
δ−CH anomerici
(GlcA, GlcNAc)
HA-Gly-OH
δ−CH₃ (GlcNAc)
δ−CH anomerici
(GlcA, GlcNAc)
δ −CH₂ α(Gly-OEt)
δ−CH₃ (Gly-OEt)
HA-Gly-OEt
δ−CH₃ (GlcNAc)
δ−CH anomerici
(GlcA, GlcNAc)
HA
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm

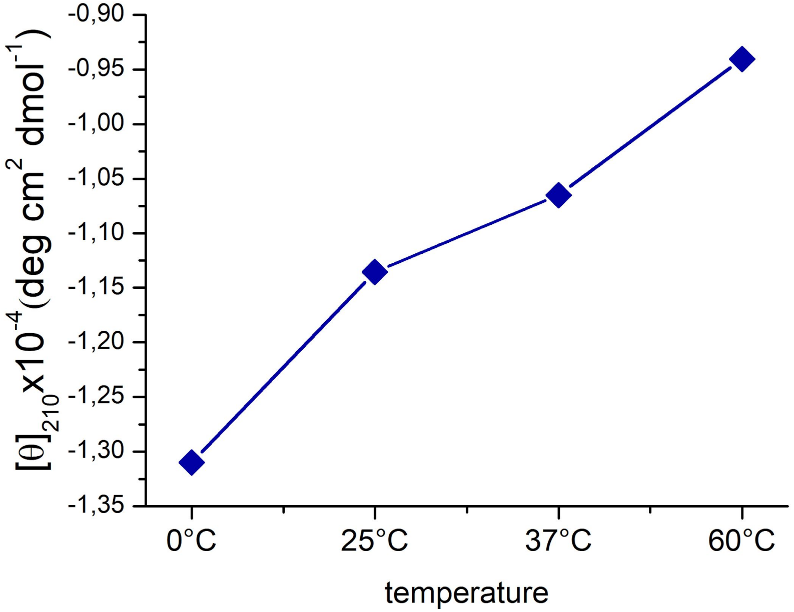
ATR-FTIR

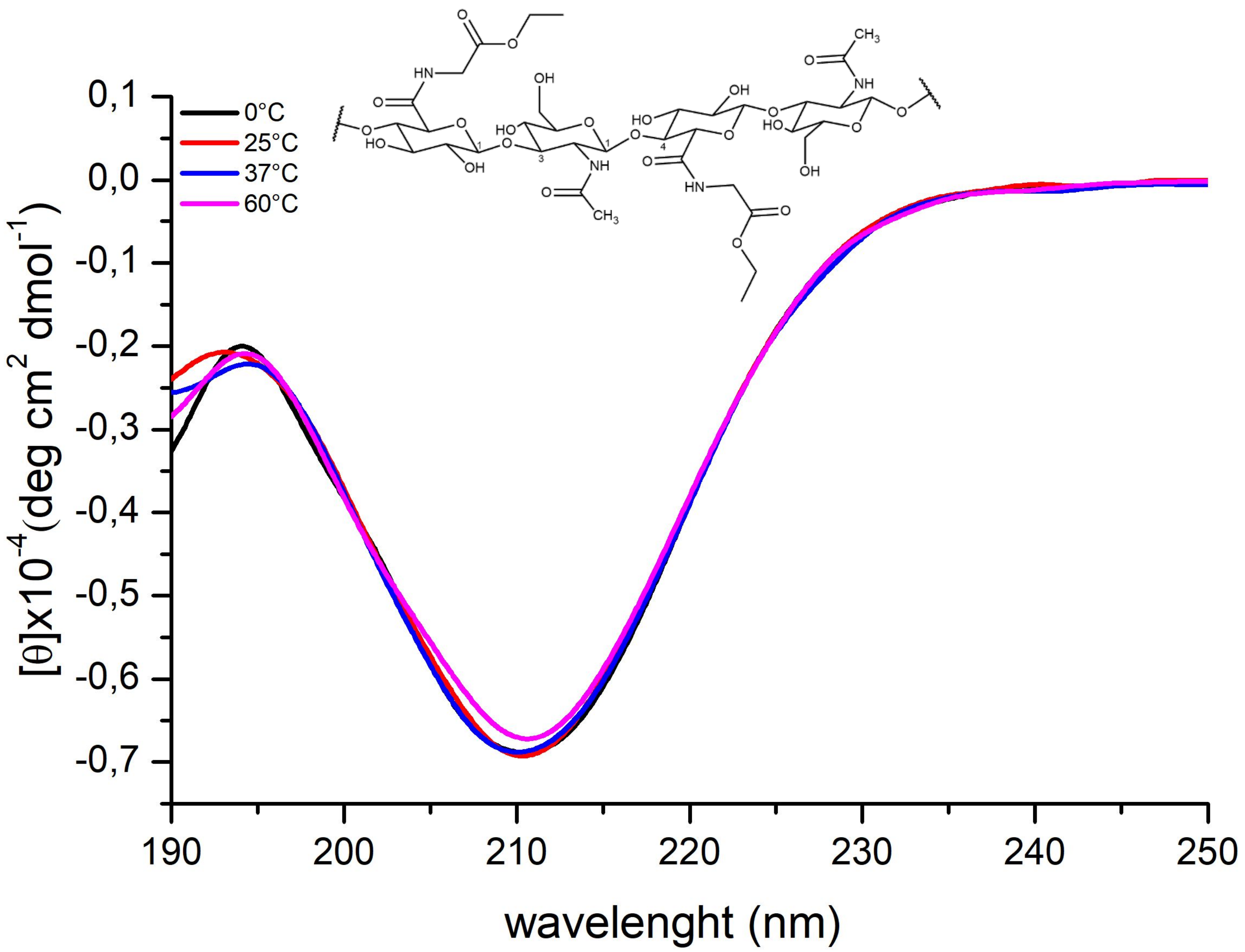


