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Chemical reaction on polysaccharides V. Pullulan chloroalkylation

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Abstract

The article discusses the synthesis of pullulan derivatives containing chloroalkyl groups by the reaction of crosslinked pullulan microparticles with different chloroalkyl chlorides in organic basic solvents. These new products may allow the attachment of various bioactive compounds by covalent bonding. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Pullulan; Chloroalkyl polysaccharides; Chloroalkyl pullulan

1. Introduction

Pullulan is a water soluble extracellular polysaccharide, consisting of a linear chain of D-glucopyranosyl units that alternate regularly between one α(1-6) and two α(1-4) linkages. Owing to its oxygen impermeability, non-toxic and non-irritating properties it is used for producing films, binders, adhesives, thickeners, viscosity improvers and coating agents. Thus, pullulan has a number of potential uses in the pharmaceutical and food industries and in other fields of biotechnology. By introducing functional groups into the pullulan macromolecule, it is possible to improve its performance and extend the fields of possible applications.

A number of publications and, especially, patents discuss a variety of pullulan derivatives and their potential applications: chlorinated (Mayer, 1990), sulphinylethylated (Imai, Shiomi & Tesuka, 1991), etherified (Fujita, Fukami, & Fujimoto, 1978; Nishijima, Niwase & Fujimoto, 1979), cyanoethylated (Onda, Muto, Joetsu & Suzuki, 1981; Murase, Fujita, Ohnishi, & Tamura, 1983), carboxylated (Tsuji, Fujimoto, Masuko & Nagase, 1976), permethylated (Keilich, Salminen & Husemann, 1971), cationized (Onishi, 1985), sulfated (Carpov, Mocanu & Mihai, 1985), acetylated (Hijiya & Shiosaka, 1974; 1975), esterified (Hijiya & Shiosaka, 1974).

Various attempts were made to perform the haloalkylation reaction on cellulose (Khvostenko, Chang & Rogovin, 1961; Chang & Rogovin, 1960; Klavinš & Prikulis, 1982; Haimovich, Sela & Dewdney, 1967), polyethylene glycol (Dal Pozzo, Donzelli & Ferruti, 1986) or polyvinyl alcohol (Carpov & Dragan, 1972). More recently, Mocanu and Carpov (1992) and Ramirez, Sanchez-Chaves and Arranz, (1994) have reported the chloroacetylation of dextran. As far as we know, no study has been reported on the chloroalkylation of pullulan.

The present article studies the chloroalkylation reaction of pullulan with chloroalkyl chlorides (C_2–C_5), in organic basic solvents at temperatures varying between 0°C and 70°C; these new derivatives obtained can be used as intermediates for chemical modifications with substances containing amine, carboxyl, hydroxyl, thiol or other groups.

2. Experimental

2.1. Materials

The materials used are:

- Pullulan microparticles crosslinked with hydroxypropyl bridges, 0.1 mm in diameter (dry) (Mihai, Mocanu, Carpov & Ghiocel, 1984). The properties of these microparticles are shown in Table 1.
- Chloroacetyl chloride p.a. (ClAcCl); 3-chloropropionyl chloride p.a. (ClPrCl); 4-chlorobutyryl chloride p.a. (ClBuCl); and 5-chlorovaleryl chloride p.a. (ClVaCl) (Aldrich).
- Solvents: *N*,*N*-dimethyl formamide p.a. dist. (DMF); *N*,*N*-dimethyl acetamide p.a. dist. (DMA); *N*-methyl pyrrolidone p.a. dist. (NMP) (Prolabo).

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Table 1
Characteristics of the pullulan microparticles crosslinked with hydroxypropyl bridges

Sample	Water uptake (g/g)	Acetone uptake (g/g)	Bed volume of swollen gel (ml/g)	Specific gravity of swollen particles (g/ml)
1	3.74	0.20	7	1.2
2	5.36	0.35	10	1.1
3	6.72	0.26	14	0.9

2.2. Methods

2.2.1. Synthesis

To 1 g of vacuum dried crosslinked pullulan microparticles, 20–30 ml of the appropriate solvent was added. The chloroalkyl chloride is added dropwise, keeping the temperature at 0°C in an ice-bath. The reaction vessel was then immersed in a thermostated bath and stirred gently for the desired duration and temperature. The products obtained were washed with acetone to remove excess of reactant and solvent, and then with water, to remove the secondary products (the absence of Cl^- was checked with 0.1 N $AgNO_3$) and finally dehydrated with methanol and vacuum dried at a maximum temperature of 60°C. All these purification operations were performed quantitatively in a glass crucible filter of G_3 porosity.

