Supporting Info

Crosslinking of chitosan with dialdehyde derivatives of nucleosides and nucleotides. Mechanism and comparison with glutaraldehyde.

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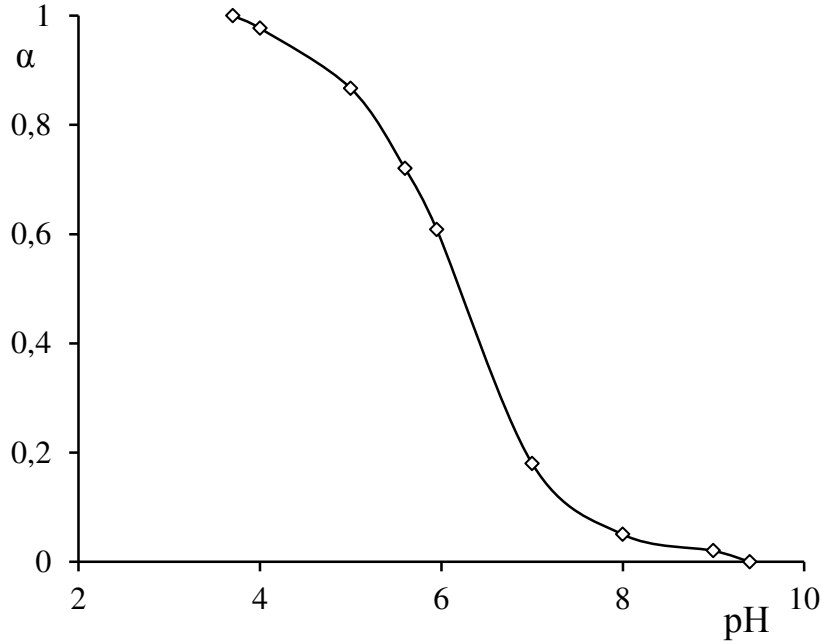


Figure 1. Degree of protonation (α) of chitosan (MW 190000) amino group at 20° C. Estimated pK = 6.2.

Rheological properties of chitosan solutions during the crosslinking reaction with the dialdehydes were studied using rotational viscometer «Rheotest 2" (Germany). Measurements were performed using a thermostatic coaxial-cylinder measuring system as a sensing element. The changing of shear stress at constant shear rate (γ = 3 c^{-1}) were followed in order to study the kinetics of chitosan gelation in the presence of dialdehydes. Measurement accuracy was ± 3%. Value of the dynamic viscosity η was calculated as the ratio of shear stress τ to shear rate γ: $\eta = \frac{\tau}{\gamma}, [Pa \cdot s]$.

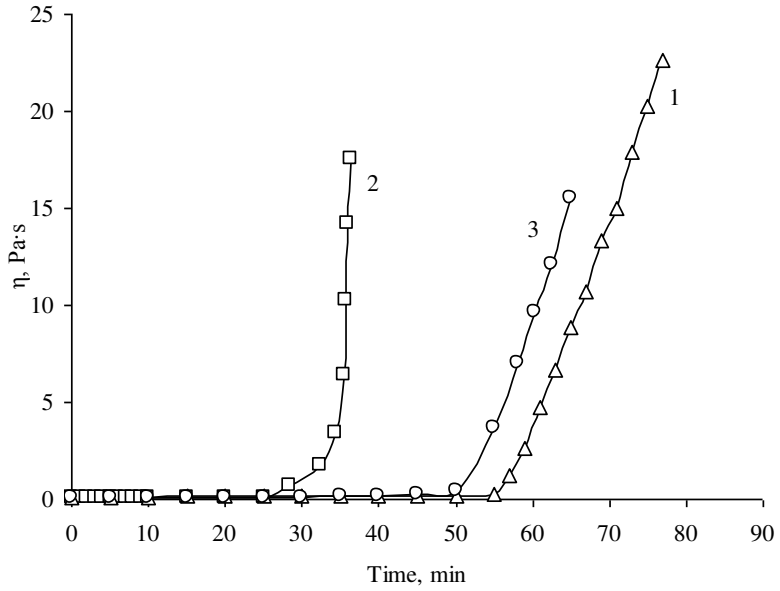


Figure 2. Kinetics of viscosity change of chitosan solutions in the presence of oUrd (1) oAMP (2) and GA (3) at 20 ^{o} C. Dialdehyde/NH_2 ratio: 0.67 mol/mol (1), 0.024 mol/mol (2 and 3).

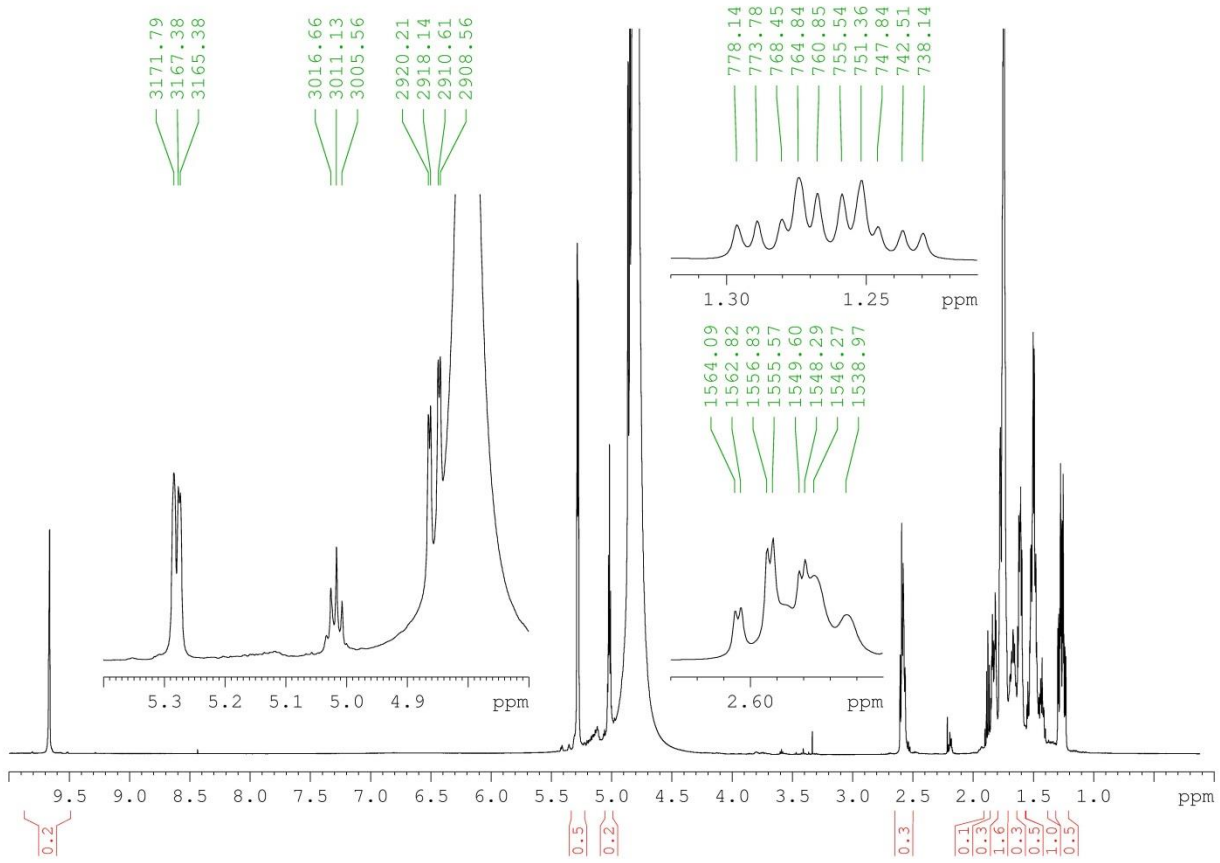


Figure 3. ^{1}H NMR spectrum (600 MHz) of a freshly prepared solution of GA in D_2O at 32 ^{o}C and pH 5.6.

