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SYNTHESIS AND CHARACTERIZATION OF DEXTRAN METHACRYLATES

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Abstract. Dextran methacrylates were received by acylation of dextran with methacryloylchloride in the presence of tertiary amines in the DMF/LiCl solution. Degree of substitution (DS) of synthesized derivatives reached 1.8 methacrylic residues per glucopyranoside unit of dextran macromolecule. Dextran methacrylates, obtained in the presence of triethylamine, with DS over 0.5 were insoluble in water. Derivatives synthesized in the presence of pyridine were separated in the form of a stable water soluble complex with pyridine chloride. These complexes maintain their water solubility up to DS = 1.8. The structurization of synthesized dextran methacrylates in water solutions initiated by redox system $(\text{NH}_4)_2\text{S}_2\text{O}_8$ - Et_3N yields active hydrogels, containing residual double bonds.

Keywords: dextran, methacrylate, macromonomer, biomaterials, hydrogel.

1. Introduction

Dextran is watersoluble polysaccharide, which consists of α -D-glucopyranoside units basically connected by α -1,6-glycoside bonds [1]. Good biocompatibility of dextran and its derivatives makes their usage as components of polymeric biomaterials perspective [2-4]. However, the subjects of particular interest are dextran derivatives which contain polymerizable functional groups. They can be applied as macromonomers in various areas of modern engineering and, first of all, for the synthesis of active hydrogels [5].

For the first time macromonomers based on dextran were synthesized by P. Edman (1980), who modified dextran with glycidylacrylate [6]. In 1995 Van Dijk-Wolthuis fulfilled the modification of dextran with glycidylmethacrylate [7]. Application of the techniques described in these works gives the possibility to obtain water soluble derivatives of dextran with DS (statistic

amount of acrylic groups per one glucopyranoside unit) that does not exceed 0.5. At higher values of DS such derivatives lose their solubility in water and cannot be used for hydrogels synthesis.

The syntheses of water soluble highly substituted dextran methacrylates as well as a reactive hydrogels on their basis are described in this article.

2. Experimental

2.1. Materials

Dextran (Serva) with MW = 15000–20000 was dried in vacuum during 5 h at 373 K. *N,N*-dimethylformamide (DMF) (Merck) and methacryloyl chloride (MAC) (Merck) with qualification “extra pure” were used without preliminary purification. Pyridine (Py) and triethylamine (TEA) (Merck) were purified according to techniques [8]. LiCl (Merck) was baked for 3 h at 573 K and stored in desiccator above CaCl_2 .

2.2. Synthesis of Dextran Methacrylates

DMA were obtained by the following technique: 1.6 g of dextran (10 mmol) were dissolved in 20 ml of DMF containing 2 g of LiCl at 373–378 K under nitrogen. The solution was cooled down to 291–293 K, the base (Py or TEA) was added, and after 10 min of stirring MAC was charged in quantities pointed in the Table. The blend has been mixed for 20 h at 291–293 K. Water soluble samples of modified dextran (samples 1, 5-8) were precipitated by acetone and purified by three-time reprecipitation. Water insoluble DMA that has been formed after the acylation (samples 2-4) were precipitated with water from their DMF solutions, and also purified by three-time reprecipitation with acetone from aqueous solution. After precipitation DMA were dried in vacuum at 323 K to the constant mass.

The amounts of the reagents and characteristics of DMAs obtained

DMA	Base	Loaded, mmol			Characteristics of DMA obtained				Solubility in	
		Dextran	MAC	Base	[C=C], mmol/g	[Cl], mmol/g	N, %	DS	Water	Methanol
1	TEA	10	5	5	1.73	-	-	0.31	+	-
2		10	10	10	3.17	-	-	0.65	-	-
3		10	20	20	4.86	-	-	1.16	-	-
4		10	30	30	6.03	-	-	1.63	-	-
5	Pyridine	10	5	5	1.86	1.23	1.88	0.41	+	+
6		10	10	10	2.31	1.73	2.43	0.57	+	+
7		10	20	20	3.54	2.56	3.58	1.22	+	+
8		10	40	40	4.05	3.19	4.16	1.82	+	+

2.3. Analysis Methods

The content of double bonds in DMA was determined by bromide-bromate method according to the technique [9], after boiling with 0.5 N alkali solution for 1 h for complete saponification of samples. The relative error of such method determined on modelling samples did never exceed 3 %.

The content of nitrogen was determined by Kieldall method [10] and of chloride-ions – by argentometry [11].

