MILORAD CAKIĆ[1]
GORAN NIKOLIĆ[1]
LJUBOMIR ILIĆ[2]
SLOBODAN STANKOVIĆ[1]

[1]Faculty of Technology, University of Niš, Leskovac, Serbia and Montenegro

[2]Pharmaceutical and Chemical Works "Zdravlje", Leskovac, Serbia and Montenegro

SCIENTIFIC PAPER

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SYNTHESIS AND FTIR CHARACTERIZATION OF SOME DEXTRAN SULPHATES

The result of the synthesis and investigation of FTIR spectra of some dextran sulphates and their sodium (Na) salts are presented. A synthesis was conducted (under different reaction parameters) of esters with various degrees of esterification (from 1.0 to 2.3). FTIR investigations were performed on sulphates of low and high molecular hydrogenated dextrans of various molecular masses (M_W of 5000, 7000, 8000, 40000 and 500000). Particular care was taken to determine the degree of linearity of the synthesized dextran sulphates, the conformation of glucopyranosyl units and the way the sulpho groups and the water were bonded in their structures.

Key words: dextran, hydrogenated dextran, dextran sulphate, IR spectra.

Many oligo– and polysaccharides form esters with inorganic acids. Sulphuric acid is the most important one [1]. The sulphates of some polysaccharides are very effective against peptic ulcers, in medicine. But since they have a pronounced anticoagulative effect, the practical difficulties in the use of these medications are considerable particularly in the bleeding phase of the ulcer [2]. Consequently, it is of considerable importance to find an anti-ulcer medication having no anti-coagulative effect (compared to heparin as a standard) and having a low degree of sulphation. One of the steps in this examination is the structural study of polysaccharide sulphates and their salts (Na, Al, ammonium, etc.) showing a high inhibitor effect on the proteolithic activity of pepsin in vitro. Polysulphate esters of carbohydrates beside their anticoagulative, have also an antilipemic and antiviral effect. Medications based on these substances are of great interest for the pharmaceutical industry. The study of their structure in correlation with pharmacological activity is interesting

Dextran sulphates are well known in the literature [3]. Dextran sulphates exist as the sodium salt forms, making them soluble and stable in water (Figure 1).

Dextran oligomers with a low content of reduction groups (RG) are of particular interest in the pharmaceutical industry. Thus, hydrogenated dextran sulphates (HDS) of low molecular masses (M_W 5000, 7000, 8000, 40000) and high molecular masses (M_W 500000), as well as their Na–salts, were synthesized with a degree of esterification ranging from 1.0 to 2.3.

The structure of these substances was studied using FTIR spectroscopy at room (RT) and liquid nitrogen temperature (LNT). The confirmation of the dextran glucopyranosyl units (before and after esterification), the way the sulphate groups bond, the

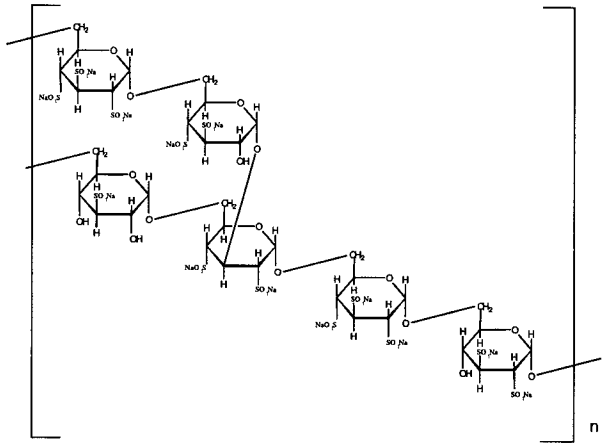

Figure 1. Structure of dextran sulphate as the sodium salt form

degree of dextran sulphate linearity (i.e. the α–(1,3) glucoside bond content) and the way water bonds to the structure were determined by this method.

EXPERIMENTAL

Dextrans (Zdravlje–Leskovac, Serbia) are derived from *Leuconostoc mesenteroides*, strain B–512. Various M_W (5000, 7000, 8000, 40000 and 500000) are produced by limited acid hydrolysis [4]. The fractionation of dextran was accomplished by size exclusion chromatography (Sephadex G–100) and ethanol fractionation when the largest Mw dextrans were precipitated first [5,6]. The molecular mass was measured by low angle laser light scattering (KMX–6, LDC). Hydrogenated low molecular dextran (HLMD) was prepared with sodium boron hydride at room temperature [7].

