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Determination of structural peculiarities of dexran, pullulan and γ-irradiated pullulan by Fourier-transform IR spectroscopy

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Abstract

Deconvoluted IR-absorbance spectra of dextran, pullulan and γ-irradiated pullulan were analyzed in order to find the most specific spectral peculiarities that allow one to obtain information about the structure and conformation of these macromolecules in solvents that exhibit different influences on the system of intra- and intermolecular interactions. The changes in intensity and width of the IR bands at about 1040, 1020 and, in the case of pullulan, also at 996 cm^{-1}, were related to changes in conformation and short-range interactions of the polysaccharides. Furthermore, certain bands within the 1200–900 cm^{-1} region were considered as a characteristic for the type of glycosidic linkage. The results of the FTIR spectroscopy study allowed one to suggest a predominant cleavage of the α-(1→4) linkages upon the radiation–chemical destruction of pullulan.

Keywords: Fourier-transform IR spectroscopy; Dextran; Pullulan; γ-Irradiation; Glycosidic linkage

1. Introduction

Pullulan and dextran are well-known neutral polysaccharides with numerous applications in the food, cosmetic and pharmaceutical industries. Moreover, these biopolymers are the subjects of theoretical studies as the representatives of macromolecules differing in the type of glycosidic linkage.[1–6] It is known that the stereochemistry of the glycosidic linkage and constraints of the chain that arise from interactions between sugar residues may introduce several restrictions on the conformational transitions in a carbohydrate chain. The α-(1→4)-linked maltooligomers were found to have higher relaxation times in comparison with the corresponding isomaltooligomers, which confirms a more flexible character of α-(1→6)-linked oligosaccharides.[7] Amylose is expected to have a relatively smaller conformational space than dextran because of the presence of the α-(1→4) linkages. Due to specific interunit bonding, this polysaccharide in the solid state is known to adopt a double-helix conformation. In the case of dextran, the occurrence of the α-(1→6)-glycosidic bond provides for an increase of chain mobility. The pullulan structure is intermediate between amylose and dextran structures because of the co-existence of both α-(1→4) and α-(1→6) linkages in a single compound. Consequently, the segmental mobility of the pullulan backbone is not uniform, with the regions of increased mobility centered on the α-(1→6) linkages.[4,8]

Important results concerning the mobility and conformation of carbohydrate chains were obtained from the analysis of these compounds by the methods of IR and Raman spectroscopy.[9,10] This is, in particular, because of the fact that steric factors and the spatial location of individual groups strongly contribute to the formation of vibrational spectra of carbohydrates. Although Fourier-transform infrared spectroscopy (FTIR) is now widely used to study the composition of complex carbohydrate systems, the molecular orientation, molecular interactions and conformational transitions of polysaccharides in solution or upon hydration,[11] the investigations into the conformation of pullulan chains by means of IR spectroscopy are rare in literature.

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In the present work we have analyzed the deconvoluted IR-absorbance spectra of dextran, pullulan and γ-irradiated pullulan in order to identify spectral markers that could be used to characterize structural differences in these compounds. We focused major attention on the bands in the 1250–900 cm^{-1} region because the absorbance pattern due to ring vibrations in this spectral range is known to be individual for each carbohydrate structure.[12] Moreover, we attempted to obtain information about the conformations of these macromolecules in solvents exhibiting a different influence on the system of intra- and intermolecular interactions. Recently, it was shown that the conformational flexibility of the pullulan chains increase substantially upon γ-irradiation of the polysaccharide.[13–15] In this paper, we have applied FTIR spectroscopy to determine spectral manifestation of the changes in the pullulan structure caused by γ-irradiation and thereby complete the structural investigation of this modified polysaccharide.

2. Experimental

Materials.—Pullulan standard P-400 (Shodex, Japan) and dextran standard T-500 (Pharmacia, Sweden) were used. The samples of native pullulan with weight-average molecular weights (M_w) of approximately 150 and 1200 kDa were synthesized from sucrose with the use of *Auerobasidium pullulans* BMP 97 strain.[16] Me_2SO purchased from Sigma was of ACS reagent grade.