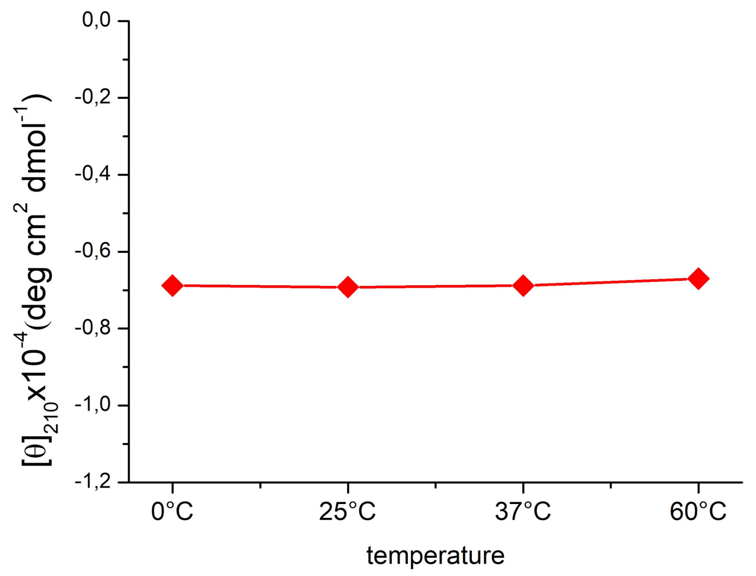
bande IR		(cm^{-1})
HA-Gly-OH		
1	ν O-H	3288
2	ν N-H	3094
3	ν C-H	2883
4	ν C=O ammide	1609
5	δ N-H	1556
6	δ C-H	1384
7	ν C-O-C, ν	1151
8	C-O, ν C-O-H	1034
HA-Gly-OEt		
1	ν O-H	3288
2	ν N-H	3094
3	ν C-H	2921
4		2852
5	ν C=O estere	1735
6	ν C=O ammide	1631
7	δ N-H	1555
8	δ C-H	1373
9	ν C-O-C, ν	1210
10	C-O, ν C-O-H	1035

