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Characteristics of films prepared from native and modified branched β-1,3-D-glucans

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The properties of films prepared from a high molecular weight (mol. wt: $7{\cdot}5 \times 10^6$ g/mol) branched β-1,3-D-glucan (schizophyllan) and a polyalcohol (mol. wt: $7{\cdot}3 \times 10^6$ g/mol) derived from schizophyllan by periodate oxidation and subsequent borohydride reduction are described. The films can only be prepared by casting from aqueous solutions, because the polymers are not thermoplastic. They have a low permeability to oxygen, but a high permeability to water vapour. The tensile strength of the films is 45–58 N/mm^{-2} for schizophyllan and 12–18 N/mm^2 for the polyalcohol, and both, but especially the polyalcohol films, have a low elongation at break. Films prepared from both polymers, under conditions where the triple helices are disrupted (>0·01 M NaOH), show lower tensile strength and elongations at break as well as higher oxygen permeabilities. A relationship exists between the water content of the films and the tensile strength.

INTRODUCTION

Fungi of the genus *Schizophyllum* (Wessels *et al.*, 1972) secrete a polysaccharide commonly named schizophyllan, which is a β-1,3-D-glucan with one 1,6-linked β-D-glucopyranosyl group attached to every third residue in the main chain (Norisuye *et al.*, 1980; Münzer, 1989). Extracellular polysaccharides with this type of structure are also produced by other fungi (Cateley, 1983), for example those of the genus *Sclerotium* (Johnson *et al.*, 1963; Rinaudo & Vincendon, 1982).

A polyalcohol can also be prepared from schizophyllan by periodate oxidation and subsequent borohydride reduction (Schulz & Rapp, 1991).

The structure of the repeat unit of schizophyllan and the polyalcohol is shown in Fig. 1. In aqueous solutions and in solid state both schizophyllan and the polyalcohol form a triple-stranded helix. In dimethyl sulfoxide, in > 0·01 M sodium hydroxide or at a temperature > 135°C the triple helix is disrupted giving a single, randomly coiled chain (Kashiwagi *et al.*, 1981; Yanaki *et al.*, 1983; Adechi *et al.*, 1990).

At present, the interest in biodegradable materials and polymers with specific properties, for example polyhydroxy alkanoates (Owen, 1985; Marchessault *et al.*, 1988) or pullulan (Yuen, 1974), is increasing. This was the motive for investigating the film-forming properties of schizophyllan and the polyalcohol. Films of these aqueous solutions cast on PVC plates were used to investigate the dependence of plasticizer and water content on mechanical characteristics and permeabilities.

EXPERIMENTAL

Materials

Schizophyllan was isolated from culture suspensions of *Schizophyllum commune* ATCC 38548 cultivated in a medium consisting (Münzer, 1989) of KH_2PO_4 (1·0 g), $MgSO_4{\cdot}7H_2O$ (0·5 g), yeast extract (3·0 g), and D-glucose·H_2O (33·0 g) per litre of deionized water. A 50-litre bioreactor (Braun-Diessel International, Melsungen, FRG) equipped with three four-bladed fan impellers was used, and the cultures were stirred at 100 rpm and aerated at 0·1 v/vm (volume of air per volume of culture suspension per minute). After cultivation for 100 h the mycelium was separated by cross flow filtration (pore size 0·2 mm) (Filtron, Karlstein, FRG) and residual hyphal fragments were removed by filtration through glass fibre prefilters (Sartorius, Göttingen, FRG). The

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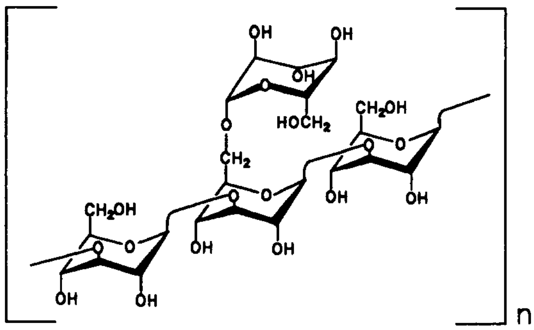
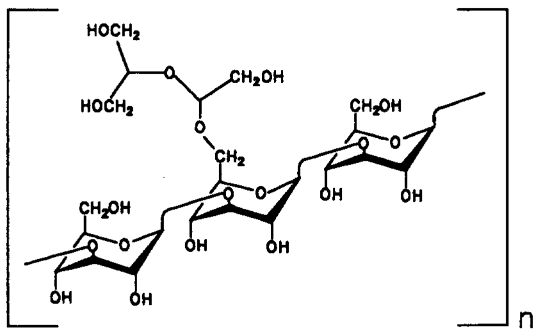


n = 11500

(a)



n = 11500

(b)