Dextrans of appropriate M_W were sulphated by an adapted Ricketts method [8]. A few types of esters were synthesized during sulphating with a pyridine–sulphur trioxide complex. By varying the esterification conditions, esters of various esterification degrees

Author address: M.D. Cakić, Faculty of Technology, University of Niš, Bulevar oslobodjenja 124, 16000 Leskovac, Serbia
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(about 1.0 and 2.3) were synthesized. The obtained dextran sulphate was salted out and dissolved in an aqueous NaOH solution at pH 7–10. The Na–salt of dextran sulphate was precipitated by adding ethanol.

Partially deuterated analogues were prepared from the dextran hydrates. Deuteration was accomplished by suspending a sample in liquid deuterium oxide (D_2O: sample 5:1 by gross weight). The mixture was kept in special fused test tubes in vacuum at 30°C for 5 days and then dried in vacuum at room temperature [9].

The IR spectra, as an average of 40 scans, were recorded at room (298 K) and liquid–nitrogen (77 K) temperature using a BOMEM MB–100 FTIR spectrometer (Hartman–Braun), equipped with a standard DTGS/KBr detector, in the range 4000–400 cm^{-1} with a resolution of 2 cm^{-1}, by Win–Bomem Easy software. The spectrometer was purged with dry N_2. A variable temperature cell Specac P/N 21525 was used for the LNT measurements. All the spectra were baseline corrected and area-normalized. A Fourier self–deconvolution based on the Griffiths/Pariente method was applied to enhance resolution in the spectral region of 4000–400 cm^{-1}. A gamma factor of 12, corresponding to a peak width of 24 cm^{-1}, was used. Deconvoluted spectra were smoothed by the 30–point Savitzky–Golay filter method.

RESULTS AND DISCUSSION

Even though polysaccharides are complicated substances for study by FTIR spectroscopy, this method is widely employed in solving many problems involving their structures. Based on the FTIR spectra of the CO deformational vibration region, the configuration and type of glucoside bond of many oligo– and polysaccharides can be determined. Therefore, the CH and OH stretching and deformation vibration regions can assist in the conformational analysis of the pyranosyl units, the determination of the degree of polysaccharide crystallinity (the degree of isotopic exchange by deuteration) and the type and energy of intermolecular bonds [10–14].

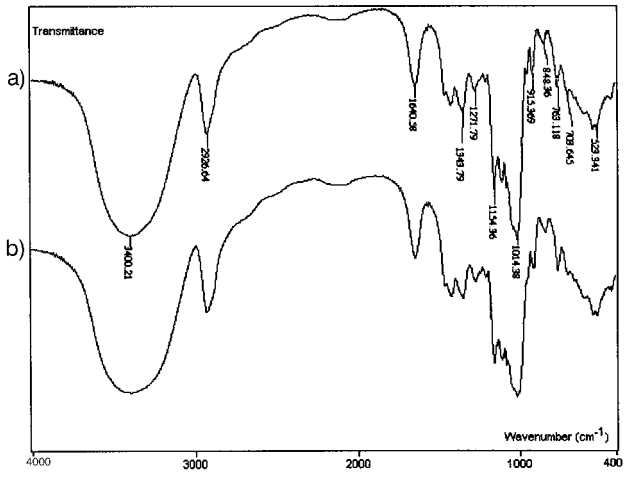

Figure 2. FTIR spectra of: (a) low molecular dextran (LMD) and (b) hydrogenated low molecular dextran (HLMD)

The analysis of low molecular dextrans (M_w 5000) and their hydrogenated analogues show very similar FTIR spectra (Fig. 2). The similarity of the spectra implies that, there is no change in dextran linearity as well as no change in the glucopyranosyl unit conformation during the hydrogeneration of the samples. Information on the conformation in the polysaccharide can be acquired in the 950–600 cm^{-1} region in which the deformational γ_{CH} vibration bands are expected. The number and position of these bands depend on the glucopyranosyl unit conformation [11,12,15]. A bond in the 885–925 cm^{-1} region shows that two CH groups are in the axial position (AA–fragment), the band in the 825–855 cm^{-1} region the equatorial–axial position (EA–fragment), the band in the 790–825 cm^{-1} region the equatorial–equatorial position (EE–fragment) and the band in the 860–885 cm^{-1} region the axial–equatorial position (AE–fragment). The bands at about 916 cm^{-1} and 850 cm^{-1} in the FTIR spectra of LMD and HLMD (Fig. 2) indicate the EA and AA fragments, i.e. the C1–glucopyranosyl conformation.

