Fine structure and oxygen permeability of silk fibroin membrane treated with methanol

Norihiko Minoura*
Industrial Products Research Institute, 1-1-4 Higashi, Tsukuba, Ibaraki 305, Japan

Masuhiro Tsukada
National Institute of Sericultural and Entomological Science, 1-2 Owashi, Tsukuba, Ibaraki 305, Japan

and Masanobu Nagura
Faculty of Textile Science and Technology, Shinshu University, 3-15-1, Tokida, Ueda 386, Japan

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Dissolved oxygen permeability, water content, crystalline structure, crystallinity, and density of silk fibroin membrane were measured as a function of immersion time of the membrane in 50 vol% aqueous methanol solution. Permeability coefficient and water content exhibited a minimum at the treatment time of 30 min. After the treatment of methanol the silk II crystalline structure appeared in the membrane. The density of the membrane decreased with increasing treatment time, although the crystallization proceeded in the membrane. These results indicate an existence of roughly molecular-packed space (void) among the crystals. The appearance of the minimum for water content was attributed to the development of the crystal and to the growth of the roughly molecular-packed space. In addition to these two effects, the chain immobilization effect associated with the crystallization was taken into account to explain the minimum for the permeability coefficient.

(Keywords: silk fibroin; membrane; permeability; crystallinity; oxygen)

INTRODUCTION

There are many reports on higher-order structures of silk fibroin membranes. Recently, Nagura *et al.*[1] found that the silk fibroin membrane obtained by casting from the silk fibroin solution has a micro phase-separated structure which consists of crystal and amorphous regions. A relationship between the unique structure of the silk fibroin membrane and a permeation of small molecules through the membrane is of particular interest because the amorphous regions govern the permeation property.

One of the authors has extensively studied on a permeability of a synthetic polypeptide membranes to gases, vapours, and ionic solutes in relation to the primary and the higher-order structure of the polypeptides[2–6]. As a development of the research, the silk fibroin membrane treated with methanol was found to have a high dissolved oxygen permeability[7]. However, the permeability depended on the immersion time of the membrane in methanol which makes the membrane water insoluble.

In the present paper, a dissolved oxygen permeability of the silk fibroin membrane is studied in detail as a function of immersion time in methanol.

EXPERIMENTAL

Preparation of membranes

Liquid silk was collected from the posterior division of the silk gland in full-grown larvae of *Bombyx mori* (one day before spinning). The aqueous solution of silk fibroin was diluted to about 1%. The silk fibroin membrane obtained by casting from the solution onto thin polyethylene film was dried at 25°C and at a relative humidity of 65%. The membrane was subsequently immersed in 50 vol% aqueous methanol solution for different periods of time at 25°C. The membrane was dried in vacuum and conditioned for 7 days at a temperature of 25°C and 65% r.h. in desiccator. The membranes treated with the methanol solution for more than 3 min were insoluble in water.

A water content of the membrane, defined as the weight percent of water in the water-swollen membrane, was determined as follows. The membrane was swollen at 20°C, and weighed repeatedly until a constant weight of the swollen membrane was obtained. The membrane was then dried to a constant weight under vacuum at 80°C. The water content of the membranes thus obtained is summarized in *Table 1*, together with the thickness of the wet membranes used for the oxygen permeation experiments. The thickness of the membrane was measured using a micrometer.

Measurement of oxygen permeability

The permeation of oxygen dissolved in water through the silk fibroin membranes was measured by using an oxygen electrode according to the experimental method developed by Minoura *et al.*[8]. The silk fibroin membranes were laminated to polystyrene membranes to avoid membrane degradation during the measurement according to the method reported in a previous paper[8]. The permeability coefficient P [$\mathrm{cm^3}$ (STP) cm $\mathrm{cm^{-2}}$ $\mathrm{s^{-1}}$ $\mathrm{cmHg^{-1}}$] was calculated from the steady state current of the permeation curve. Prior to measurements, the membranes were immersed in distilled water for about 3

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* To whom correspondence should be addressed

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Table 1 Thickness of wet membrane used for permeation experiments and water content of membrane (20°C)