Fig. 1. Repeat units for (a) schizophyllan and (b) the polyalcohol.

aqueous schizophyllan solution was subsequently diafiltrated (cut off 100 000 daltons) and concentrated to 5 g/litre. The polyalcohol was prepared by oxidizing schizophyllan with periodate followed by reduction with sodium borohydride, using the method of Goldstein (Goldstein *et al.*, 1965). The resulting solutions were diafiltrated and also concentrated to 5 g/litre.

The triple helices of schizophyllan and the polyalcohol were degraded to single, randomly coiled chains by increasing the pH to 13 using NaOH. The resulting solutions were neutralized, dialyzed and concentrated to 5 g/litre.

Glycerine and maltitol were used as plasticizers.

Preparation of the films

Appropriate amounts of plasticizer were added to the aqueous stock solutions of schizophyllan and the polyalcohol followed by vigorous stirring, yielding plasticizer contents of 5–25% (wt/wt). The resulting solutions were degassed, cast on PVC plates (400 cm^2) and dried for approximately 100 h under reduced pressure (10^4 Pa) at 40°C (70–80% relative humidity).

Water content

The water content was determined using a Karl Fischer Titrator DL 18 (Mettler, Darmstadt, FRG).

Percentage elongation at break and tensile strength

Measurements were carried out using a Schenck Triebel RME stress frame (Schenck Triebel, Ratingen, FRG) according to DIN 53455.

Oxygen permeability

These data were obtained with a Mocon Oxtran (Modern Control Inc., Minneapolis, USA) in accordance with DIN 53380, part 3.

Water vapour permeability

Measurements were conducted according to DIN 53122, part 1.

All measurements mentioned above were carried out three times with at least two different samples of each film.

RESULTS AND DISCUSSION

Preparation of the films

Different materials were tested as plates. Glass was useless, because the films could not be removed. On silylated glass and metal plates the films were more easily removed but the best materials for film removal were Teflon and polyvinyl chloride (PVC). All further investigations were carried out with PVC plates. The use of glycerine or maltitol as plasticizers gave equivalent results.

The best drying conditions were a temperature of 40°C and a pressure of 10^4 Pa. These conditions resulted in a relative humidity of 70–80% in the drying chamber. More drastic drying conditions resulted in fragile films with a lower water content — 0·01 mm was the minimum thickness of film that could be handled. For the following investigations the films were dried for 4 days under the conditions mentioned above, resulting in a thickness of 0·03 mm.

Water content of the films

Films prepared from schizophyllan in the triple helical conformation contained about 10% H_2O, whereas the single chain schizophyllan films had a water content of 7·2%. The triple helical polyalcohol films had 8·5% H_2O and the single chain polyalcohol film had 7·2% H_2O. The plasticizer concentration in the film did not affect the final water content. In other words, both films

Table 1. Properties of schizophyllan and polyalcohol films

Polymer[a]	S	S	S^{SC}	P	P	P^{SC}	Pu[b]
Plasticizer [%]	10	25	10	10	25	10	10
Water [%]	9·8	9·9	7·2	8·5	8·5	6·3	4·3
Elongation [%]	3·8	4·6	0·7	1·0	1·5	0·7	10–21
Tensile str. [N/mm^2]	54·9	57·9	9·3	12·6	18·2	9·2	7–8
O_2-perm. (0·03 mm) [ml/m^2 per d per bar]	1·8	4·2	9·2	31·7	31·8	39·8	2
H_2O-perm. (0·03 mm) [g/m^2 per d per bar]	487	557	510	513	448	494	>800
Solub. [%]	100	100	100	21	10	100	100
after [h]	168	72	144	672	672	672	<0·5

[a]S = schizophyllan, S^{SC} = single chain schizophyllan, P = polyalcohol, P^{SC} = single chain polyalcohol, Pu = pullulan.
[b]Yuen, 1974.

contain 25% less water if the polymer was in single, randomly coiled conformation. This result can be interpreted as the loss of water contributing to the crystalline triple helix conformation (Itou *et al.*, 1986) or a removal of water that could be located between the triple helices.