It is well known that the natural and pharmacological properties of dextran depend on three basic factors: the molecular structure, the average molecular mass and the distribution of the molecular masses [16]. Dextrans with a low degree of molecular branching are in clinical use. Such dextrans have more pronounced antigenic properties. The α–(1,3) glucoside bond content (the degree of linearity) in the studied macromolecules can be deduced by the 794 cm^{-1} band intensity [10]. In this IR range of the spectra LMD and HLMD (Fig. 2) do not have the characteristic bands, but a weak shoulder at 800 cm^{-1} can be noticed. This information proves that the α–(1,3) glucoside bond content is less than 5%, showing a high degree of linearity.

The synthesized dextran sulphates of various molecular masses and a 17% ester bonded sulphur content (degree of esterification 2.3) were studied by FTIR spectroscopy. Commercially available preparations of dextran sulphate were used that had nominal M_w values of 5000 and 500000. The spectral–structural characteristics of dextran sulphates of M_w between these two extremes were examined. This involved the synthesis of a suitable species by the sulphation of dextrans with different, commercially available, molecular weights. The major structural features of this polysaccharide group are an alternating sequence of (1,3)–linked and (1,4)–linked glucopyranosyl residues containing various degrees and sites of sulphation [3]. Figure 3 shows the IR spectra of dextran sulphates M_w 7000 (with esterification degrees about 1 and 2.3), M_w 40000 and 500000.

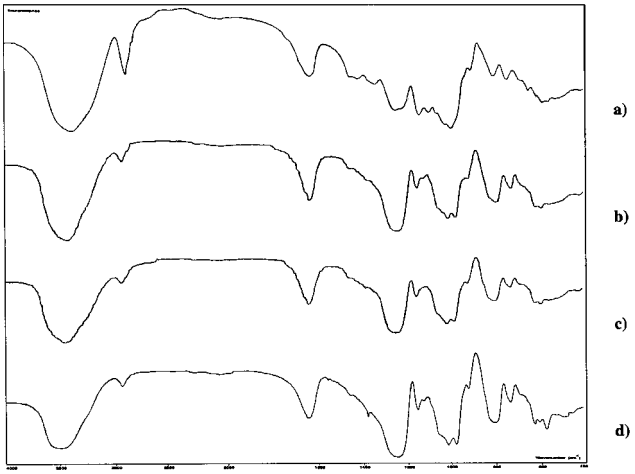

Figure 3. FTIR spectra of dextran sulphates with molecular masses of: (a) M_w 7000 and an esterification degree of about 1; (b) M_w 7000 and an esterification degree of about 2.3; (c) M_w 40000, and an esterification degree of about 2.3; (d) M_w 500000 and an esterification degree of about 2.3.

The presence of the sulpho group (SO_2) can be determined by the bands in the FTIR spectra at about 1245 cm^{-1} and 988 cm^{-1}, originating from ν_{as} (S=O) and ν_s (S=O) vibrations, respectively, as well as the bands at 809 cm^{-1} and 580 cm^{-1} originating from ν_{as} (O–S–O) and ν_s (O–S–O) vibrations. The intensity of these bands increases with the esterification degree (Fig. 3a and 3b). Study of the various forms of chondroitinsulphate by FTIR spectroscopy showed that the band position of the ν_{as} (O–S–O) vibration is sensitive to the position of the sulpho group in relation to the glucopyranose ring [13]. For a sulpho group in the axial position, a band at about 850 cm^{-1} and in the equatorial position at about 820 cm^{-1} are characteristic. Such a criterium was proposed to determine the sulpho group position in other polysaccharide polysulphate esters. Based on this criterium and the bond position at 810 cm^{-1} of the ν_{as} (O–S–O) vibration (Fig. 3), it can be assumed that the sulpho groups are bonded in the equatorial position of the glucopyranose ring in the studied dextran sulphates.