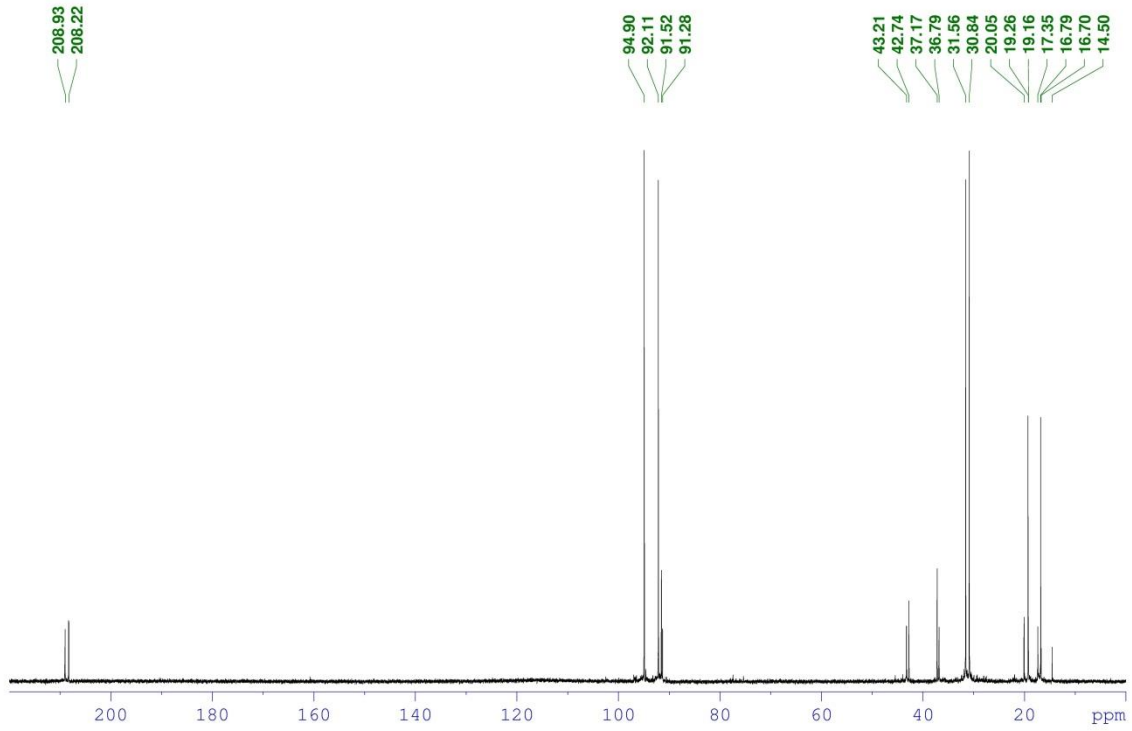


Figure 4. ^{13}C NMR spectrum (150 MHz) of a freshly prepared solution of GA in D_2O at 32 ^{o}C and pH 5.6.

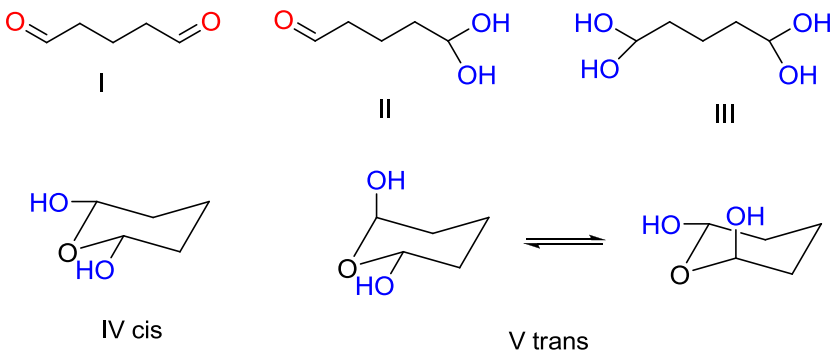


Figure 5. Structures of GA forms in aqueous solutions.

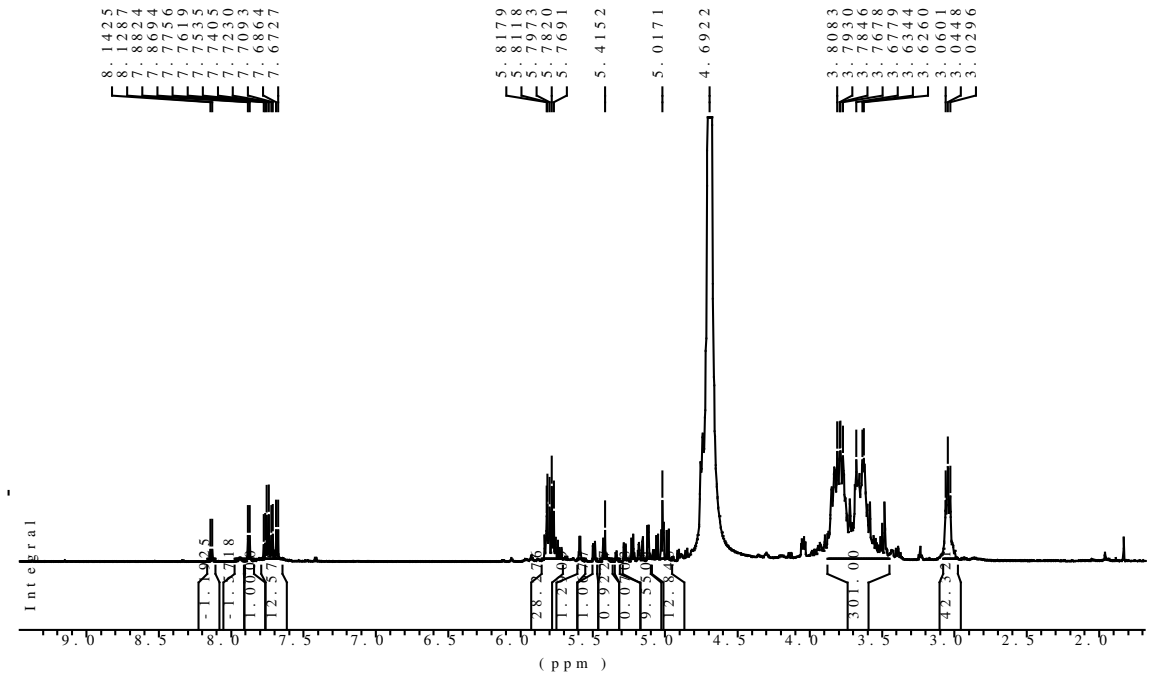


Figure 6. 1H NMRspectrum (600 MHz) of oUrd (0.05 M) in D_2O at 22 °C, pH 7.0.

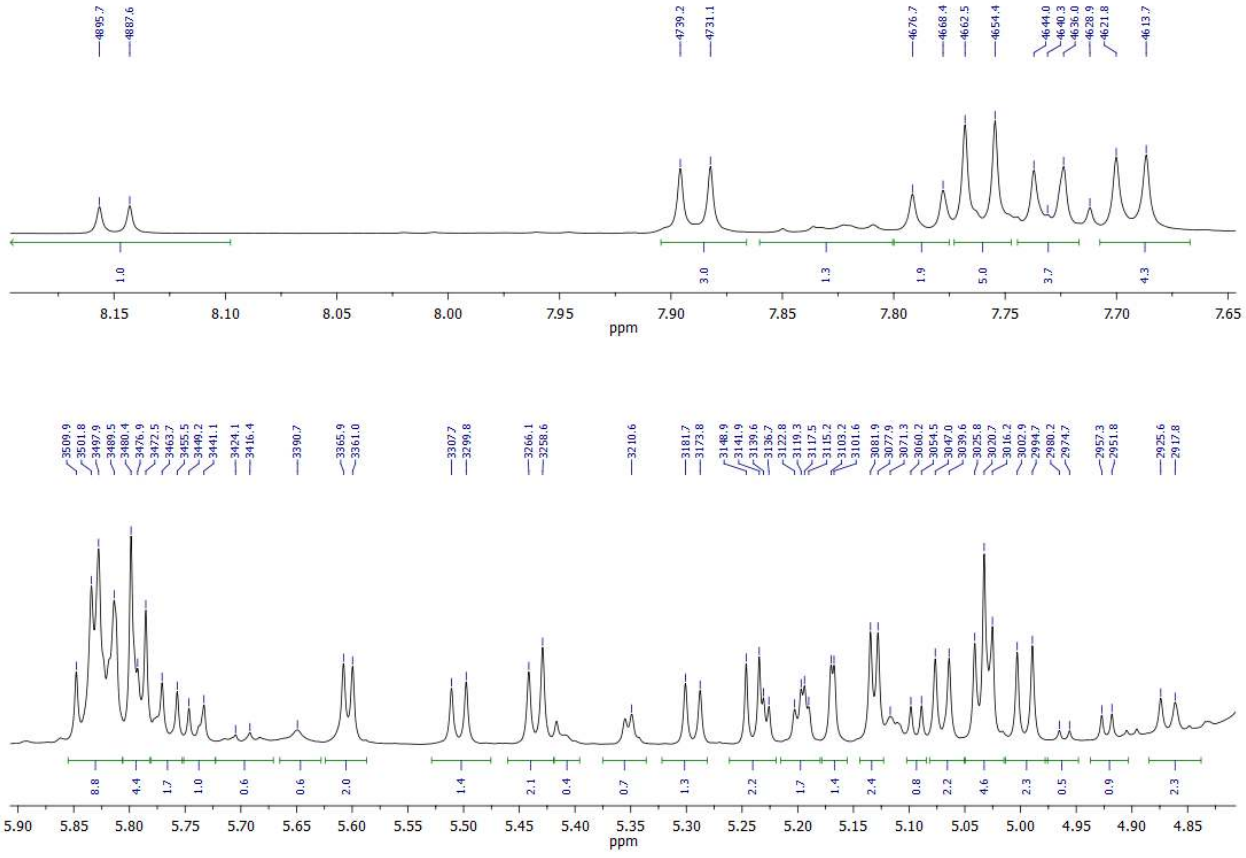


Figure 7. Low-field regions of ^1H NMR spectrum (600 MHz) of oUrd (0.05 M) in D_2O at 22 °C, pH 7.0.