Sample preparations.—Aqueous solutions of native pullulan with $M_w \approx 1200$ kDa and dextran T-500 (6 g/dL) were radiolyzed as described elsewhere.[13,14] The samples of γ-irradiated polysaccharides and native pullulan with $M_w \approx 150$ kDa were separated into fractions on a preparative gel-filtration column (120 × 5 cm i.d.) packed with Sephadex G-200 gel. The fractions collected were then lyophilized.

Molecular-weight characteristics (M_w, polydispersity index M_w/M_n) of the fractions were estimated by means of analytical size-exclusion chromatography (SEC). SEC was carried out using a liquid chromatography system comprising a type 600E solvent delivery system and a type 410 differential refractometer (all from Waters). Two TSK G-4000 PW_{XL} (TosoBiosep) columns operated in series were used. A P-82 set of pullulan standards (Shodex) was used to construct universal calibration curve.[13] SEC analysis revealed that the fractions have almost unimodal molecular-weight distributions (MWD) and their M_w/M_n values are in the range of 1.1–1.2.

Lyophilized pullulan and dextran samples (2 mg) were mixed with KBr (100 mg), stored at 80 °C for 6 h and compressed into tablets under vacuum. To obtain a melted pullulan sample, the fraction of pullulan with $M_w = 70$ kDa were kept for 1 week in a closed desiccator over saturated solution of KNO_3 under conditions of 85% relative humidity at 25 °C. The resulting material was dried at 25 °C for 2 days, ground into powder, then mixed with KBr and compressed into a pellet (2 mg in 100 mg of KBr).

Samples of P-400 and T-500 were dissolved in the water–Me_2SO mixtures with different content of Me_2SO (water–Me_2SO, v/v): 100/0, 90/10, 70/30, 50/50, 30/70, 0/100. The solutions (0.25%, w/v) were cast on KRS-5 crystals[17,18] to obtain thin films and dried at 45 °C for 1 day. A final drying was performed at 80 °C for 12 h. The dryness of the film was controlled by the band at ca. 1648 cm^{-1}, which is associated with the deformation vibrations of the OH bond from water molecules.[19]

FTIR spectroscopy.—The FTIR absorbance spectra as averages of 128 scans at a resolution of 4 cm^{-1} were recorded in triplicate on either Nicolet Protégé 460 or Midac M2000 spectrometers equipped with standard DTGS detectors. Prior to use, both spectrometers were purged with dry N_2. In the region of 1250–800 cm^{-1}, all spectra were baseline corrected and area normalized. A Fourier self-deconvolution based on the method described by Griffiths and Pariente[20] was applied to enhance resolution in the spectral region of 1250–900 cm^{-1}. A gamma factor of 12, corresponding to a peak width of 24 cm^{-1}, was used. Deconvoluted spectra were smoothed by the 9-point Savitzky–Golay filter method.

3. Results and discussion

Band assignments.—In the 1175–975 cm^{-1} region the spectra of pullulan and dextran comprise a number of highly fused bands (Figs. 1(a) and 2(a)). The enhancement of resolution by using a Fourier self-deconvolution allows bands to be more accurately detected (Figs. 1(b) and 2(b)). The main bands found in the deconvoluted spectra of pullulan and dextrans at ca. 1155, 1107, 1080, ca.1020 and 1000 cm^{-1} are due to valent vibrations of the C–O and C–C bonds and deformational vibrations of the CCH, COH and HCO bonds.[21] In order to avoid erroneous results of deconvolution, it is necessary first to establish the degree to which the detected bands correlate with those reported for other polysaccharides.