DS of dextran methacrylates were calculated according to relationship which connects the contents of double bonds and nitrogen with the degree of substitution of –OH groups in glucopyranoside unit of dextran:

$$DS = \frac{[C=C]}{616.8 - 0.42 \cdot [C=C] - 50.82 \cdot [N]}$$

where [C=C] – contents of double bonds, mmol/g; [N] – nitrogen contents, %, in samples received in the presence of pyridine; for the samples received in the presence of TEA [N] = 0.

2.4. Spectroscopic Research

The IR-spectra were taken with “Specord M-80” for the suspension of samples in the vaseline oil at the range of 600–3800 cm⁻¹. The UV-spectra – with “Specord M-40” equipment for water solutions of samples at the range of 200–500 cm⁻¹.

2.5. Preparation of Hydrogels

1.5 g of water soluble DMA were dissolved in 10 ml of the borate buffer (pH 8.1) and the solution of 0.27 g (NH₄)₂S₂O₈ and 60 μl TEA, previously dissolved in 1 ml of the buffer, was added. The mixture has been kept for 20 h at 293 K and 50 ml of acetone were added. Coagulated polymer was separated by centrifugation, washed with acetone and dried in vacuum at 323 K to constant mass.

Degree of conversion of double bonds was calculated by using the following formula:

$$n = \left(1 - \frac{[C=C]_1}{[C=C]_0} \right) \cdot 100\%$$

where [C=C]₁ and [C=C]₀ – contents of double bonds in obtained cured polymers and initial DMA, respectively, which were also determined by bromide-bromate method.

3. Results and Discussion

Acylation of dextran with chloroanhydrides of aliphatic carboxylic acids is an effective method to obtain dextran esters and allows achieving high degrees of substitutions in OH-groups. As a rule the process is carried out in formamide, dimethylsulfoxide (DMSO) [11, 12], or in DMF/LiCl system [13, 15] in the presence of organic basis for linkage of HCl. It is known that solutions of dextran in formamide have high viscosity [11] that complicates agitation of the reaction mixture. On the other hand, conducting the process in strongly diluted solutions is undesirable because of difficulties connected with isolations of products. DMSO is capable of oxidizing dextran during heating [14]. Besides, the attempt of dextran acylation with methacryloylchloride in DMSO was not successful. Apparently, the optimum solvent is the DMF/LiCl system, which we used for synthesis of DMA. The process could be represented by Scheme 1.

The characteristics of obtained DMA are presented in the Table.

The DS of DMA vs. initial mole ratio MAC–dextran is shown in Fig. 1.

The character of this dependence testifies that the efficiency of the MAC usage practically does not depend on the nature of the base used and falls when *n* increases. Therefore it is necessary to use the significant excess of MAC in order to achieve higher DS.

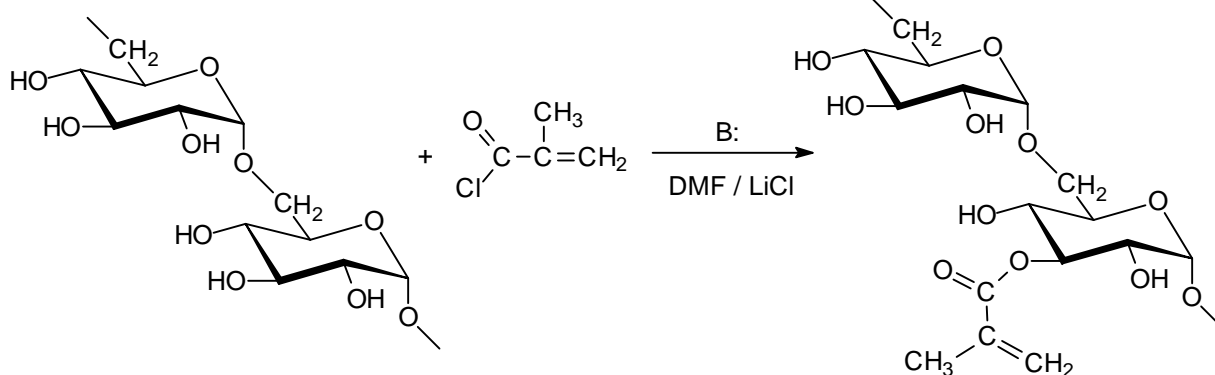
S. Kim and co-workers [13] showed that during the acylation of dextran with bromoacetyl bromide the efficiency of the acylation agent usage grows symbatically

to DS and practically reaches 100 % at $n = 3$ (Fig. 1c). Such difference during course of acylation can be explained by various solvation of dextrans acylated with bromoacetyl bromide and MAC that creates various steric hindrances for the interaction of hydroxyl groups of dextran and appropriate acylation agents.