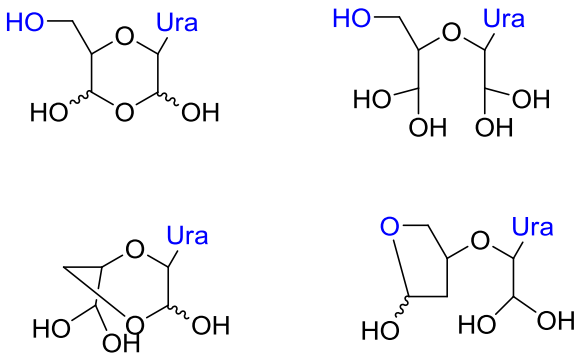


Figure 8. Proposed structures of hydrated species of oUMP in aqueous solution.

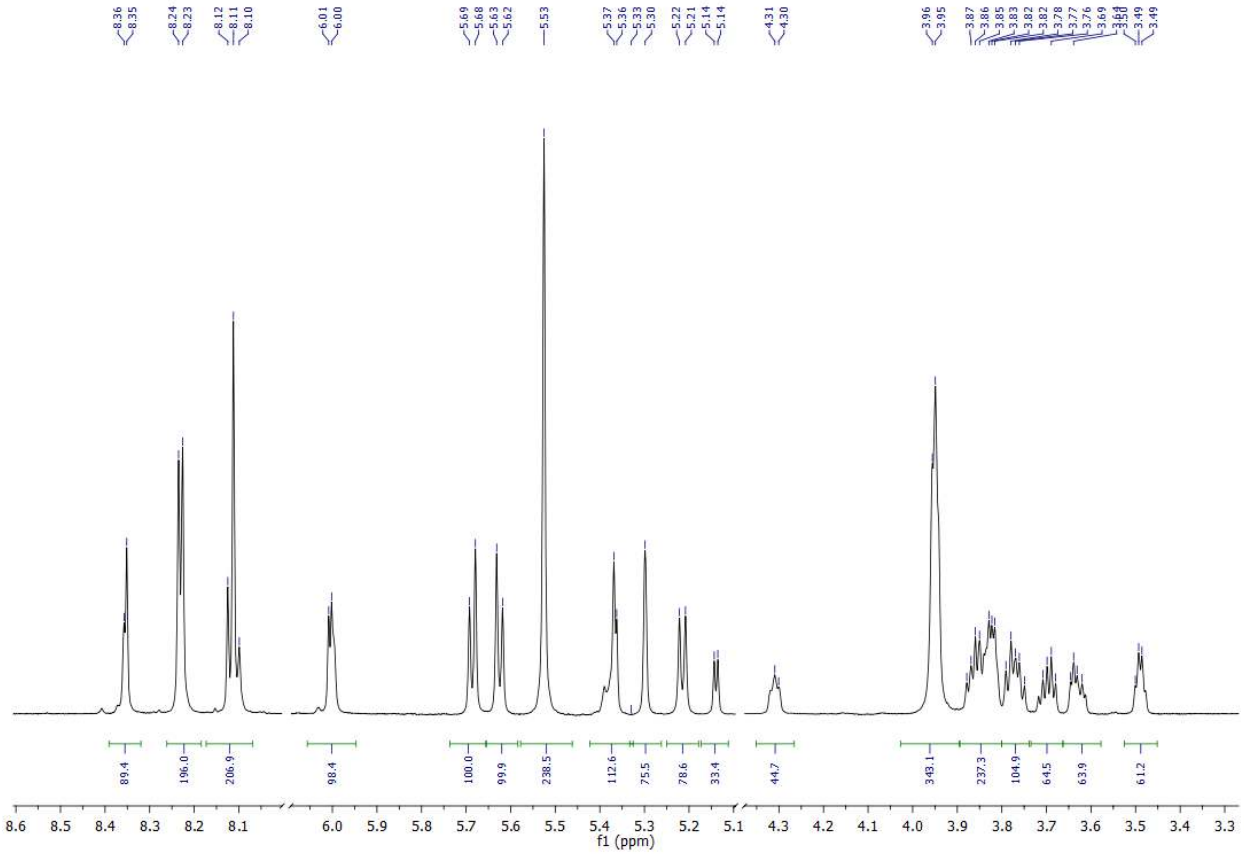


Figure 9. ^{1}H NMRspectrum (600 MHz) of oAMP (0.05 M) in D_2O at 22 °C, pH 7.0.

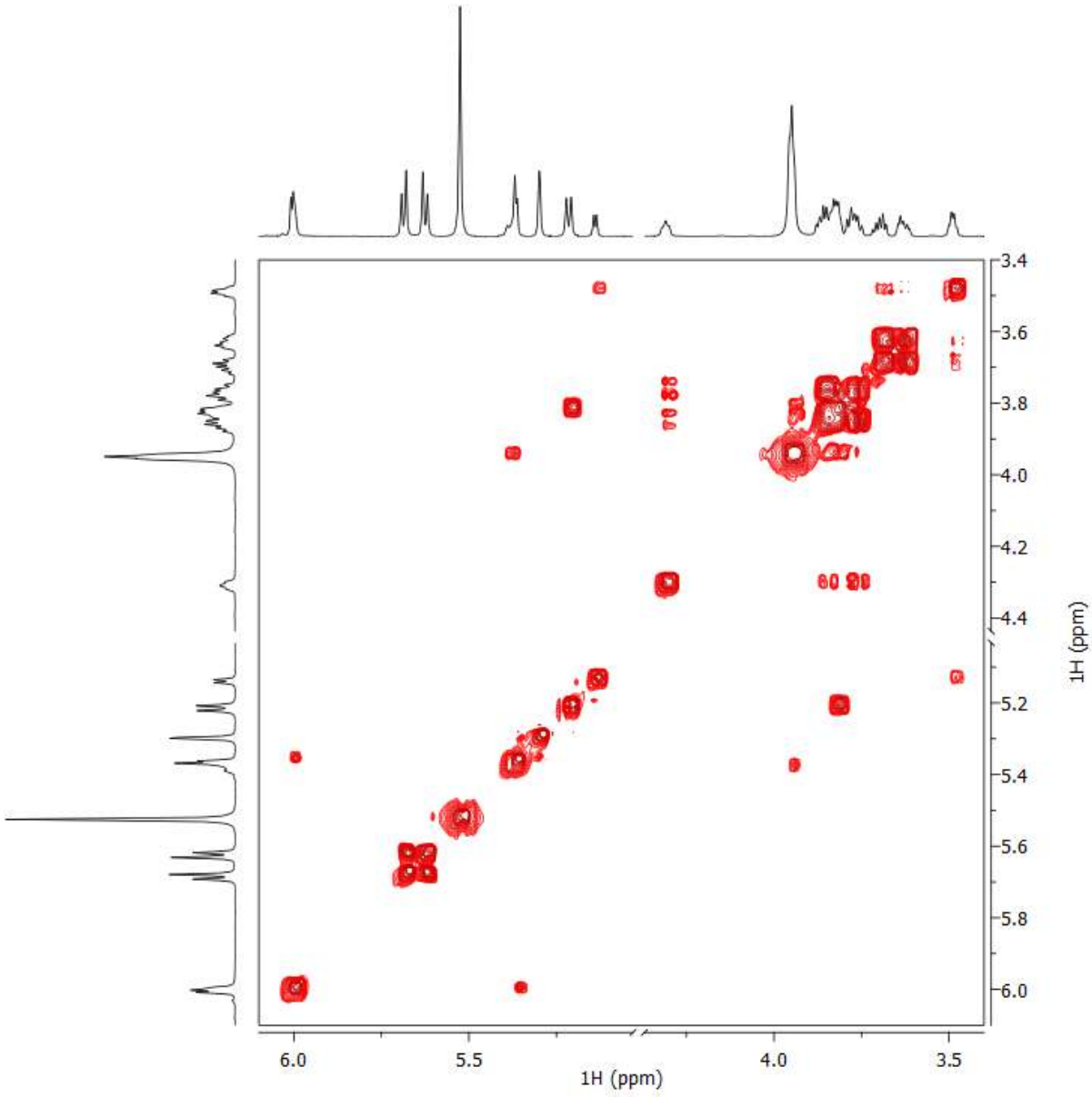


Figure 10. COSY spectrum (600 MHz) of oAMP (0.05 M) in D_2O at 22 °C, pH 7.0.