Considering the glucopyranosyl unit conformation, an explanation was previously given that the bands in the NMD FTIR spectra (Fig. 3) at about 916 cm^{-1} and 850 cm^{-1} show a C1 dextran glucopyranosyl unit conformation. The spectra (in this region) of the sulphate esters (Fig. 3) and the original substances (Fig. 2) are similar. The band at about 920 cm^{-1} and the clearly evident shoulder at about 850 cm^{-1} are proof of a C1–glucopyranosyl unit conformation. In such a conformation the OH groups are in the equatorial position. A weak shoulder at about 790 cm^{-1}, in accordance with the above criterium, shows a high degree of ester linearity, i.e. a content of α–(1,3) glucoside bonds of less than 5%. On the basis of the C1 conformation of the glucopyranosyl units and the low content of of α–(1,3) glucoside bonds, it can be estimated that the esterification proceeds via the OH group in the C_2, C_3 and C_4 positions in the glucopyranose ring (Fig. 1).

The FTIR spectra of the dextrans and synthesized dextran sulphates in the valence OH region are particularly changed, as expected. The centroid of the complex band, positioned in the spectra of LMD and HLMD (Fig. 2) at about 3430 cm^{-1}, is shifted by about 70 cm^{-1} towards higher frequencies in the IR spectra with a high degree of esterification (Fig. 3). This is logical because the conditions for hydrogen bonding disappear in esters, as opposed to dextrans or samples with a low degree of esterification, the OH of which groups are involved in hydrogen bonding [12]. That is the reason why the FTIR spectra in the ν_{OH} region of the original dextrans (Fig. 2b) and the dextran sulphates with a degree of esterification of about 1.0 (Fig. 3a) are similar.

The band intensity of ν_{CH} vibration from the CH_2 group (at about 2950 cm^{-1}) is less than in the IR spectrum of dextran sulphates with an esterification degree of about 2.3 versus the dextran esterification degree of about 1 (Fig. 3). Samples with a high degree esterification have considerably lower contents of OH groups which can form hydrogen bonds. This enables a change of the torsion angle around the C_1–C_6 glucopyranosyl bonds, which is followed by a decrease in the ν_{CH} vibration intensity of the CH_2 groups.

The FTIR spectra of a dextran sulphate (M_w 8000) and a dextran sulphate Na–salt (M_w 500000), with a degree of esterification of about 2.3 are shown in Fig. 4. The FTIR spectra of partially deuterized analogues are shown for comparison in the same Figure 4. The FTIR spectra of these samples are very similar to the previously discussed polysaccharides even though the to their different M_w and form. This fact points to their similarity. The isotope exchange method by deuteration (D_2O) shows that the dextran sulphates are crystallohydrates.

In the FTIR spectrum of the deuterated dextran sulphate M_w 8000 (Fig. 4b) two bands appear at about 2580 and 2310 cm^{-1} in the ν_{OD} region, while two bands at about 2598 cm^{-1} and 2320 cm^{-1}, the intensities of which show a low degree of exchange, appear in the FTIR spectrum of the deuterated dextran sulphate Na–salt M_w 500000 (Fig. 4d). Its partners in the ν_{OH} region of the RT–FTIR spectrum can not be determined because there is one intense and wide band at about 3500 cm^{-1}, with a shoulder at the lower frequency side. Therefore, band separation in the LNT–FTIR spectra (Fig. 5) appears with temperature decrease. This is the reason for three bands in the ν_{OH} region at about 3380, 3230 and 3140 cm^{-1}. The bands at about 3230 and 3140 cm^{-1} originate from the ν_{OH} vibrations of water molecules (the shift factor was 1.36). The band at about 3380 cm^{-1} is the result of the unesterified OH groups from dextran. The band from the water molecule ν_{OH} vibrations at about 1650 cm^{-1} is sensitive to deuteration,