Elongation percentage and tensile strength

All films prepared from schizophyllan and the polyalcohol showed very low elongations at break (Table 1). For schizophyllan in the triple helix conformation the elongation changes from 3·8 to 4·6% as the maltitol concentration increases from 5 to 25% (Fig. 2). In the case of single chain schizophyllan the elongation at break is only 0·7% at 10% maltitol. The elongation at break of films prepared from the polyalcohol is much lower, increasing from 1% at 5% maltitol to 1·5% at 25% maltitol. Films prepared from single chain polyalcohol show only 0·7% elongation at break.

The tensile strengths of the triple helical schizophyllan films are also higher than that of the triple helical

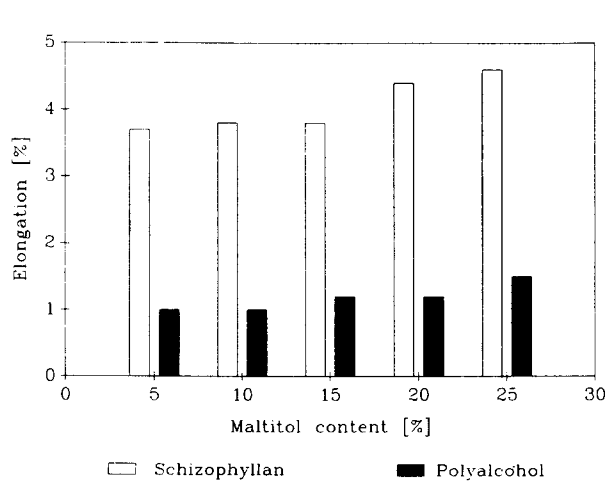


Fig. 2. Elongation percentage of schizophyllan and polyalcohol films at different maltitol contents.

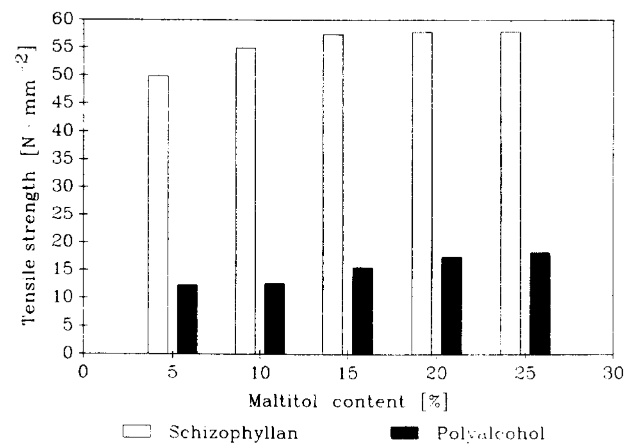


Fig. 3. Tensile strength of schizophyllan and polyalcohol films at different maltitol contents.

polyalcohol ones and increase with plasticizer content from 49·8 N/mm^2 to 57·9 N/mm^2. In the case of the polyalcohol it alters from 12·4 N/mm^2 to 18·2 N/mm^2 (Fig. 3). Films prepared from the single chain polymer show much lower tensile strengths [9·3 N/mm^2 (schizophyllan) and 9·2 N/mm^2 (polyalcohol)] (Table 1).

Tensile strength and elongation are related to the water content of the films. The higher the water content the greater the tensile strength and the elongation at break. It also appears that films of higher strengths are formed by triple helical polymers.

Thus, pullulan (Yuen, 1974), a polysaccharide with α-1,6-linked maltotriose units, which exists as a random coil (Itou *et al.*, 1987) forms films with a tensile strength of only 7–8 N/mm^2 at 4·3% water (Table 1). The higher elasticity at break of 10–21% elongation is due to the higher flexibility of the α-1,6-linkage (Brant & Burton, 1981; Kato *et al.*, 1982).