	Treatment time (min)							
	3	6	15	20	30	40	50	60
Thickness of wet membrane (mm)	0.070	0.093	0.078	0.092	0.091	0.108	0.090	0.040
Water content (%)	31.5	32.4	34.0	29.6	25.0	32.0	31.2	30.4

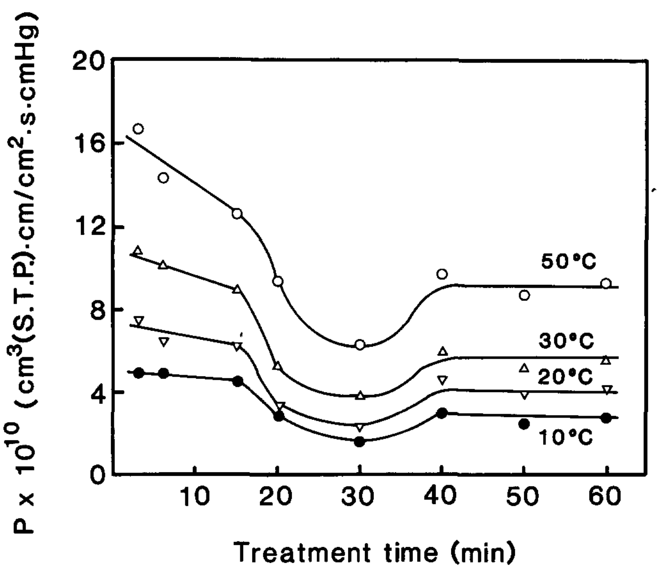

Figure 1 Oxygen permeability coefficient of the wet silk fibroin membrane at different temperatures as a function of the immersion time in aqueous methanol

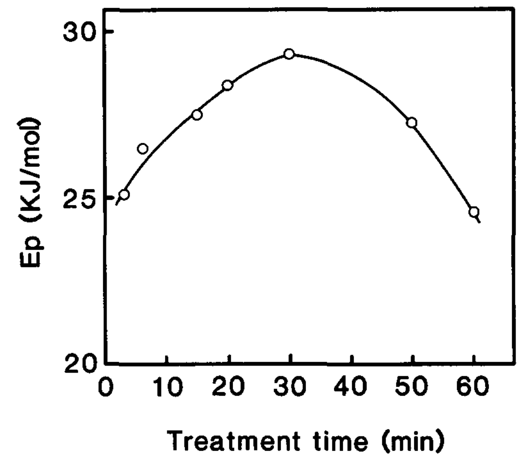

Figure 2 The apparent activation energy E_P for permeation as a function of the immersion time in aqueous methanol

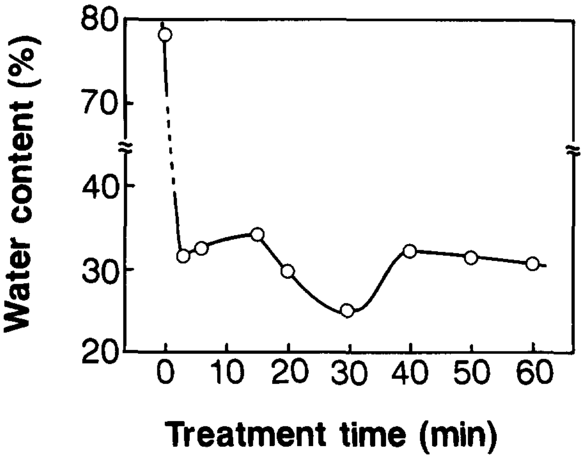

Figure 3 Water content of the silk fibroin membrane as a function of the immersion time in aqueous methanol

days. Sorption equilibrium with respect to water is assumed.

Structure analysis

Density of the membrane was measured by the density gradient column method in a heptane/carbon tetrachloride mixture system at 20±0.05°C.

X-ray diffraction patterns were obtained using a diffractometer (Rigaku Denki Co., Ltd. Ru-200) with Cu–K$_\alpha$ radiation. The voltage and current of the X-ray source were 40 kV and 20 mA, respectively. The diffraction intensity curves were measured by a diffractometer (Rigaku Denki Co., Ltd. Geigerflex 2028) at a scanning rate of 1 degree min^{-1}, time constant of 1 s, scanning region of 5–45°. Crystallinity was evaluated from the X-ray diffraction curves according to the method proposed by Hermanns[9].