In the IR-spectra of DMA (Fig. 2) there are intensive characteristic absorption bands of valence oscillations of esteric C=O group at 1732 cm^{-1} , and also

C=C bonds at 1638 cm^{-1} , which confirm the presence of methacrylic fragments in DMA structure.

From the Table, it can be observed that the acylation of dextran in the presence of pyridine results in obtaining water- and methanol soluble DMA even at high values of DS. The presence of nitrogen and chloride-ions in these DMA specifies that they are precipitated from the reaction mixture with pyridine chloride, the amount of which does not decrease even after repeated reprecipitation of DMA.



Scheme 1

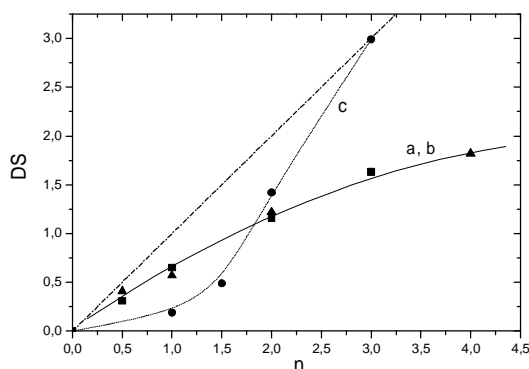


Fig. 1. Dependence of the DS of DMA on initial mole ratio of MAC–dextran (n), achieved in the presence of TEA (a) and pyridine (b). The same for acylation of dextran with bromoacetyl bromide (c)

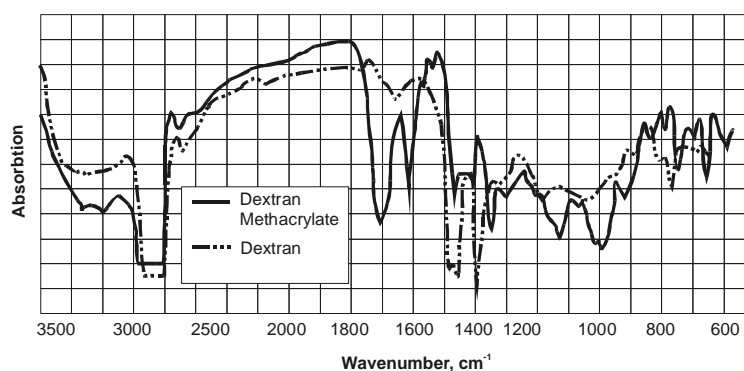


Fig. 2. The IR-spectra of DMA (DS = 0.57) and initial dextran

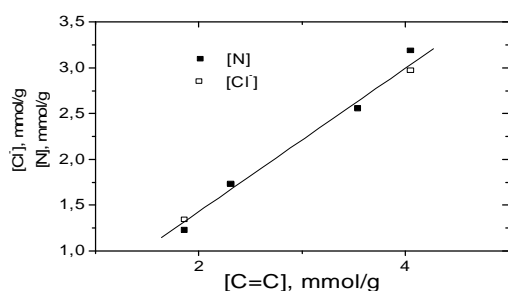


Fig. 3. Content of nitrogen and chloride-ions vs. content of methacrylic fragments in DMA

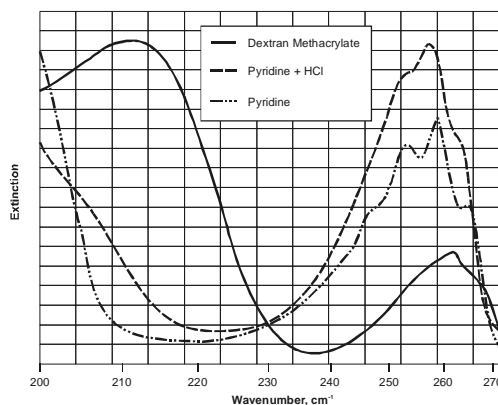


Fig. 4. UV-spectra of DMA (sample 6, Table 1), pyridine chloride and pyridine

Fig. 3 represents the correlation between the contents of nitrogen, chloride-ions and methacrylic fragments in DMA obtained in the presence of pyridine (samples 1-4). Both nitrogen and chloride-ions contents linearly depend on the amount of double bonds, have identical slopes and, consequently, are described by the same straight line. It testifies that the content of pyridine chloride is directly proportional to the content of methacrylic fragments, and in all samples the content of pyridine chloride equals 0.75 mol with respect to 1 mol of methacrylic groups.