2.2.2. Physico chemical characterization of products

The total chlorine content was determined according to Schöniger (1955; 1956). The solvent uptake at equilibrium was determined by centrifugation, using Pepper's method (Pepper, Reichenberg & Hale, 1952).

IR spectra were recorded on a Perkin-Elmer 577 Spectrophotometer. ^{1}H NMR DMSO-d_6 spectra of the synthesised linear chloroacetyl pullulan were recorded on a Jeol-L-60HL (60 MHz) spectrophotometer.

The degree of substitution with chloroalkyl groups (DS) was calculated from the total chlorine content, using the following relationship:

$$\mathrm{DS} = \frac{162 \times \mathrm{Cl}\%}{35.5 \times 100 - M_{\mathrm{RCl}} \times \mathrm{Cl}\%} \quad (1)$$

where Cl% – total chlorine content (g%) and M_{RCl} – 76.5 for ClAcCl; 90.5 for ClPrCl; 104.5 for ClBuCl; and 118.5 for ClVaCl.

DS represents the degree of substitution with chloroalkyl groups on the glucopyranosic unit of pullulan, neglecting the initial small amount of crosslinking with hydroxypropyl bridges.

Taking into account that, by chloroalkylation, one introduces $-CO(CH_2)_nCl$ groups ($n = 1$–4), the weight increase (WI) can be calculated from the DS values, using the relationship:

$$\mathrm{WI\ (g/g)} = \frac{M_{\mathrm{RCl}} \times \mathrm{DS}}{162}. \quad (2)$$

If all purification procedures are performed quantitatively, and no secondary, unforeseeable reaction occurs, the weight increase (determined after each reaction) must correspond to that calculated from Eq. (2).

3. Results and discussion

The chloroalkylation reaction of pullulan develops according to the following reaction:

$$\mathrm{Pull{-}OH + Cl{-}CO(CH_2)_nCl \rightarrow Pull{-}O{-}CO(CH_2)_nCl + HCl}$$

$(n = 1 - 4)$

The reaction was performed in basic aprotic solvents,

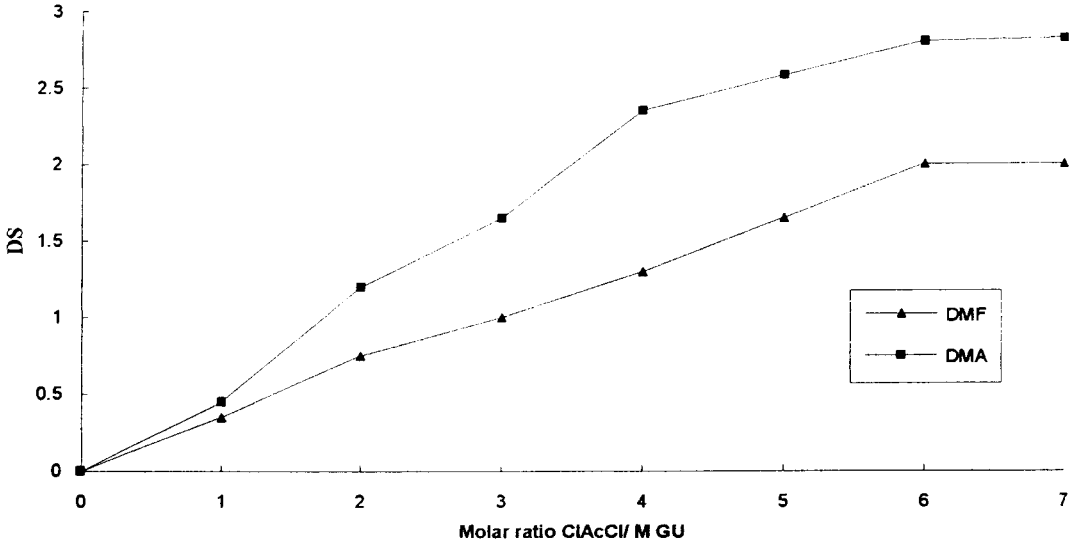

Fig. 1. Effect of molar ratio ClAcCl/M_{GU} on DS with chloroacetyl groups of sample 3. Reaction conditions: temperature, 50°C; reaction time, 6 h.