Oxygen permeability

The oxygen permeabilities of schizophyllan films were very low and those with a maltitol content up to 10%

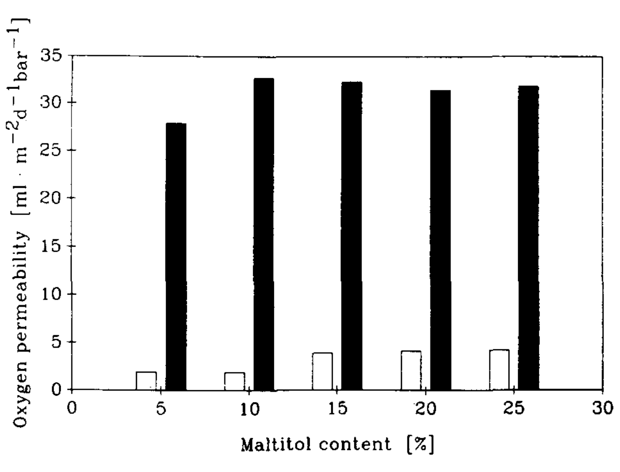


Fig. 4. Oxygen permeability of schizophyllan and polyalcohol films (thickness 0·03 mm) at different maltitol contents.

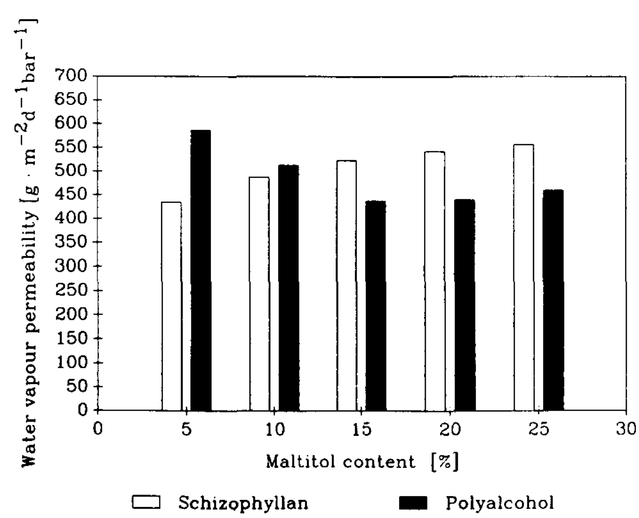


Fig. 5. Water vapour permeability of schizophyllan and polyalcohol films (thickness 0·03 mm) at different maltitol contents.

had only 1·8–1·9 ml/m^2 per d per bar; those containing 25% maltitol had permeabilities of 4·2 ml/m^2 per d per bar (Fig. 4). The permeability of the polyalcohol films is considerably higher. It increases from 27·9 ml/m^2 per d per bar to 32·7 ml/m^2 per d per bar and then decreases to 31·8 ml/m^2 per d per bar with increasing maltitol content. This points to a greater distance between the triple helices caused by the side chains of the polyalcohol in contrast to schizophyllan with its closed glucopyranose residues. The oxygen permeability of films prepared from the single chain species is much higher (schizophyllan 9·2 ml/m^2 per d per bar and polyalcohol 39·8 ml/m^2 per d per bar) compared with the values for the films formed from the triple helical forms. This observation leads to the assumption that the triple helical conformation needs less space for packing than the randomly coiled single chains.

The oxygen permeability for pullulan films is about 2 ml/m^2 per d per bar (Yuen, 1974).

Water vapour permeability

The high water vapour permeabilities are typical for water-soluble materials, because the transfer is caused mainly by a solubility–diffusion mechanism (Greener & Fennema, 1989). Table 1 shows a dependence between the rate of solubilization in water and the water vapour permeability of the films. For schizophyllan films the rate of solubilization in water and the water vapour permeability increases with rising maltitol content, while these values decrease for polyalcohol films (Fig. 5). In the case of schizophyllan films, these observations could be explained by insertion of maltitol molecules into the hydration shell causing greater distance between the strains. For the polyalcohol a stronger linkage between hydroxyl groups of the side chains and the plasticizer is proposed.

The water vapour permeability of films prepared from the more soluble pullulan is higher than for schizophyllan and the polyalcohol, which confirms the relationship between solubility and water vapour permeability.

CONCLUSION

The aim of this work was to investigate the physicochemical characteristics of films formed from schizophyllan and a chemical modified polymer based on schizophyllan. The special characteristics of the films are a high water solubility and a very low oxygen permeability. Therefore, its use as an oxygen barrier film, which could be easily removed by water, is conceivable. Unfortunately, the low elasticity may lead to problems in practical use.

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