RESULTS AND DISCUSSION

Oxygen permeability

The oxygen permeability coefficient of the wet silk fibroin membrane is plotted in *Figure 1* as a function of the immersion time in 50 vol% aqueous methanol solution. At every temperature studied, the permeability coefficients decrease with increasing treatment time up to 30 min, and then increase to reach a constant value for the treatment time of about 50 min. When the logarithm of permeability coefficient is plotted against the reciprocal of absolute temperature (the Arrhenius plot) for the membranes of different periods of immersion time, a straight line can be drawn in the temperature range of 7–35°C. Therefore, apparent activation energy E_P for permeation was calculated using the following equation by the least squares method:

$$P = P_0 \exp(-E_P/RT) \quad (1)$$

where P_0 is the pre-exponential factor and R is the gas constant. *Figure 2* shows the apparent activation energy E_P as a function of the treatment time. The E_P shows a maximum value at the treatment time of 30 min. It is noteworthy that the treatment time at the maximum E_P coincides with that at which the minimum permeability coefficient appeared (*Figure 1*).

In order to explain the minimum for the permeability coefficient and the maximum for the apparent activation energy, a water content of the membrane was measured. *Figure 3* shows the water content of the silk fibroin membrane treated with 50 vol% aqueous methanol as a function of the treatment time. The water content of the membrane decreases sharply within the short period of