Apparently, pyridine chloride in DMA forms a complex with methacrylic fragments. There are two typical absorption bands of the pyridinium aromatic ring – K-line (211 cm^{-1}) and B-line (259 cm^{-1}) at the UV-spectrum of this complex (Fig. 4). Significant bathochromic shift of these bands in comparison with the spectrum of pyridine chloride water solution as well as the absence of pyridine chloride in the products of dextran acylation by chloroanhydrides of saturated carboxylic acids [11, 12] allows to assert that the complex is formed due to coordination of pyridinium nucleus and double C=C bond of methacrylic fragment [18]. Nevertheless, at any DS approximately 25 % of methacrylic groups cannot participate in the complex formation process, apparently due to steric hindrances.

In our opinion, as a result of interaction between the pyridine nucleus and methacrylic group a charge transfer complex has been formed (Fig. 5), where the electron-deficient pyridine ring acts as an acceptor of electron density and the double-bond conjugated system of methacrylic fragment plays the role of a donor.

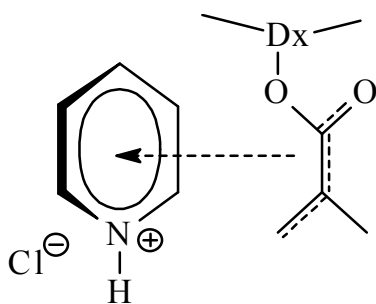


Fig. 5. Prospective structure of a charge transfer complex

It is known that pyridine chloride is characterized by a high ability for hydration [16], therefore the introduction of its fragments to DMA essentially raises their hydrophilicity and, as a consequence, water solubility of samples. DMAs synthesized in the presence of TEA have practically lost their ability to dissolve in water when their DS achieved the value at 0.5.

Obtained water soluble complexes of highly substituted DMA and pyridine chloride are the point of interest for synthesis of reactive hydrogels. In the complex with pyridine chloride methacrylic fragments keep ability to polymerize to form hydrogels, which contain residual double bonds, and also polar fragments of pyridine chloride. Their amount can vary depending on the DS of initial DMA and the degree of conversion of methacrylic fragments in the gel formation process. During the structurization of DMA with DS = 0.41 within 20 h the degree of conversion of double bonds in obtained hydrogel was 89 %. The highest degree of conversion (up to 95 %) were observed also by Van Dijk-Wolthuis and Stenekes [7, 17] during the structurization of modified dextrans containing insignificant amount of methacrylate fragments. During the structurization of DMA with DS = 1.22 the hydrogel is formed when double bonds conversion reaches 40 %. The residual 60 % of the amount of methacrylic fragments can take part in other types of reactions, for example they can attach low molecular reagents or act as centers of graft copolymerization.

4. Conclusions

The method of synthesis of unsaturated dextran derivative with controlled DS was developed.

It was shown that the DMAs received in the presence of pyridine form a complex with pyridine chloride, which remains water soluble even at high degrees of substitution.

Structurization of DMA in water solution initiated by Red-Ox system $(\text{NH}_4)_2\text{S}_2\text{O}_8\text{-Et}_3\text{N}$ results in formation of hydrogels which possess residual active double bonds.

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СИНТЕЗ І ХАРАКТЕРИСТИКИ ДЕКСТРАНМЕТАКРИЛАТІВ

Анотація. Ацилюванням декстрану метакрилоїлхлоридом в присутності третинних амінів у розчині ДМФА/LiCl одержані декстранметакрилати. Визначено, що ступінь заміщення (DS) синтезованих похідних досягає значення до 1.8 метакрилатних фрагментів в розрахунку на одну елементарну ланку полісахариду. Показано, що декстранметакрилати, синтезовані в присутності триетиламіну при досягненні DS 0.5 втрачають розчинність у воді, а одержані в присутності піридину виділяються у вигляді стабільного водорозчинного комплексу з гідрогенхлоридом піридину, причому водорозчинність зберігається навіть при досягненні DS 1.8. Структурування одержаних декстранметакрилатів у водних розчинах під дією Red-Ox системи $(\text{NH}_4)_2\text{S}_2\text{O}_8\text{-Et}_3\text{N}$ веде до утворення реакційноздатних гідрогелів, які містять залишкові подвійні зв'язки.

Ключові слова: декстран, метакрилат, макромономер, біоматеріали, гідрогель.