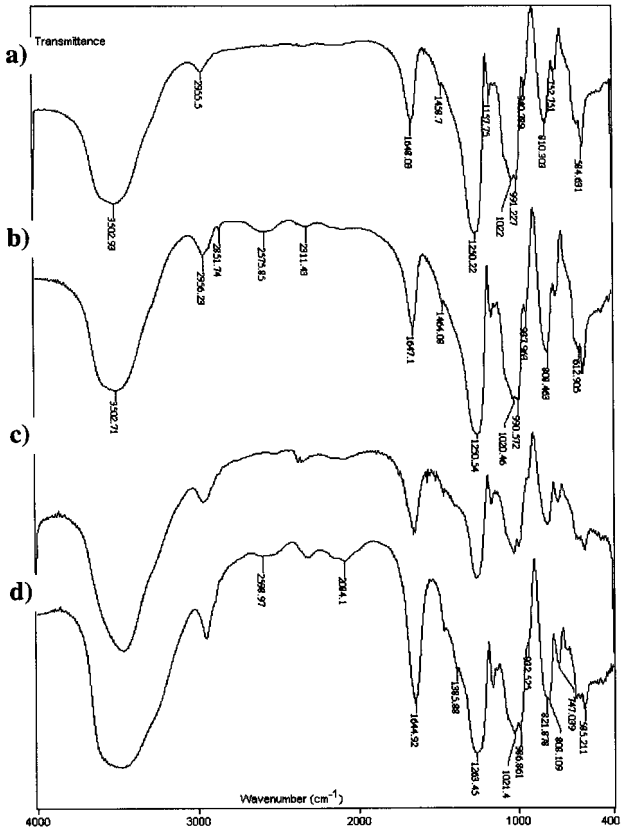

Figure 4. FTIR spectra of: (a) dextran sulphate M_w 8000, (b) partially deuterated dextran sulphate M_w 8000, (c) the Na–salt of dextran sulphate M_w 500000, (d) the partially deuterated Na–salt of dextran sulphate M_w 500000

having a partner in the spectrum of the partially deuterated form at about 1140 cm^{-1} (Fig. 4).

On the basis of the appearance of the FTIR spectra (Fig. 4) in the (OH region, by the criteria of Falk [17], it can be concluded that the studied dextran sulphates have one type of water in their structure. The water protons are involved in the forming of two different relatively weak hydrogen bonds. On the basis of the Berglunds [18] correlation, the O_W···O distance (using the ν_{OD} vibrations) was estimated. For the dextran sulphate MW 8000 these distances are 296 and 267 pm. For the Na–salt dextran sulphate M_W 500000 the O_W···O distances are 298 pm and 270 pm.

CONCLUSION

Dextran sulphates of various molecular mass were synthesized and subsequently characterized by RT and LNT FTIR spectroscopy. The C1–glucopyranosyl unit conformation in the dextran molecule was determined. A high degree of linearity of the polysaccharides, with a α–(1,3) glucoside bond content of less than 5%, was also determined. It was also seen that the esterification proceeded via the OH groups at the C_2, C_3 and C_4 positions in the glucopyranose ring. The sulpho group in the dextran sulphate is bonded at the equatorial position in relation to the glucopyranosyl unit.

Isotope exchange showed that the studied samples were crystallohydrates, which have one type of water molecule in their structures. The water protons are involved in the formation of two different relatively weak hydrogen bonds (the estimated O_W···O distances being 298 pm, 296 pm, 270 pm and 267 pm).