The band at about 1150 cm^{-1} has been previously assigned to valent vibrations of the C–O–C bond and glycosidic bridge.[12] Another assignment was given, however, by Bose et al.[22] who proposed to ascribe the band at 1150 cm^{-1} to the exocyclic C–O stretching vibrations. The latter finding is in agreement with the observations that the frequency position of this band may vary for different polysaccharides, depending on

the repeating units, and axial or equatorial orientation of the OH groups.[11,12] It was recently shown that the appearance of several new bands in the 1175–1140 cm^{-1} region should be considered as a manifestation of the formation of the glycosidic linkage, rather than the single band at 1150 cm^{-1}.[23]

The broad peak at 1107 cm^{-1} should be most likely ascribed to the vibration of the C–O bond at the C-4 position of a glucose residue.[24] Complex vibrations involving the stretching of the C-6–O-6 bond with participation of the deformational vibrations of the C-4–C-5 bond result in an appearance of the band at 1080 cm^{-1}.[25] The band at 1080 cm^{-1} in the spectra of dextran is less pronounced than in the spectra of pullulan (Figs. 1(b) and 2(b)). The main difference between dextran and pullulan structures is a type of glycosidic linkages, i.e., solely α-(1→6)- in the case of dextran and

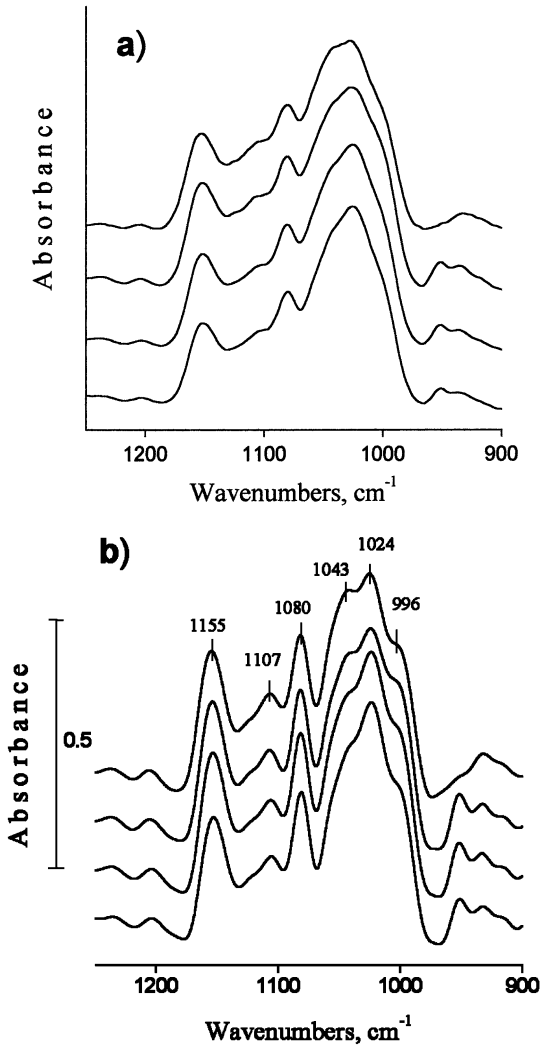

Fig. 1. Original (a) and deconvoluted (b) spectra of pullulan P-400 samples cast in films on KRS-5 crystals from water–Me_2SO mixtures (100/0; 90/10; 50/50; 0/100 from top to bottom). Spectra were recorded using a Midac M2000 FTIR spectrometer.