Table 2
Influence of the basic solvent used in the chloroacetylation reactions (sample 3) of crosslinked pullulan. Reaction conditions: temperature, 50°C; molar ratio, ClAcCl/M_{GU} 6/1; reaction time, 6 h

Sample	Solvent[a]	Chlorine content (g%)	DS	Weight increase (g/g)	
				Found	Calculated from DS
PCl 19	DMF	24.01	2.27	0.95	1.07
PCl 18	DMA	24.82	2.44	1.23	1.15
PCl 20	NMP	24.06	2.28	0.62	1.08

[a] 30 ml/g.

which act both as solvating agents for the tridimensional network of pullulan microparticles and as HCl acceptors.

Fig. 1 plots graphically the influence of the molar ratio of ClAcCl against the glucopyranosyl unit weight ($M_{GU} = 162$) of pullulan on DS with chloroacetyl groups in the chloroacetylation reaction of sample 3 (Table 1). From the data plotted, one can observe that the DS values increase, as the molar ratio is higher; both in DMF and in DMA, DS does not increase significantly over a ratio of 6/1. The maximum DS value obtained is about 2.8. Also, one can observe that the DS value attained is higher in DMA, than that in DMF, for the same molar ratios used, because of the higher basicity of the former.

As the reaction is accompanied by the evolution of HCl, it is to be expected that the nature of the basic solvent influences the development of the reaction. In order to check this, chloroacetylation of sample 3 of crosslinked pullulan microparticles was performed in different basic dipolar solvents. The results obtained are presented in Table 2.

As can be seen from this data, DS increases in the following order: DMF < NMP < DMA, which corresponds to the basicity series of the corresponding solvents (Badea, 1973).

A special case is represented by the pullulan chloroacetylation reaction in dimethylsulfoxide (DMSO), which is a good solvent for this polysaccharide. As is known, DMSO can act both as reagent and as solvent (Agami, 1965). With acid chlorides, DMSO reacts violently forming paraformaldehyde (Allan, Moks & Nelson, 1967; Michelot & Tchoubar, 1966). Also, pullulan hydroxyls react very easily with chloroacetyl chlorides. Hence, by performing the pullulan chloroacetylation in DMSO, two parallel reactions may occur: DMSO–ClAcCl and pullulan–ClAcCl.

Chloroacetylation of linear and crosslinked microparticles of pullulan in DMSO was attempted. The reaction was found to be exothermic and the formation of a white deposit (of paraformaldehyde) in the reflux condenser occurred sometimes.

Following the dropwise addition of chloroalkyl chlorides, the reaction was continued at 50°C for 6 h and then the mixture was poured into acetone (in which the linear polymer precipitates), filtered, washed with cold water (to remove the secondary reaction products) followed by warm water (to remove the paraformaldehide formed) and then dried from methanol.

The characteristics of the products obtained are presented in Table 3. From the data presented and considering that the polymers have no sulfur content (hence no incorporation of $(H_3C)_2S$ is possibly), the following conclusions may be drawn:

- the main reaction occurs between DMSO–ClAcCl (the DS with chloroacetyl groups is small); and
- an additional crosslinking reaction occurs, owing to the presence of formaldehide in status nascendi in acidic medium, with $-CH_2-$ bridges. This assumption is based on the facts that the weight increase found is higher than the weight increase (WI) calculated from the DS (Eq. (2)). The chloroacetylated linear pullulan is insoluble in any solvent, although the DS is very low; the water uptake decrease of crosslinked pullulan microparticles is higher than the one corresponding to such a small DS.