CD

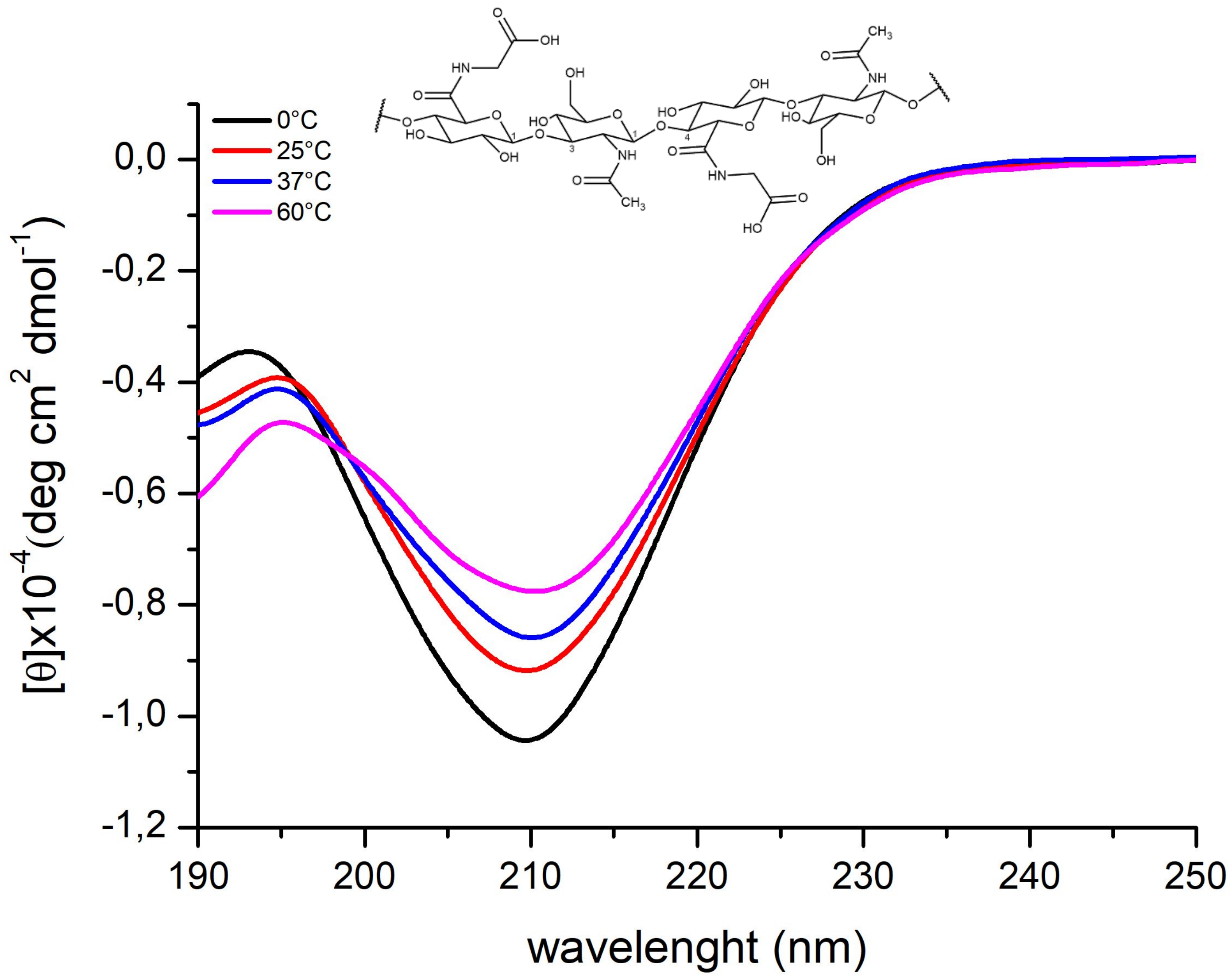

0°C
25°C
37°C
60°C
[θ]x10^{-4}(deg cm^2 dmol^{-1})
Wavelength (nm)
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
-1,4
190
200
210
220
230
240
250


[θ]$_{210}$x10^{-4}(deg cm^2 dmol^{-1})
temperature
0°C
25°C
37°C
60°C
-0,90
-0,95
-1,00
-1,05
-1,10
-1,15
-1,20
-1,25
-1,30
-1,35

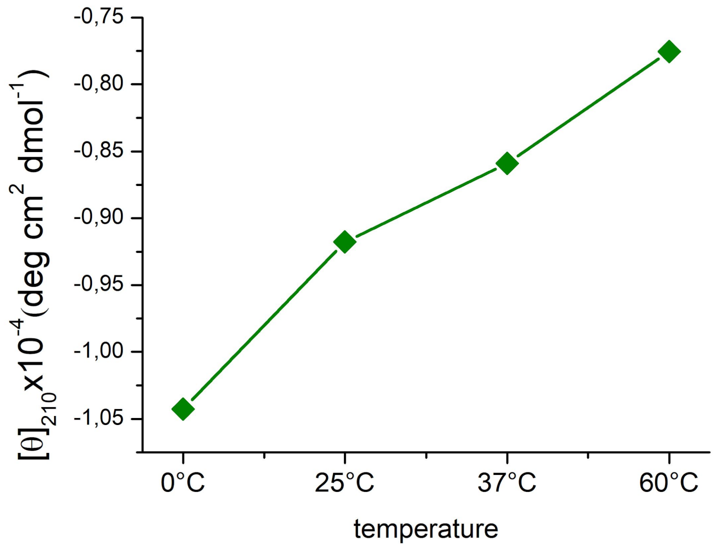

0,1
0,0
-0,1
-0,2
-0,3
-0,4
-0,5
-0,6
-0,7
0°C
25°C
37°C
60°C
[θ]x10^{-4}(deg cm^2 dmol^{-1})
190
200
210
220
230
240
250
wavelenght (nm)
HN
NH
OH
HO
CH$_3$


0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
[θ]$_{210}$x10^{-4}(deg cm^2 dmol^{-1})
0°C
25°C
37°C
60°C
temperature

CD



0°C
25°C
37°C
60°C
[θ]x10^-4(deg cm^2 dmol^-1)
0,0
-0,2
-0,4
-0,6
-0,8
-1,0
-1,2
190
200
210
220
230
240
250
wavelenght (nm)



[θ]210x10^-4(deg cm^2 dmol^-1)
-0,75
-0,80
-0,85
-0,90
-0,95
-1,00
-1,05
0°C
25°C
37°C
60°C
temperature

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