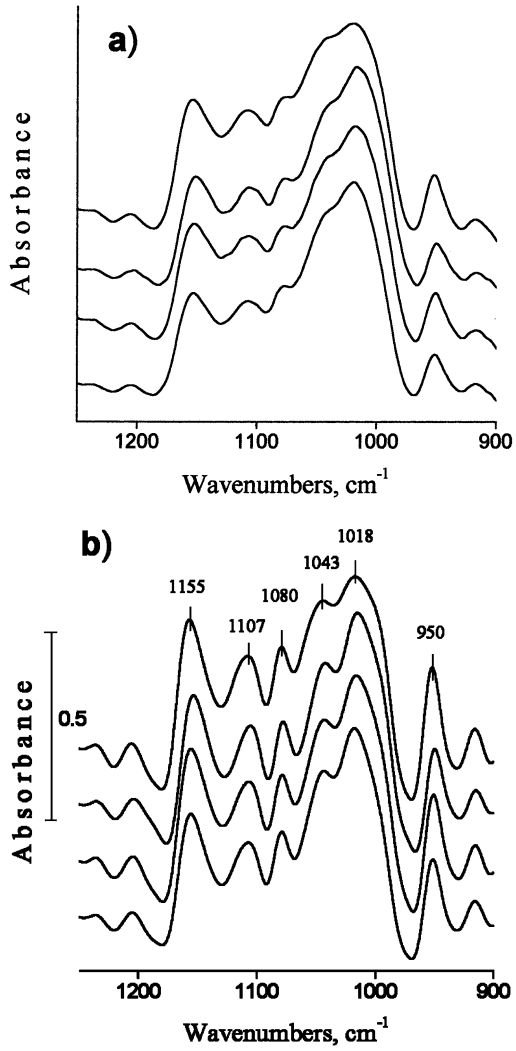

Fig. 2. Original (a) and deconvoluted (b) spectra of dextran T-500 samples cast in films on KRS-5 crystals from water–Me_2SO mixtures (100/0; 90/10; 50/50; 0/100 from top to bottom). Spectra were recorded using a Midac M2000 FTIR spectrometer.

α-(1→4) and α-(1→6) linkages in a ratio of 2:1 in the case of pullulan; therefore, one can suppose that this band is sensitive to such a structural difference. In the pullulan macromolecule there is a large proportion of the primary C–OH groups at the C-6 position, and an intense band at 1080 cm^{-1} is observed (Fig. 1(b)). In the case of dextran, all C-6 atoms participate in the formation of the C-6–O–C-1 linkages; as a result, the band intensity at 1080 cm^{-1} for dextran is reduced (Fig. 2(b)). These findings suggest that the 1080 cm^{-1} band can be considered as a characteristic for the type of interunit links.

The bands at about 1047 and 1022 cm^{-1} found for both polysaccharides in the spectra of starch were shown to relate to the crystalline and amorphous phases, respectively.[26–28] The changes in intensity of these bands are strongly associated with the alterations

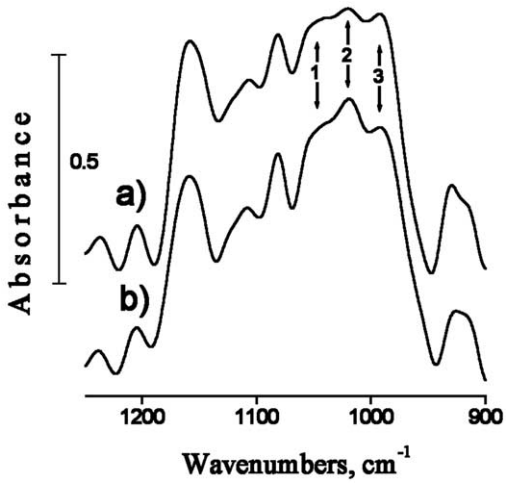

Fig. 3. Deconvoluted FTIR spectra of pullulan sample before (a) and after (b) water-induced plasticisation. Bands: (1) 1043 cm^{-1}; (2) 1020 cm^{-1}; (3) 996 cm^{-1}. Original absorbance spectra of the samples in KBr pellets were recorded using a Nicolet Protégé 460 spectrometer.

in the macromolecular order.[29–31] Although dextran and pullulan appear to be less structurally organized than starch because of the great chain flexibility around the α-(1→6)-glycosidic bonds,[4,8] an existence of the conformational short-ordering is well documented for both polymers.[32–34] Therefore, the bands at 1043 and ca. 1020 cm^{-1} in the spectra of dextran and pullulan may be responsible for the more- and less-ordered structures, respectively. The finding that the band at 1040 cm^{-1} in the spectra of dextran in KCl splits into two components at 1030 and 1050 cm^{-1} [35,36] further confirms a high sensitivity of this band to conformational transitions of the polysaccharide molecule.

The band seen in the case of pullulan at 1024 cm^{-1} appears in the dextran spectra at 1018 cm^{-1} and, in contrast with pullulan, becomes separated from the band at 1043 cm^{-1} by a well-distinguished valley at 1035 cm^{-1} (Fig. 2(a) and (b)). Another interesting feature is that the band at 996 cm^{-1}, found for pullulan upon deconvolution as a high-frequency shoulder of the 1024 cm^{-1} band, is absent in the spectra of dextran (Figs. 1(b) and 2(b)). It was shown that the band at 994 cm^{-1} associated with C–OH bending vibrations at the C-6-position in the case of amylose indicates the strength of the interchain interactions via hydrogen bonding.[37] The primary hydroxyl groups at the C-6-position are available only in the pullulan structure; therefore, an appearance of the band at 996 cm^{-1} in the spectra of pullulan upon deconvolution is thought to be quite normal.