From these preliminary experiments it can be concluded that chloroacetylation of pullulan in DMSO is a complex reaction which requires further extensive study.

Fig. 2 plots the influence of the duration of chloroacetylation reaction on the DS, for sample 2. As can be seen, the

Table 3
Physico-chemical characteristics of the reaction products of pullulan (linear and crosslinked) with ClAcCl in DMSO. Reaction conditions: molar ratio, ClAcCl/M_{GU} 6/1; molar ratio, ClAcCl/DMSO 0.145/1; temperature, 50°C; reaction time, 6 h

Sample	Chlorine content (g%)	DS	Weight increase (g/g)		Water uptake (g/g)
			Found	Calculated from DS	
A[a]	2.75	0.13	0.10	0.06	2.30
B[b]	2.66	0.13	0.19	0.06	2.42

[a] A – linear pullulan.
[b] B – crosslinked pullulan microparticles (sample 1).

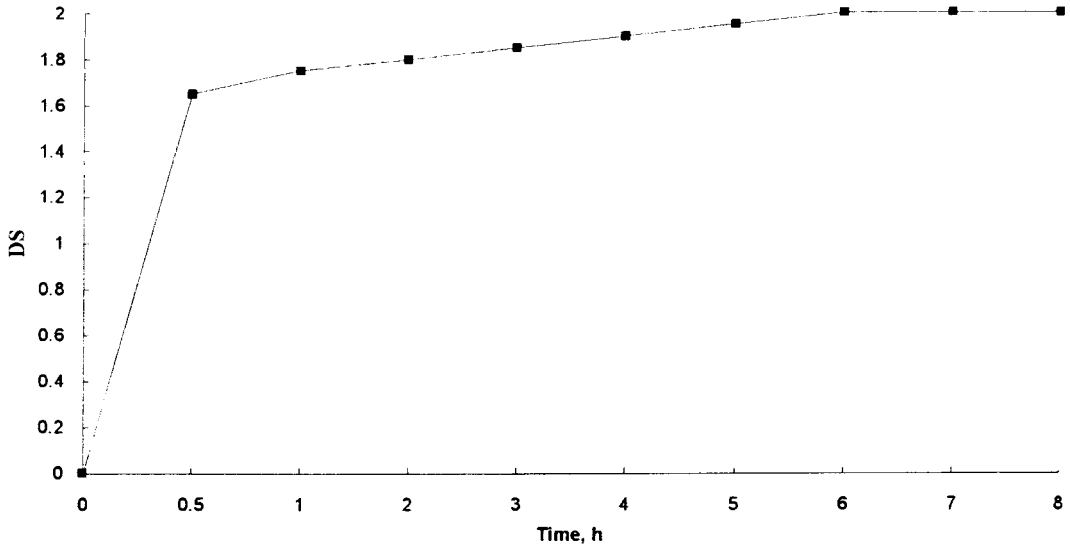

Fig. 2. Effect of the reaction duration on the DS with chloroacetyl groups of sample 2. Reaction conditions: temperature, 50°C; molar ratio, ClAcCl/M_{GU} 6/1; solvent, DMF.

reaction rate is high enough, reaching the DS value of 1.5, 10 min after the reaction's initiation.

Unlike dextran where chloroacetylation does not occur below 40°C (Mocanu & Carpov, 1992), temperature influences only to a small extent the progress of the chloroacetylation reaction of crosslinked pullulan microparticles (Fig. 3). Therefore, one can conclude that pullulan reactivity in the chloroacetylation reaction is higher than that of dextran. This difference in reactivity correlates with the higher content of primary hydroxyl groups of pullulan in comparison with the dextran chain.

Using chloroalkyl chlorides with longer aliphatic chains (ClPrCl, ClBuCl, ClVaCl), it can be observed that DS increases considerably as the temperature rises from 25°C to 70°C (Table 4). By comparing the reactivities of the series of acyl chlorides employed it can be appreciated that ClAcCl is the most reactive, while the others (ClPrCl, ClBuCl, ClVaCl) have almost the same reactivities.