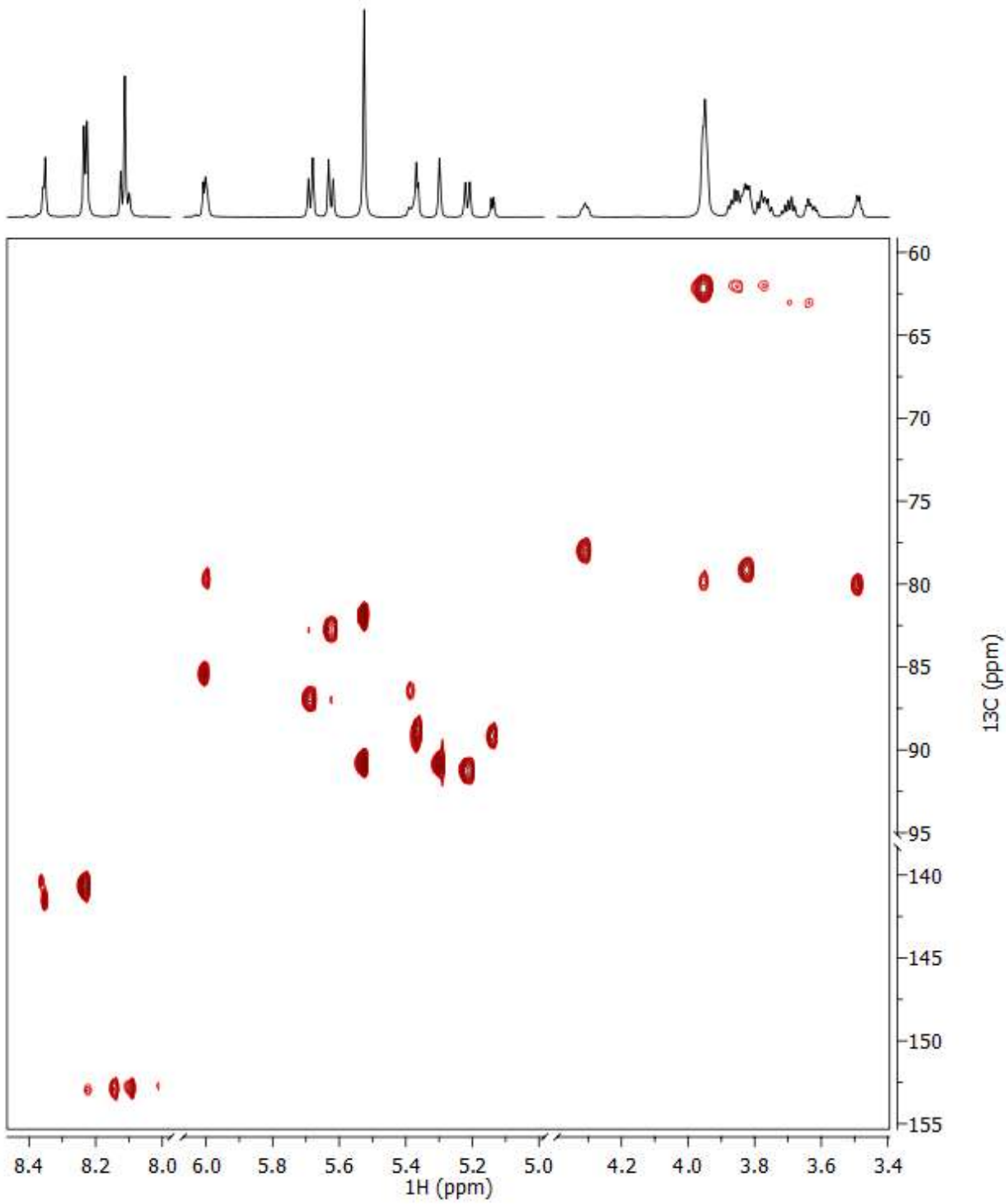
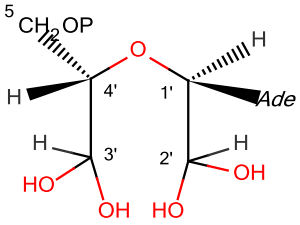
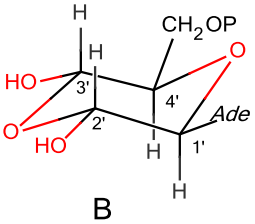


Figure 11. HSQC spectrum 600 MHz) of oAMP (0.05 M) in D_2O at 22 °C, pH 7.0.

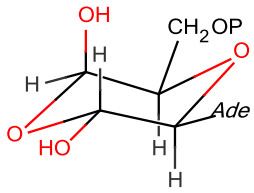
Table 1.Hydrated forms of oAMP in aqueous solutions and their NMR spectra .



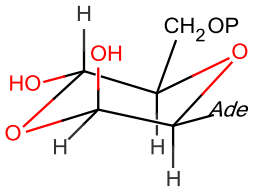
A



B



C



D

Compound	Group	^{1}H	^{13}C	$^{3}J_{HH}$
A	1'	6.01	85.4	4.0
	2'	5.37	88.9	4.0
	3'	5.14	89.2	4.9
	4'	3.50	80.0	4.9
	5'	3.64	63.0	m*
	5''	3.69	63.0	m
B	1'	5.53	90.8	7.9
	2'	5.53	81.9	7.9
	3'	5.22	91.3	8.1
	4'	3.82	79.2	8.1
	5'	3.96	62.2	m
	5''	3.96	62.2	m
C	1'	5.68	86.9	7.9
	2'	5.62	82.7	7.9
	3'	5.30	90.8	1.2
	4'	4.31	78.0	m
	5'	3.72	62.1	m
	5''	3.85	62.1	m
D	1'	6.00	79.7	m
	2'	5.36	88.6	m
	3'	5.39	86.4	8.0
	4'	3.95	79.8	8.0
	5'	3.95	62.2	m
	5''	3.95	62.2	m

* m denotes “multiplet” and corresponds to the cases where the splitting is too complex to extract the exact values of J-coupling constants.

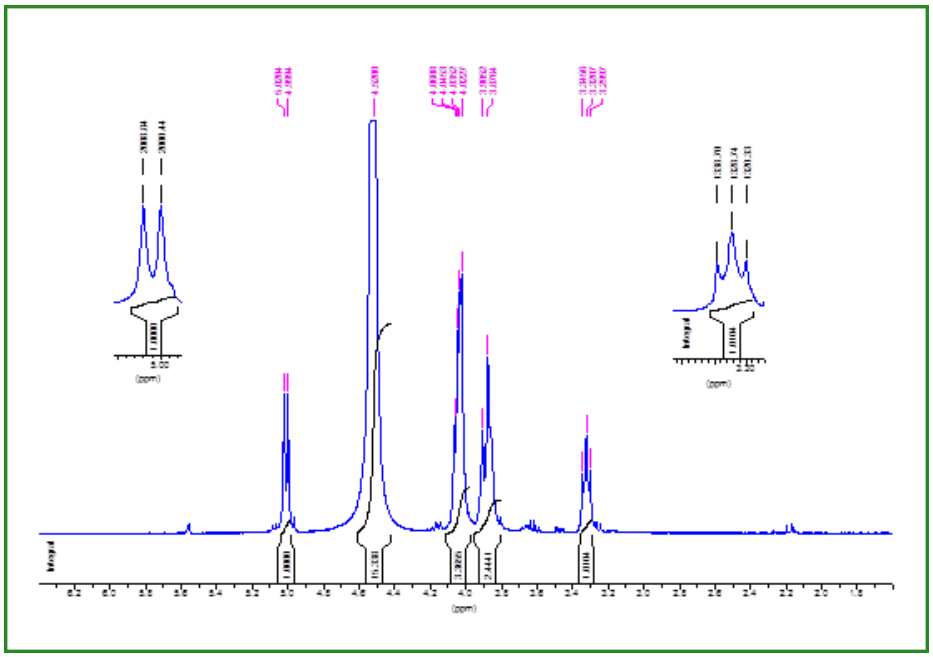


Figure 12. ^{1}H NMR spectrum (400 МГц) of low molecular weight chitosan (MW 12200) in D_2O at 47 °C (320 K) and pH 4.1