It was shown[38,39] that the primary hydroxyl groups in the (1→4)-linked anhydroglucose units of pullulan form hydrogen bonds, similarly as known for amylose. Intermolecular hydrogen bonds may be additionally formed by drying,[3] but they are not available in an aqueous solution where they become replaced by hydrogen bonds with water molecules. Therefore, a decrease of this band in the spectrum of the lyophilized pullulan sample upon melting (Fig. 3), as a result of water-induced disruption of hydrogen bonds,[40] further confirms validity of the above assignment. It appears that the intensity of the 996 cm^{-1} band in the pullulan spectra may indicate the extent of the interchain association.

Influence of Me_2SO.—The main differences observed in the spectra of polysaccharides upon addition of Me_2SO are changes in band intensities and band narrowing (Figs. 1(b) and 2(b)). The band at 1043 cm^{-1} decreases compared with a neighboring band at ca. 1020 cm^{-1} for both pullulan and dextran. This is presented in Table 1 as a variation of the intensity ratios of these two bands, R 1018/1043 and R 1024/1043, at different contents of Me_2SO. The ratios R 1018/1043 for dextran and R 1024/1043 for pullulan increase shortly from 0 to 10% of Me_2SO and remain almost constant at higher concentration of Me_2SO (Table 1). It is known that the solvent properties of Me_2SO are determined by its ability to break hydrogen bonds in the native carbohydrate structure. Therefore, considering the bands at 1043 cm^{-1} and ca. 1020 cm^{-1} as the order-sensitive ones, we assumed that the initial

Table 1
Influence of the content of Me_2SO on the most characteristic intensities ratios estimated from the deconvoluted spectra of pullulan P-400 and dextran T-500 samples

Me_2SO (%)	Pullulan		Dextran		
	R 1024/1043	R 1043/1070	R 1018/1043	R 1018/1035	R 1043/1070
0	1.09	2.77	1.17	1.21	1.96
10	1.16	3.18	1.36	1.43	2.60
30	1.20	3.20	1.32	1.45	2.65
50	1.23	3.35	1.32	1.40	2.55
70	1.24	3.32	1.31	1.43	2.60
100	1.23	3.35	1.32	1.43	2.60

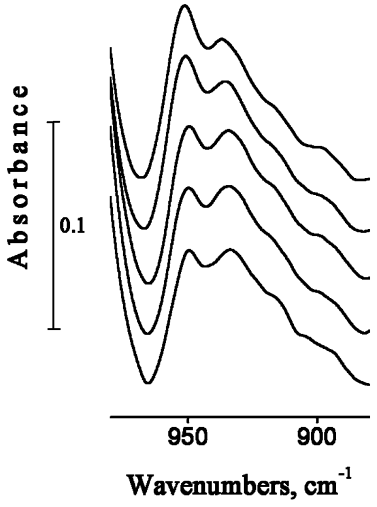

Fig. 4. Changes of the bands at 950 and 935 cm^{-1} in the spectra of pullulan sample upon increase of the content of Me_2SO in the water–Me_2SO mixtures (100/0; 90/10; 70/30; 50/50; 0/100 from top to bottom).

short-range order of the polysaccharide molecules is lost with increasing content of Me_2SO. The band at 996 cm^{-1} in the pullulan spectra preserves its intensity as the solvent is changed from water to Me_2SO, possibly because intermolecular interactions via the C-6–OH groups is not pronounced in either solvents in the polysaccharide concentration used for films formation. Destruction of the intermolecular hydrogen bonds by water, as it was seen for the melted pullulan sample (Fig. 3), decreases a friction between polysaccharide chains and facilitates segmental motions,[40] which should lead to the broadening of the IR bands. However, spectral manifestation of the destruction of hydrogen bonds in the presence of Me_2SO appears to be different from that induced by water molecules (Fig. 3) because the changes in band intensities in the case of Me_2SO–polysaccharide systems are accompanied by the band narrowing.