Also, from the data presented in Tables 2 and 4, it can be seen that the weight increases found correspond fairly well with the values calculated from DS, suggesting that no degradation or unexpected secondary reaction occurs.

The influence of the initial crosslinking degree with hydroxypropyl bridges (which is inversely proportional to their water uptake – Table 1), on the DS with chloroacetyl groups was also followed (Table 5). From the data presented in Table 5 one can observe that the DS values increase, as

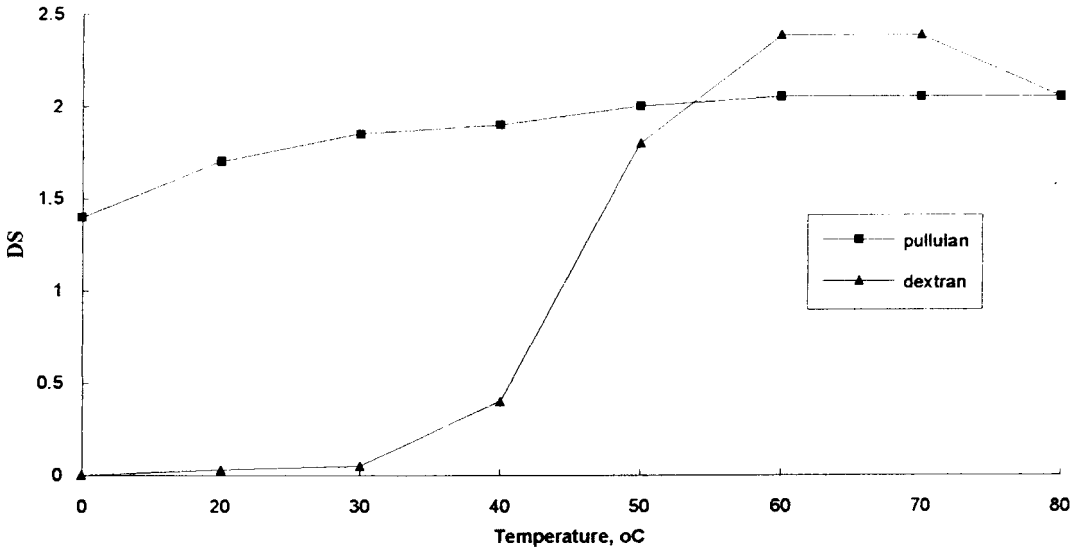

Fig. 3. Effect of the chloroacetylation temperature on the DS with chloroacetyl groups of crosslinked pullulan (sample 2) and of the crosslinked dextran microparticles. Reaction conditions: molar ratio, ClAcCl/M_{GU} 6/1; reaction time, 6 h; solvent, DMF.

Table 4
Influence of chloroalkyl chloride type used in chloroalkylation reactions (sample 3) of crosslinked pullulan. Reaction conditions: reaction time, 6 h; solvent, DMF

Sample	ClAlkCl[a]	Temperature (°C)	Molar ratio: ClAlkCl/M_{GU}[b]	Chlorine content (g%)	DS	Weight increase (g/g)	
						Found	Calculated from DS
PCl 27	ClAcCl	50	3.75/1	18.16	1.36	0.55	0.64
PCl 31	ClPrCl	50	3.75/1	16.91	1.36	0.50	0.77
PCl 32	ClBuCl	50	3.75/1	12.90	0.95	0.58	0.61
PCl 25	ClVaCl	50	3.75/1	14.48	1.28	0.85	0.95
PCl 9	ClAcCl	25	6/1	21.30	1.80	0.67	0.85
PCl 38	ClPrCl	25	6/1	12.06	0.79	0.38	0.44
PCl 39	ClBuCl	25	6/1	12.71	0.93	0.59	0.60
PCl 26	ClAcCl	50	6/1	22.57	2.00	0.88	0.94
PCl 44	ClPrCl	50	6/1	16.13	1.25	0.70	0.70
PCl 45	ClBuCl	50	6/1	16.81	1.52	0.86	0.98
PCl 7	ClAcCl	70	6/1	22.94	2.07	0.95	0.98
PCl 40	ClPrCl	70	6/1	19.08	1.70	0.70	0.96
PCl 41	ClBuCl	70	6/1	18.51	1.86	1.07	1.20

[a] Chloroalkyl chloride.
[b] Molar ratio chloroalkyl chloride/M_{GU}.