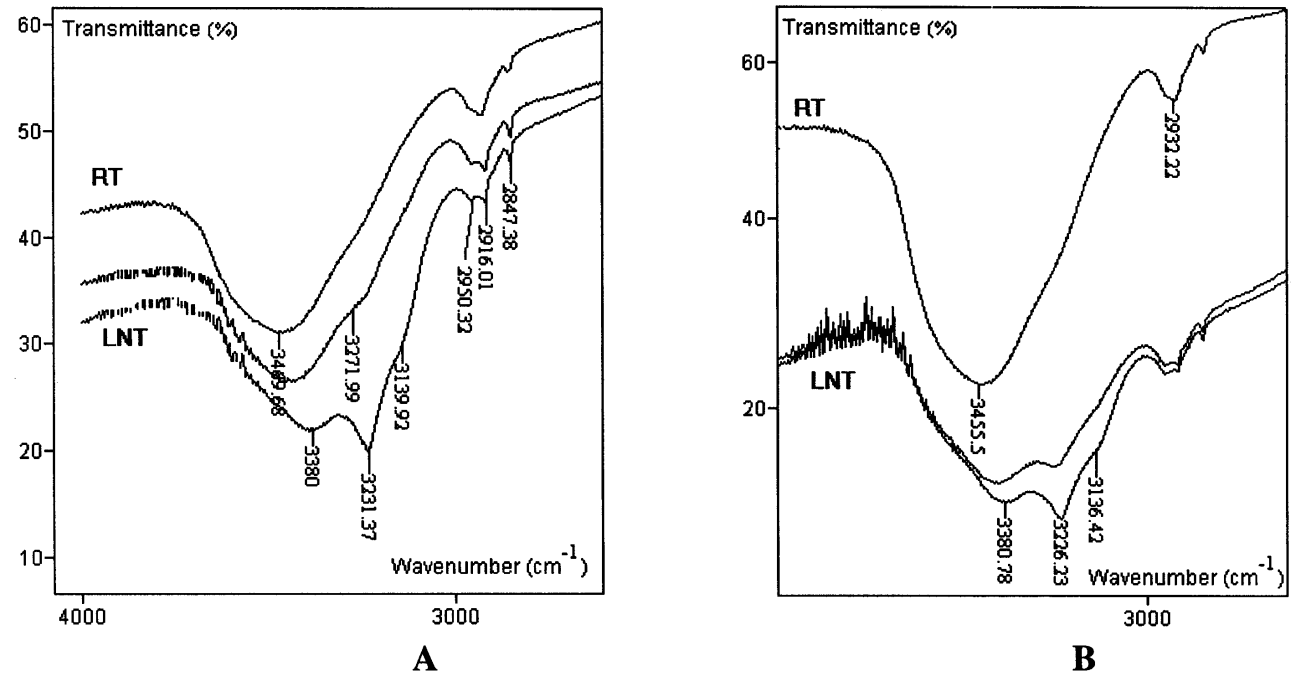

Figure 5. Stretching OH region from the RT and LNT–FTIR spectra of: dextran sulphate M_w 8000 (A), the Na–salt of dextran sulphate M_w 500000 (B)

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IZVOD

SINTEZA I FTIR KARAKTERIZACIJA NEKIH DEKSTRAN SULFATA

(Naučni rad)

Milorad Cakić[1], Goran Nikolić[1], Ljubomir Ilić[2], Slobodan Stanković[1]
[1]Tehnološki fakultet, Univerzitet u Nišu, Leskovac, Srbija i Crna Gora
[2]Farmaceutsko hemijska industrija "Zdravlje", Leskovac, Srbija i Crna Gora

U radu su prikazani rezultati sinteze i FTIR spektri dekstran sulfata različitih molarnih masa, kao i njihove odgovarajuće Na–soli. Sinteza je izvođena pod različitim reakcionim uslovima pri čemu su dobijeni estri dekstrana različitog stepena esterifikacije (1,0 i 2,3). FTIR spektroskopijom ispitivani su sulfati nisko i visokomolarnih hidrogenovanih dekstrana različitih molarnih masa (M_W 5000, 7000, 8000, 40000 i 500000), kao njihove Na–soli. Analizirani su FTIR spektri uzoraka snimani na sobnoj i temperature tečnog azota. Parcijalnim deuteriranjem odgovarajućih analoga hidrata dekstrana određivan je stepen izotopske izmene. Pažnja u radu je usmerena na određivanje stepena linearnosti sintetizovanih dekstran sulfata, utvrđivanje konformacije glukopiranozne jedinice, kao i način vezivanja sulfo grupa i molekula vode u strukturi dekstran sulfata. Kriterijumom Falka utvrđeno je da su ispitivani dekstran sulfati kristalohidrati sa jednim tipom molekula vode i da su molekuli vode uključeni u obrazovanje dve različite vodonikove veze. Berglundovom korelacijom određena su $O_w \cdots O$ rastojanja.

Ključne reči: dekstran, hidrogenovani dekstran, dekstran sulfat, IR spektri.