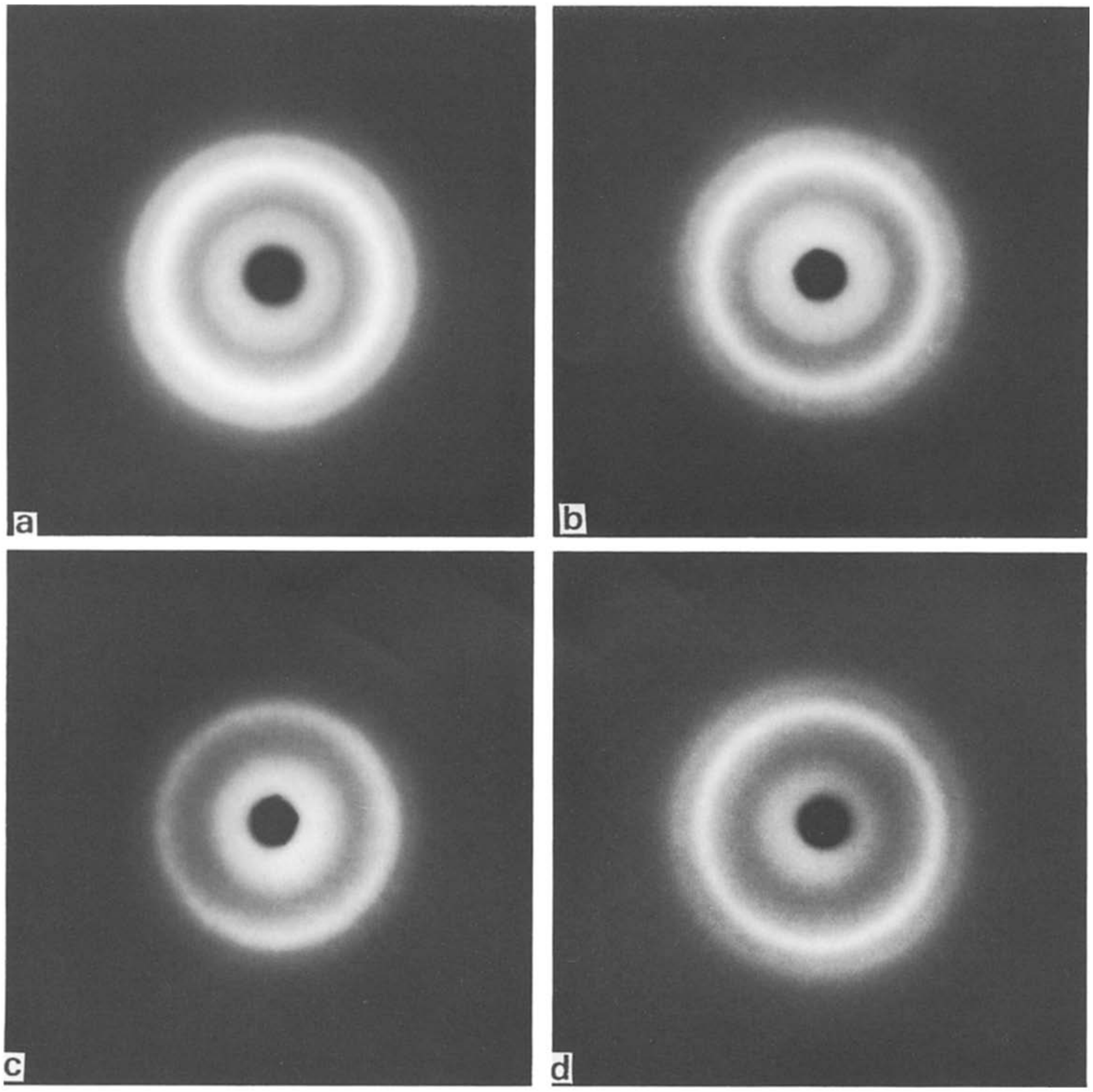

Figure 4 X-ray diffraction patterns of the silk fibroin membrane immersed in aqueous methanol for different periods of time: (a) 3 min, (b) 15 min, (c) 30 min, (d) 60 min

the immersion time (0–3 min) from 78% down to 32% and the membranes treated for above 3 min become insoluble in water. The striking characteristic that the water content of the membrane also shows a minimum at the treatment time of 30 min is observed. It is worthwhile to mention that the trend of the oxygen permeability coefficients shown in *Figure 1* agrees well with that of the water content in *Figure 3*.

The specific behaviour of the water content, the permeability coefficient, and E_P along with an increase of the treatment time suggest that the structural change of the membrane proceeds gradually when the membrane is immersed in aqueous methanol.

Structural change induced by the treatment with methanol

When silk fibroin membrane is immersed in methanol, crystallization is reported to proceed in the membrane[10].

X-ray diffraction patterns of the silk fibroin membrane treated with methanol for not less than 3 min are shown in *Figure 4*. The diffraction pattern of the membrane immersed within 3 min is the same as that of the original membrane as cast (not shown here), demonstrating the spacings of 7.55, 4.56, 3.67, and 3.20 Å attributed to the silk I crystalline structure[11]. With increasing treatment time, the diffraction pattern changes into another diffraction pattern corresponding to the spacings of 4.36 and 3.67 Å, which are characteristic of the silk II crystalline structure[11], although the diffraction pattern corresponding to the spacing of 7.55 Å remains. As seen in *Figure 4*, the intensity of the diffraction associated with the silk II crystalline structure becomes gradually stronger. That associated with the silk I crystalline structure remains although its intensity becomes relatively weak. Thus the silk II crystalline structure appears in the membrane after the treatment of methanol. Because a direct transition from the silk I crystalline structure to the silk II crystalline structure by the methanol treatment is not actually observed yet, it is considered that the random-coil fraction of the molecular chain in the amorphous region of the membrane changes into the silk II crystalline structure.

It is important to analyse the change of the crystallinity in the course of the methanol treatment of the silk fibroin membrane in relation to the crystalline structure. A

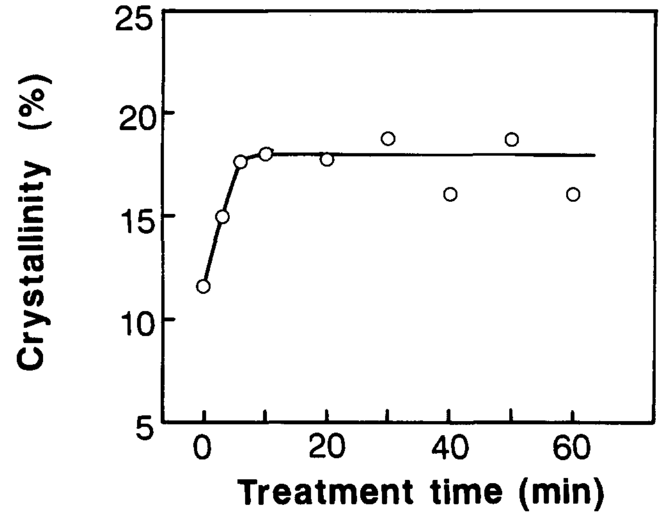

Figure 5 Crystallinity estimated from the X-ray diffraction curves according to the method proposed by Hermanns as a function of the immersion time in aqueous methanol