Table 5
Influence of the pullulan crosslinking on DS with chloroacetyl groups. Reaction conditions: temperature, 50°C; reaction time, 6 h; molar ratio, ClAcCl/M_{GU}, 6/1; solvent, DMF

Sample of ClAcPL[a]	Sample of PC[b]	Chlorine content (g%)	DS	Weight increase (g/g)	
				Found	Calculated from DS
PCl 22	1	22.31	1.96	0.67	0.92
PCl 5	2	22.64	2.00	0.91	0.94
PCl 19	3	24.01	2.27	0.95	1.07

[a] Chloroacetylated crosslinked pullulan.
[b] Crosslinked pullulan (Table 1).

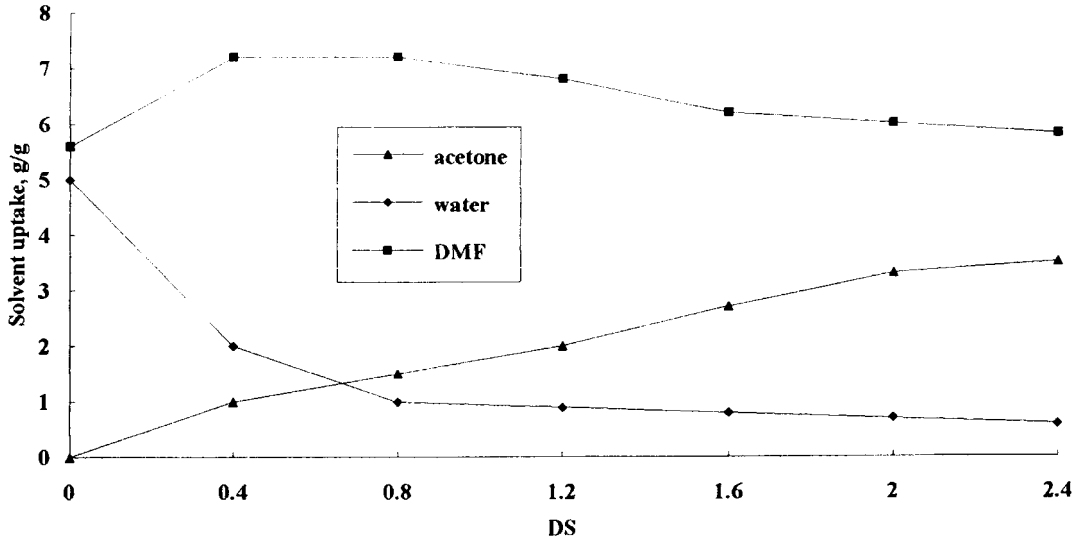

Fig. 4. Effect of DS with chloroacetyl groups of crosslinked pullulan (sample 2) on the solvent uptake.

the initial crosslinking degree of pullulan microparticles is smaller.

The IR spectrum of chloroacetylated pullulan presents a characteristic ester band at 1720 cm^{-1} and at about 700 cm^{-1} for the C–Cl bond.

The ^1H NMR DMSO-d_6 spectrum of a sample of linear chloroacetylated pullulan (prepared under the same conditions) with DS 2.8 shows, besides the signals attributed to glucopyranosic protons between 3 and 4 ppm, a signal at about 4.2–4.3 ppm, which can be attributed to the $-CH_2Cl$ (2H) protons, introduced as a result of chloroacetylation.

The chloroalkylated pullulan crosslinked microparticles are new products, which can be used for many subsequent chemical modifications. Hence, it is important to know their behavior in various solvents. The data presented in Fig. 4 show that the chloroacetylation reaction changes swelling of microparticles in different solvents: they become hydrophobic, while the acetone uptake increases with increasing of DS.

4. Conclusions

The article studies the chloroalkylation of pullulan with C_2–C_5 chloroalkyl chloride. New functionalized products to be used as intermediates in nucleophilic substitutions with various bioactive compounds were obtained.

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