As a band becomes narrower, an absorbance measured at the nearest minimum tends to decrease. Therefore, to better illustrate the band-narrowing process, we have listed in Table 1 the ratios R 1018/1035, R 1043/1070 as a function of Me_2SO concentration. These ratios were determined using peak intensities of the corresponding bands and absorbances at the nearest minimum (valley). Since the valley at 1035 cm^{-1} appears to be non-resolved, only the ratio R 1043/1070 was evaluated for pullulan. As can be seen in Table 1, the 10%-content of Me_2SO is high enough for both polysaccharides to provoke a noticeable narrowing of the 1043 cm^{-1} band. A narrowing of the bands as a result of uniform distribution of bonds energies indicates a loss of conformational mobility of the polysaccharide chains in Me_2SO. Earlier, Pavlov and co-workers found that the swelling parameter, ε, for pullulan in solution of Me_2SO is 0.180, whereas in aqueous solution $\varepsilon = 0.112$.[41] Moreover, the exponent in Mark–Houwink equation $a = 0.75$ estimated for pullulan chains in Me_2SO exceeded the value $a = 0.66$ obtained for aqueous solutions. The higher values of ε and a for pullulan in Me_2SO may be attributed to a more pronounced chain expansion effect. Thus, segmental interactions in Me_2SO become less effective, and polysaccharide chains explore a restricted range of conformations. This is observed in FTIR spectra as a narrowing of the band at 1043 cm^{-1} (Table 1).

As the configuration of the glucopyranose ring of maltotriose units is stable in Me_2SO,[42] an increase of the anisotropy reported for the pullulan chain in Me_2SO[41] can be most likely be interpreted as being due to changes of mutual orientation of the pyranose rings in such a polar solvent. It is worth mentioning here that the comparison of the IR-spectra of the pullulan samples prepared from the solutions containing different concentration of Me_2SO allowed us to detect a pronounced decrease of the band at 950 cm^{-1} in comparison with the nearest band at 935 cm^{-1} (Fig. 4). This band belongs to the structure-sensitive region, and together with the band at 935 cm^{-1}, characterizes the type of interunit bonds and angles. The band at 935 cm^{-1} was recently used to discover the co-existence of α-(1→6)- and α-(1→4)-glycosidic linkages in the pullulan structure.[16] A decrease of the band at 950 cm^{-1} indirectly confirms an occurrence of the conformational transitions in polysaccharide–Me_2SO systems owing to rotational isomerism of pyranose rings about the glycosidic bond.

Spectral peculiarities of γ-irradiated pullulan.—Previous papers have shown that the radiation–chemical destruction of pullulan results in a decrease in molecular weight of the polysaccharide.[43] This process is accompanied by a narrowing of molecular-weight distribution and a chemical modification of macromolecules due to formation of carbonyl and carboxyl groups.[13,15] A kinetic study of pullulan radiolysis[43] revealed that modification of macromolecules upon irradiation takes place predominantly for low-molecular-weight chains when the destruction becomes less pronounced. Consequently, a concentration of the carbonyl and carboxyl groups increases with decreasing molecular weight of fractions of γ-irradiated pullulan sample.[13,15] Noticeable changes of IR band relative intensities at 1043 and 1080 cm^{-1} were observed in the FTIR spectra of the γ-irradiated pullulan fractions differing in their M_w (Fig. 5). In accordance with the above interpretation, an increase of the 1043 cm^{-1} band most likely originates from more ordered structural organization of the low-molecular-weight chains, which agrees with the previous observations.[21,33] The band at 1080 cm^{-1} decreases in comparison with