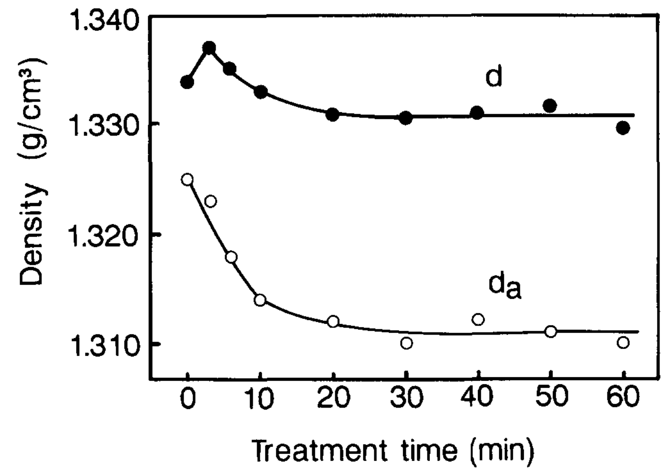

Figure 6 Density d of the silk fibroin membrane and density d_a of the amorphous phase as a function of the immersion time in aqueous methanol

degree of crystallinity of the membrane was estimated from the X-ray diffraction curve according to the method proposed by Hermanns (*Figure 5*). With increasing treatment time the crystallinity increases to reach a constant value of 18%. The increase of the crystallinity corresponds to the appearance of the silk II crystalline structure in the membrane as described previously.

To obtain further evidence on the structural change induced by the immersion in aqueous methanol, the density, which is related to the crystallinity of the membrane, was measured (*Figure 6*). The density of the membrane treated for 3 min is higher than that of the original membrane as cast. However, the density of the membrane treated for above 3 min decreases with increasing treatment time. The decrease of the density seems to be in conflict with the result of the crystallinity measurement.

The density d of the membrane can be related to the density d_a of the amorphous phase by the following equation:

$$d = v_a d_a + v_{c1} d_{c1} + v_{c2} d_{c2} \qquad (2)$$

where v_a, v_{c1}, and v_{c2} are volume fractions of the amorphous phase, the silk I crystalline phase, and the silk II crystalline phase, respectively, and d_{c1} and d_{c2} are the density of the silk I crystalline phase and the silk II crystalline phase, respectively. Using the values of density of the silk I crystalline phase (1.40 g cm^{-3}) (ref. 12) and the silk II crystalline phase (1.45 g cm^{-3}) (ref. 13) and the values of each crystallinity, d_a was calculated from equation (2). As seen in *Figure 6*, d_a decreases with increasing treatment time to reach a constant value. This result suggests the appearance and growth of roughly molecular-packed space (voids) among the crystals along with growing spherulites. The volume fraction of the void was estimated from d_a of the untreated membrane and d_a of the membrane treated for 60 min, and was only 0.01.

Thus, the minimum for the water content shown in *Figure 3* can be interpreted in terms of the appearance of the silk II crystalline structure and the roughly molecular-packed space in the membrane induced by the methanol treatment. That is, the increase of the crystallinity contributes to decrease the water content, while the roughly molecular-packed space contributes to increase the water content.

The specific characteristic of the oxygen permeability shown in *Figure 1* is explained according to these experimental facts concerning the structural change induced by the methanol treatment. In gas permeation through crystalline polymers, the measured permeability coefficient P is generally related to a permeability coefficient P_a in a completely amorphous polymer by the following equation because the polymer crystal is impermeable[14]:

$$P = K v_a P_a \qquad (3)$$

where K is a structure factor and v_a is a volume fraction of the amorphous phase. The structure factor K was calculated from the following equation[14]:

$$K = \frac{1}{v_a}\left(1 - \frac{3v_c}{2 + v_c - 0.306v_c^4 - 0.0134v_c^8}\right) \qquad (4)$$

where v_c is a volume fraction of the crystalline phase. In the present study v_a is regarded as equal to the volume fraction of water in the water-swollen membrane. Thus P_a can be calculated from P divided by K and v_a for each membrane. *Figure 7* shows P_a as a function of the treatment time using the value of the permeability coefficient measured at 20°C. The P_a decreases with