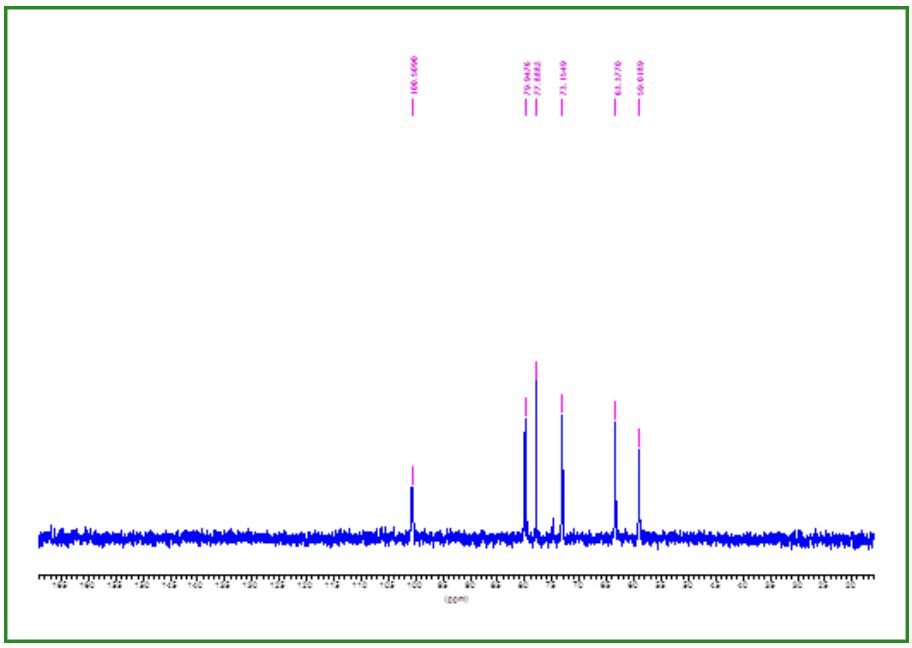


Figure 13. ^{13}C NMR spectrum (100 MHz) of low molecular weight chitosan (MW 12200) in D_2O at 47 °C (320 K) and pH 4.1.

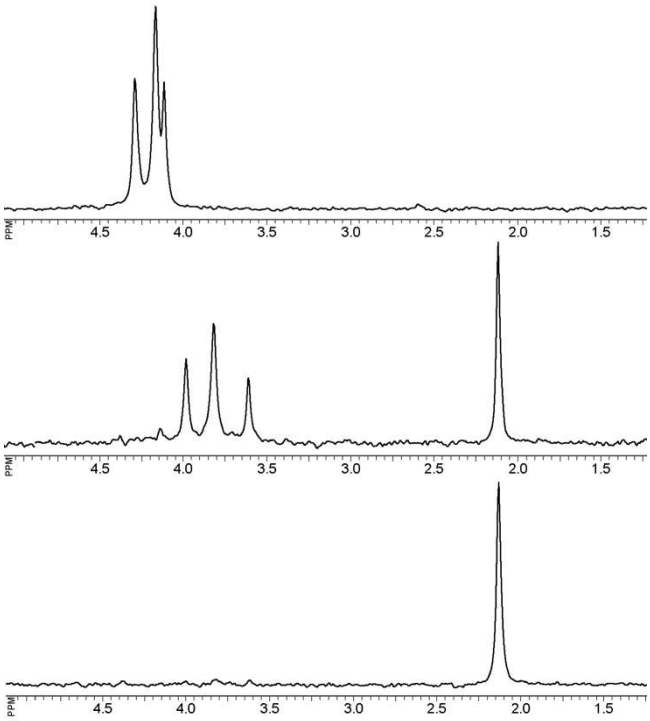


Figure 14. 243 MHz ^{31}P NMR spectra of oAMP ($5.0 \cdot 10^{-2}$ M) in D_2O at 32 °C (305K), pH 7.0. A – initial spectrum. B – after 7 hrs, C – after 23 hrs.

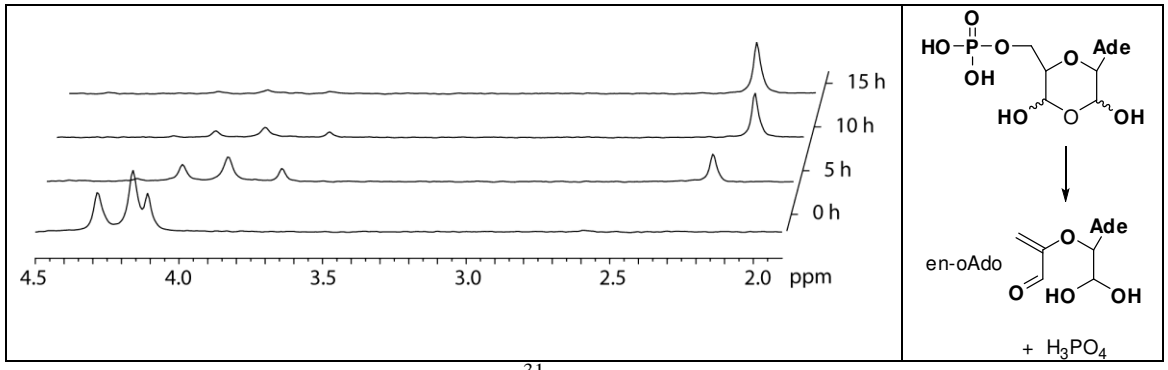


Figure 15. The time dependence of the ^{31}P-NMR spectra line shape of oAMP in D_2O at 32° C and pH 7.0. The low field signal corresponds to inorganic phosphate according to the data of HMBC spectroscopy (no cross-peaks with the proton signals of the 5'-CH_2-group were observed).

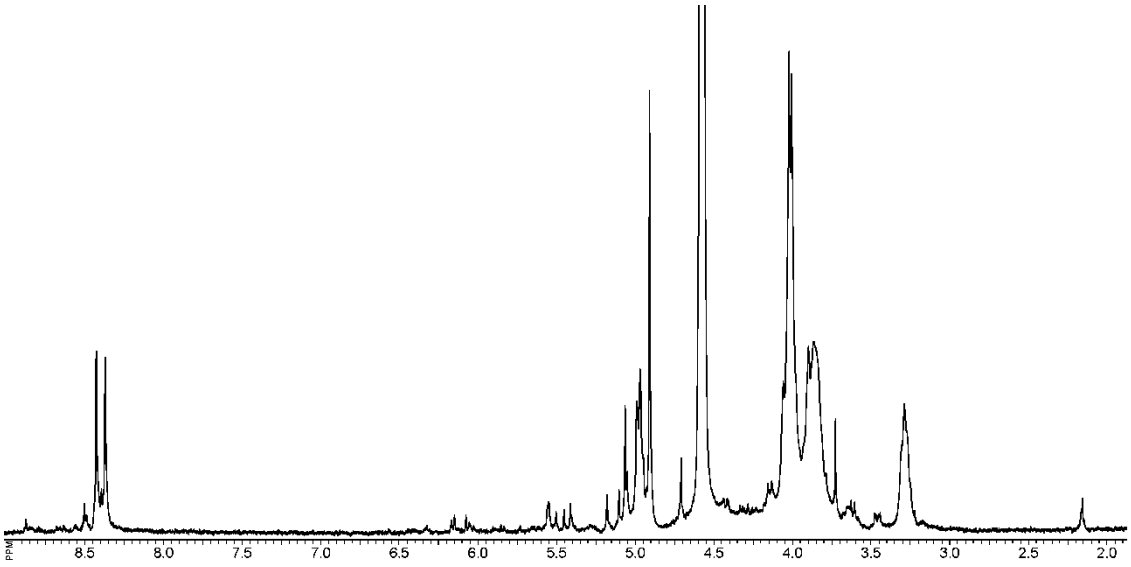


Figure 16. 600 MHz ^{1}H NMR spectrum of oAMP (0.005 mmol) in D_2O in the presence of low molecular weight chitosan (avg. MW 12200 with deacetylation degree 98%) registered after 5 hrs at 52 °C (325 K), pH 4.0. Ratio of NH_2/C=O-group equal 1mmol/mmol.