neighboring band at 1107 cm^{-1} as the M_w of γ-irradiated pullulan decreases (insertion in Fig. 5). This phenomenon was not observed in the case of γ-irradiated dextran. Calculation of the absorbance ratio R 1080/1107 gives the values of 1.10 for the native dextran (α-(1→6)-linked glucose residues) and 1.73 for native pullulan molecule, where α-(1→6) linkages represent one third of all glycosidic bonds. Therefore, taking into account the above assignment of this band, the decrease of the band at 1080 cm^{-1} means that a different proportion of the α-(1→4) and α-(1→6) linkages is formed in pullulan chains after radiolysis. The ratios between the α-(1→6) and α-(1→4) linkages calculated for the fractions of γ-irradiated pullulan from FTIR data are plotted in Fig. 6. For the fractions with $M_w < 50$ kDa, the ratio α-(1→6)/α-(1→4) is higher than that expected from the strict sequence of two α-(1→6) linkages and one α-(1→4) linkage in the pullulan structure.[44] The possible explanation of this discrepancy is that the low molecular weight fractions comprise an increasing content of the dimeric fragments linked by the α-(1→6) linkages, which contributes to the overall occurrence of this linkage in a sample. Thus, these findings suggest that the α-(1→4) linkages are destroyed predominantly upon pullulan radiolysis. It must be pointed out that although similar results were obtained recently via NMR measurements of the oligomeric products of the acid hydrolysis of pullulan,[45] further studies should be carried out to confirm these preliminary data.

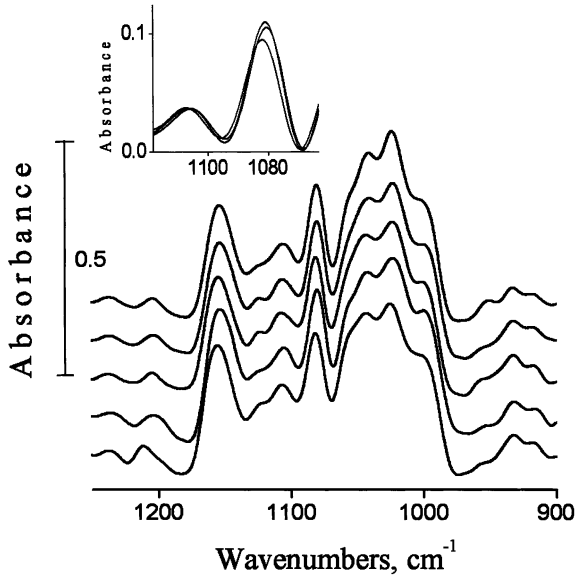

Fig. 5. Deconvoluted FTIR spectra of the most representative specimens of γ-irradiated pullulan fractions with M_w of 193, 114, 52, 28, and 8 kDa (from top to bottom). Decrease of the band at 1080 cm^{-1} shown in the insertion.

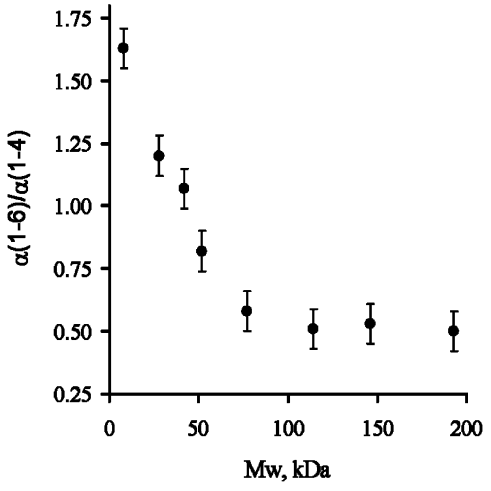

Fig. 6. The ratio between the α-(1→6)- and α-(1→4) linkages calculated for the fractions of γ-irradiated pullulan from FTIR data.

In summary, we have determined the spectral peculiarities of dextran and pullulan that allow one to evaluate structural features and conformational properties of these macromolecules in different solvents. On the basis of the FTIR data, it was concluded that the Me_2SO-induced structural alterations include the disruption of the system of hydrogen bonds and the conformational modification of polysaccharide chains accompanied by the changes in mutual orientation of anhydroglucose units. Moreover, it was shown that the band at 1080 cm^{-1} indirectly indicates a number of α-(1→6) linkages in the polysaccharide structure. The decrease of this band upon radiolysis of pullulan was interpreted as a result of the predominant cleavage of the α-(1→6) linkages.

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