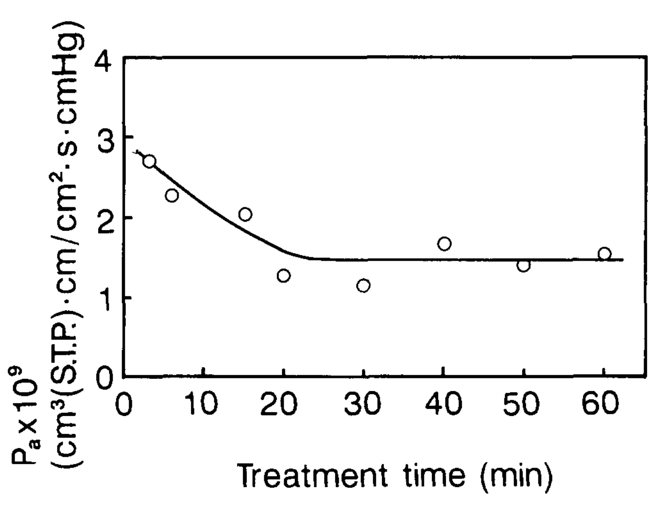

Figure 7 P_a as a function of the immersion time in aqueous methanol

increasing treatment time up to 20 min to reach a constant value, though P_a should be constant. Michaels *et al.*[15] interpreted the structure factor K as the reciprocal of the product of a geometrical impedance factor and a chain immobilization factor. The impedance factor took into account the reduction in diffusion constant due to the necessity of molecules to bypass crystallites and move through amorphous regions. In the vicinity of crystalline particles segments of polymer chain were thought to be less free to undergo rotatory and other movements than when they are further from the crystallite surfaces. In the present study, because the impedance factor has been already considered as equation (4), the decrease of P_a may be mainly attributed to the chain immobilization factor associated with the appearance and growth of the silk II crystalline structure.

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REFERENCES

1 Nagura, M., Ueki, T., Hiragi, Y., Tagawa, H., Kataoka, M., Izume, Y., Muroga, Y. and Amemiya, Y. *J. Polym. Sci., Phys. Edn.* 1987, **25**, 2567
2 Minoura, N. and Nakagawa, T. *J. Appl. Polym. Sci.* 1979, **23**, 2729
3 Nakagawa, T., Fujiwara, Y. and Minoura, N. *J. Membrane Sci.* 1984, **18**, 111
4 Minoura, N. *Makromol. Chem.* 1988, **189**, 2425
5 Minoura, N. and Fujiwara, Y. *Makromol. Chem. Rapid Commun.* 1982, **3**, 739
6 Minoura, N., Aiba, S. and Fujiwara, Y. *J. Appl. Polym. Sci.* 1986, **31**, 1935
7 Minoura, N., Tsukada, M. and Nagura, M. *Biomaterials* in press
8 Minoura, N., Fujiwara, Y. and Nakagawa, T. *J. Appl. Polym. Sci.* 1979, **24**, 965
9 Hermanns, P. H. and Weidinger, A. *J. Appl. Phys.* 1948, **19**, 491
10 Magoshi, J., Magoshi, Y. and Nakamura, S. *J. Polym. Sci., Phys. Edn.* 1981, **19**, 185
11 Shimizu, M. *Bull. Exp. Station* 1941, **10**, 475
12 Hirabayashi, K., Ishikawa, H., Kakudo, M. and Go, Y. *Sen-i Gakkaishi* 1968, **24**, 397
13 Marsh, R. E., Corey, R. B. and Pauling, L. *Biochim. Biophys. Acta* 1955, **15**, 1
14 Barrer, R. M. 'Diffusion in Polymers' (Ed. J. Crank and G. S. Park), Academic Press, London, 1968, Ch. 6, pp. 211–215
15 Michaels, A. S. and Bixler, H. J. *J. Polym. Sci.* 1961, **50**, 413