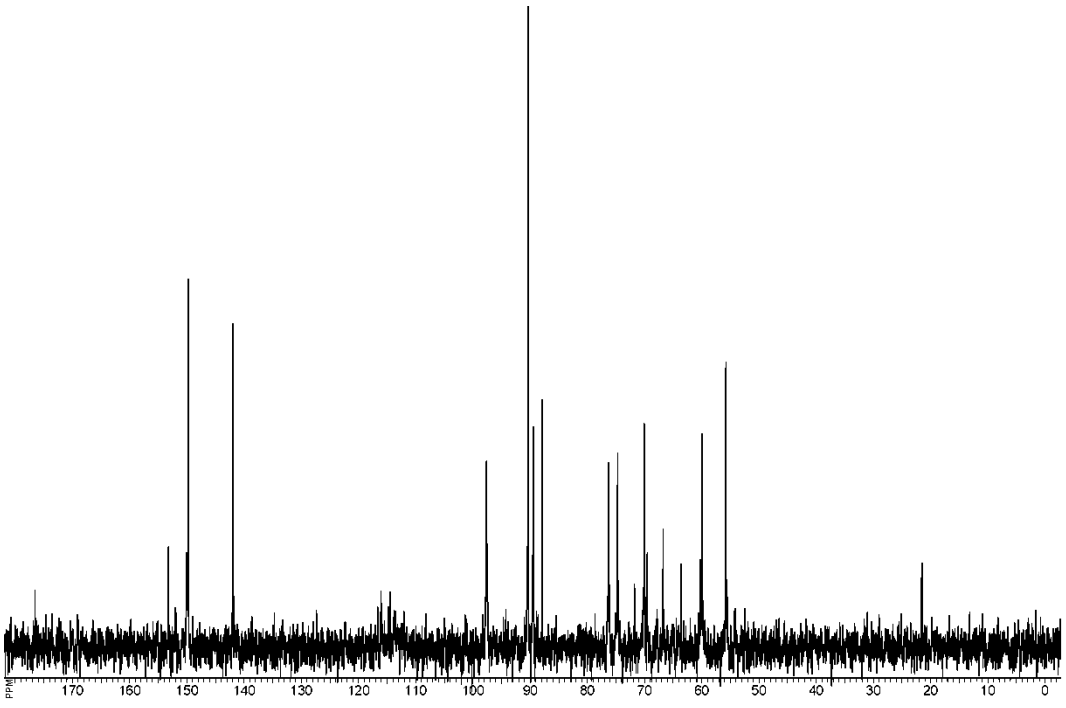


Figure 17. 150 MHz ^{13}C NMR spectrum of oAMP (0.005 mmol) in D_2O in the presence of low molecular weight chitosan (avg. MW 12200 with deacetylation degree 98%) registered after 5 hrs at 52 °C (325 K), pH 4.0. Ratio of NH_2/C=O-group equal 1mmol/mmol.

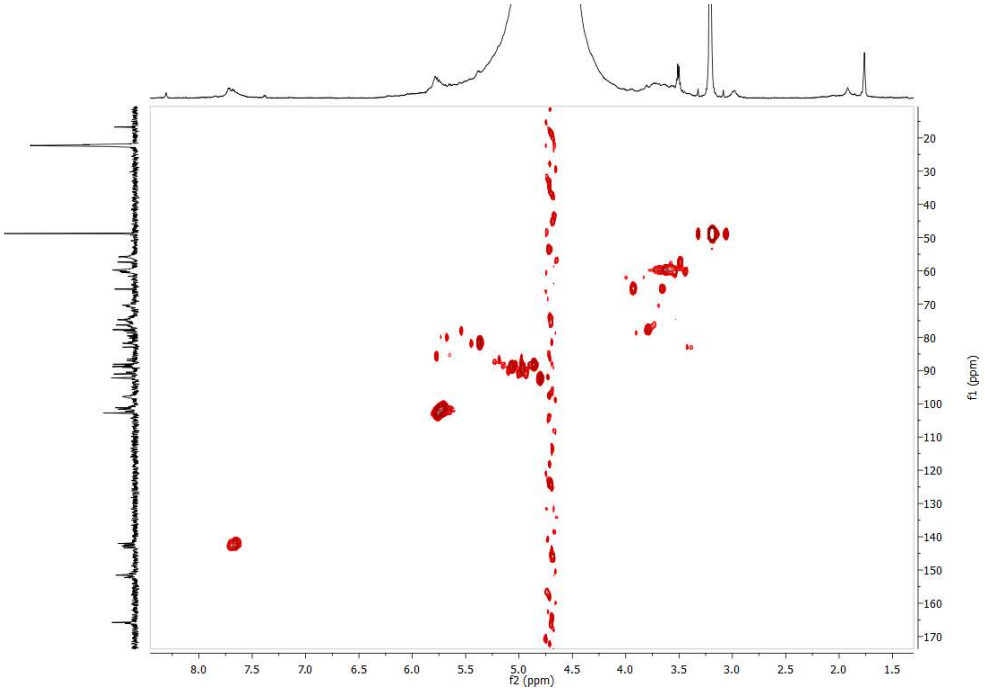


Figure 18. 2D ^{1}H-^{13}C correlation HSQC NMR spectrum of oUrd (0.005 mmol) in D_2O in the presence of high molecular weight chitosan (avg. MW 190000), pH 4.1.

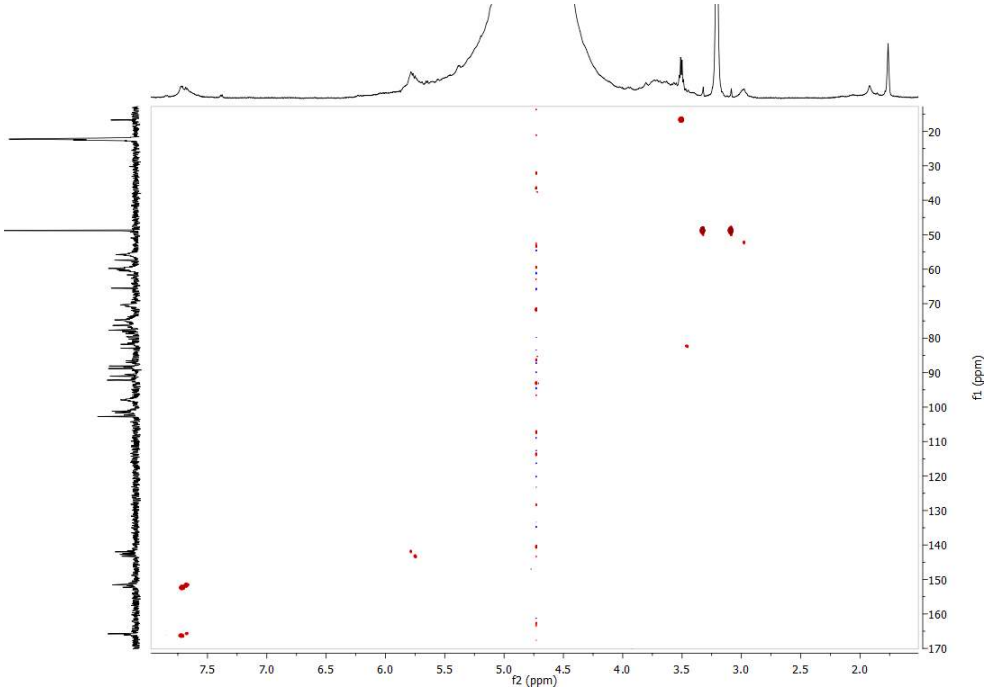
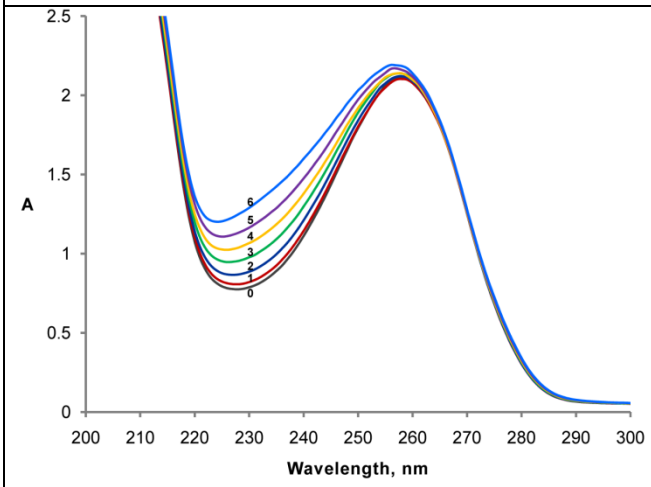


Figure 19. 2D ^{1}H-^{13}C long-range correlation HMBC NMR spectrum of oUrd (0.005 mmol) in D_2O in the presence of high molecular weight chitosan (avg. MW 190000), pH 4.1.

A



B

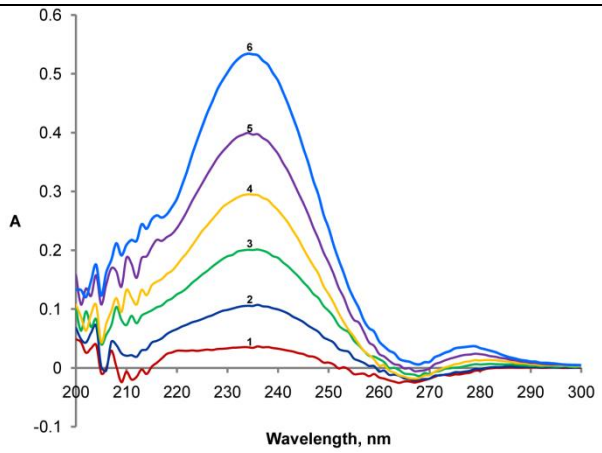
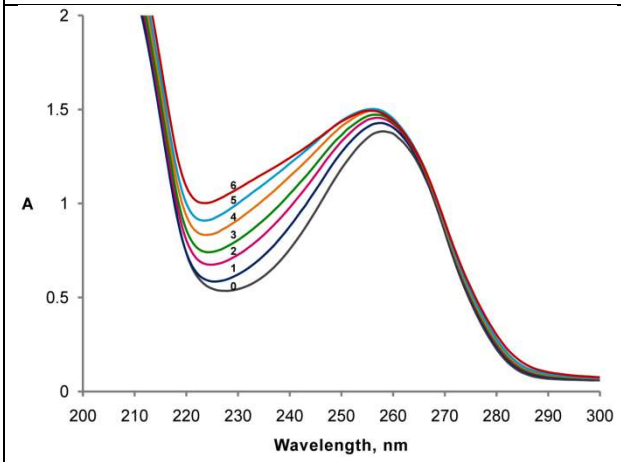


Figure 20. **A**. UV spectra of 1.41•10 4 M **oAMP** in 0.05 M phosphate buffer solution (pH 5.6, 37°C): 0 – initial spectrum, 1 – 1h, 2 – 3 h, 3 – 6 h, 4 – 10 h, 5 –15 h, 6 – 40 h. **B**. UV difference spectra obtained by subtracting from all curves the initial UV spectrum (curve 0)

A



B

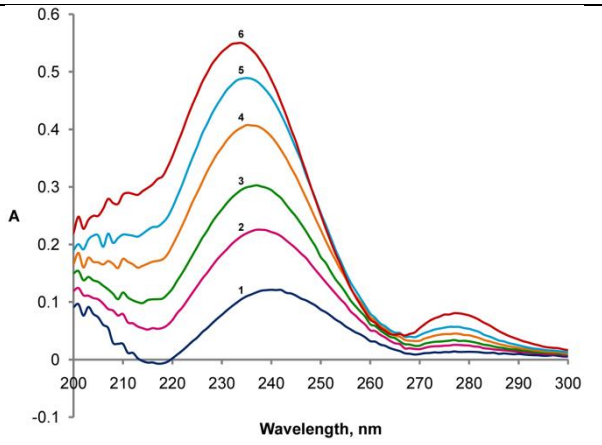


Figure 21. **A.** UV spectra of $9.20 \cdot 10^{-5}$ M **oAMP** in presence of chitosan (NH_2/COH-group ratio 5.0 mol/mol, 20°C, pH 5.6): *0* –initial spectrum, *1* – 1 h, *2* – 2 h, *3* – 3 h, *4* – 5 h, *5* – 8 h, *6* – 20 h after mixing oAMP and chitosan. **B.** UV difference spectra obtained by subtracting from all curves the initial UV spectrum (curve *0*)

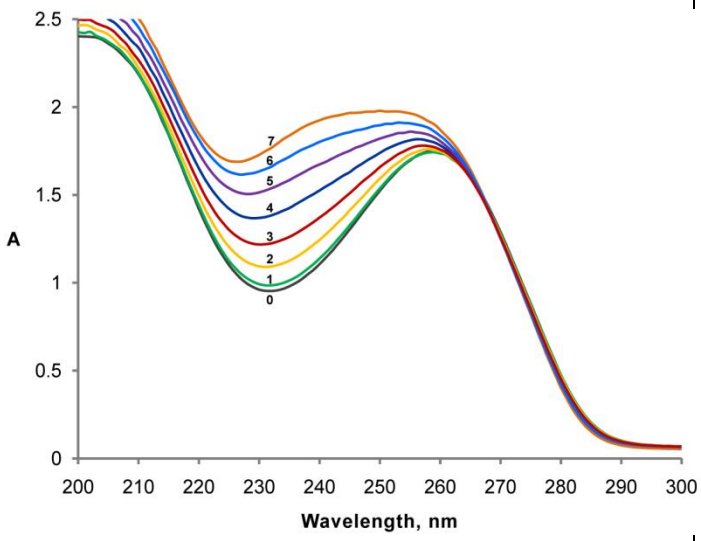
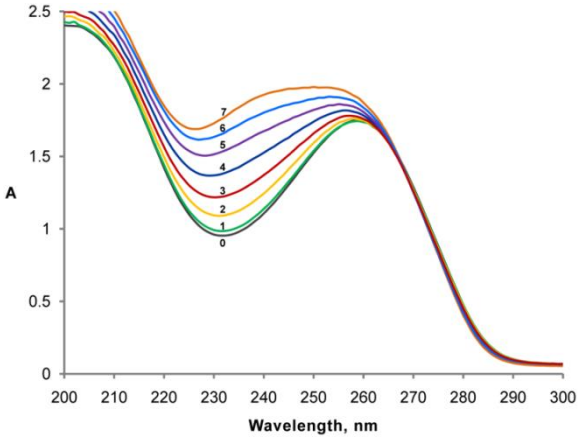
A	B
	

Figure 22. **A.** UV spectra of $1.75 \cdot 10^{-4}$ M **oUMP** in 0.05 M phosphate buffer solution (pH 5.6, 37°C): *0* – initial spectrum, *1* – 30 min, *2* – 2 h, *3* – 3 h, *4* – 7 h, *5* –11 h, *6* – 16 h, *7* – 46 h. **B.** UV difference spectra obtained by subtracting from all curves the initial UV spectrum (curve *0*)

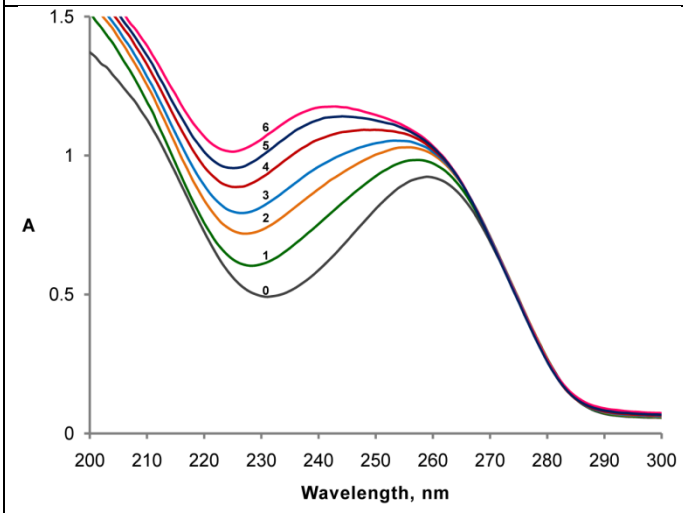
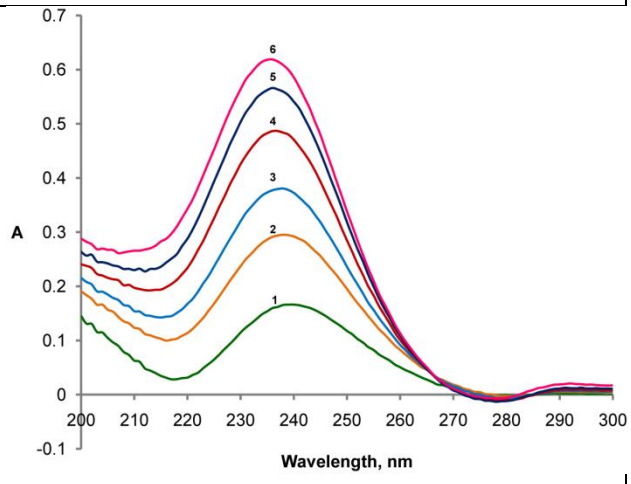
A	B
	

Figure 23. **A.** UV spectra of $9.23 \cdot 10^{-5}$ M **oUMP** in presence of chitosan (NH_2/COH-group ratio 5.0 mol/mol, 20°C, pH 5.6): *0* –initial spectrum, *1* – 1 h, *2* – 2 h, *3* – 3 h, *4* – 5 h, *5* – 8 h, *6* – 18 h after mixing oUMP and chitosan. **B.** UV difference spectra obtained by subtracting from all curves the initial UV spectrum (curve *0*)

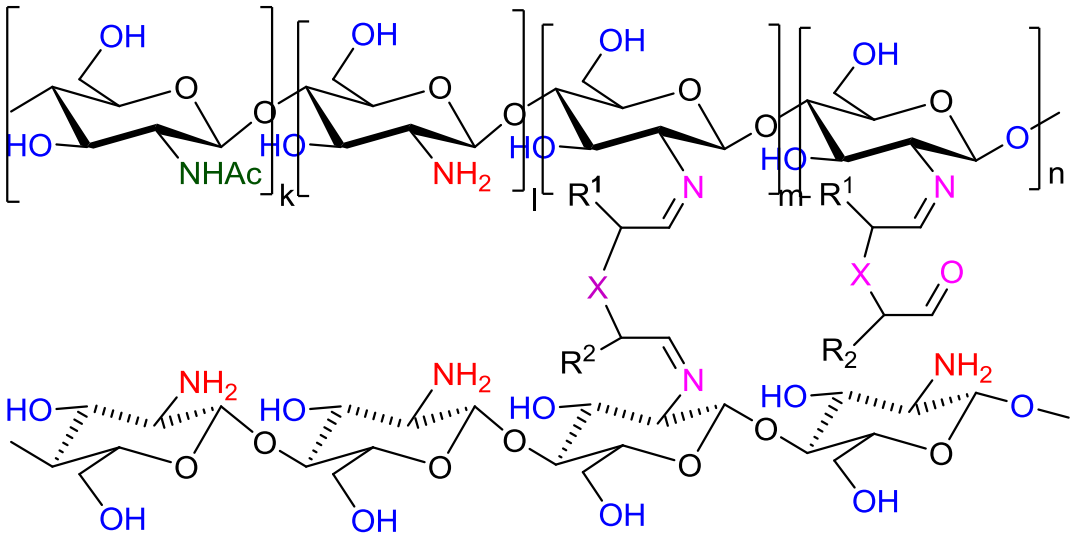


Figure 24. Crosslinking of chitosan (average MW 190000 with degree of deacetylation 87%) with GA (R^1=R^2, X=CH_2), oUMP (R^1=Ura, R^2=CH_2OH, X=O) and oAMP (R^1=Ade, R^2=CH_2OH, X=O). Number crosslinks m which is needed to cause gelation: k=13, l+n>86 , m<1.

IR spectroscopy.

IR spectra were recordered on a Bio-Rad FTS-40 spectrometer (Digilab) with 4 cm^{-1}resolution. The processing of spectra (normalization and base line correction) was carried out using the Win-IR v.4 software package (Bio-Rad, Digilab Division). Samples were prepared as KBr pellets. For the convenience of comparison, the spectra were normalized using the 1153 cm^{-1} band of valence vibrations of the C–O glycoside bond of the chitosan chain as an internal standard. In order to decrease the spectroscopic contribution of the initial compounds (chitosan and crosslinking regents) the sequential subtraction approach was used. The resulting difference spectra were enriched by spectroscopic information about the reaction products.

The samples of the crosslinked chitosan for IR spectroscopy were prepared as follows. The finely crushed gel was neutralized with 1M NaOH so that the pH was raised to pH 8.0. The residue was intensively washed with bidistilled water and finally dried in *vacuo*.

The typical IR spectra of chitosan crosslinked with oUrd are shown on Fig 25. The characteristic absorption band of pyrimidine heterocycle is located near 1680 cm^{-1} (characteristic total stripe valance and deformation vibrations of functional groups of uracil residue) which allows identifying and evaluating the content of oUrd bound to chitosan. The intensity of absorption peak is increased by conducting crosslinking at pH 5.6 in comparison with pH 4.1.

In order to decrease the spectroscopic contribution of the initial compounds (chitosan and crosslinking regents) the sequential subtraction approach was used. The resulting difference spectra were enriched by spectroscopic information about the reaction products. Figure 26 shows the

spectrum of oUrd (1) and difference spectrum of the crosslinking product (2) in which the characteristic peak of 1632 cm^{-1} corresponds to the absorption of the aldimine group (valence vibrations of the C=N bond).

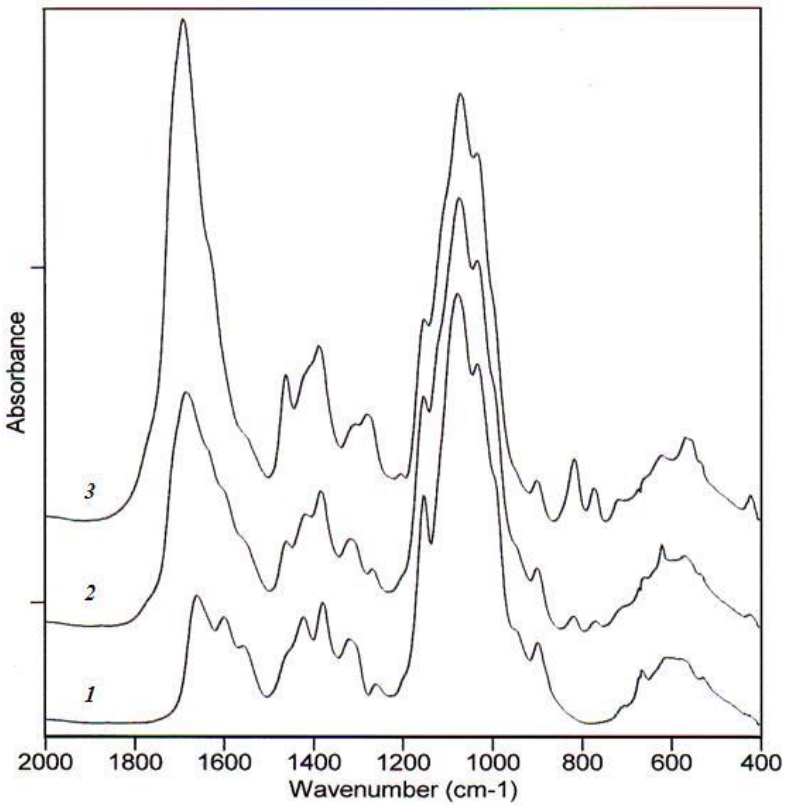


Figure 25. FTIR spectra of samples of chitosan (1) and of chitosan cross-linked by oUrd at pH 4.1 (2) and 5.6 (3).

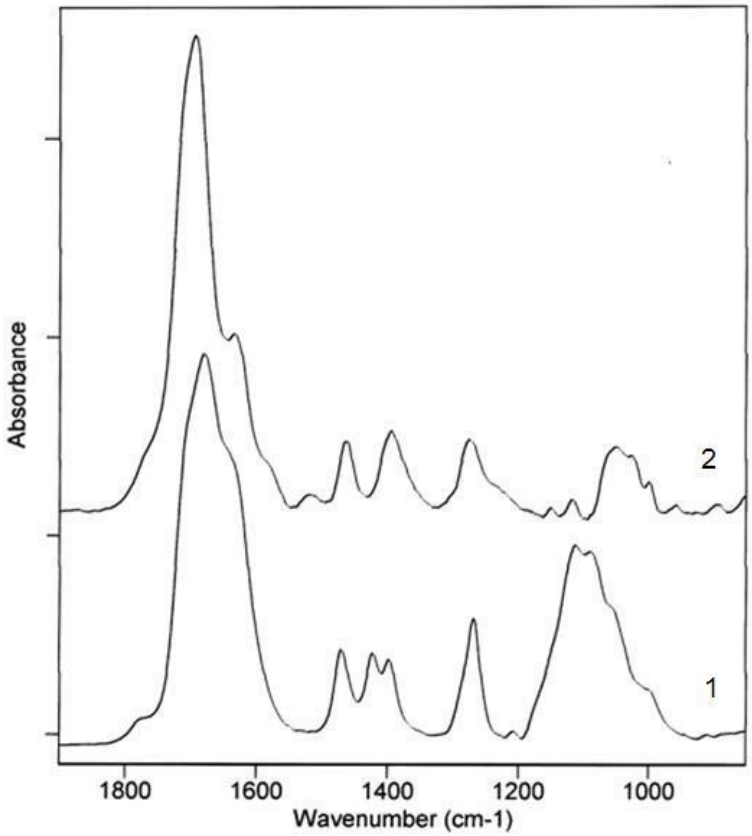


Figure 26. FTIR spectra of samples of oUrd (1) and its difference spectra (2) with chitosan cross-linked oUrd.