Improvements of the Physical Properties of Fibroin Membranes with Sodium Alginate

CHUAN XIN LIANG* and KIYOSHI HIRABAYASHI

Department of Biopolymer Engineering, Faculty of Technology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan

SYNOPSIS

The effects of sodium alginate on the physical properties of fibroin membranes, such as rupture elongation, rupture strength, water absorption, and thermal properties, were investigated. The experimental results showed the β-form crystal and β-form molecular conformation increased due to the increase of intermolecular hydrogen bonds, such as C=O---HN and C=O---HO. The kind of intermolecular interaction and β-form molecular conformation of polymer blends were clarified by ^{13}C-NMR and infrared spectroscopy. The increase in β-form crystal was examined by X-ray analysis. Further, membranes were characterized by thermogravimetric analysis (TGA) and by determining their water contents and tensile properties.

INTRODUCTION

The physical properties of fibroin membranes mainly depend on the intermolecular hydrogen bonds, because these bonds will stabilize the β-structure.[1,2] Hydrogen bonds may be formed in intra- and intermolecules of amino acids, peptides, and proteins.[3-5] However, in pure silk fibroin, only a few β-structure and intermolecular hydrogen bonds are present. In general, to increase the β-structure of fibroin, physical and chemical treatments are necessary.[6-9] On the other hand, few of the reported methods are perfect, because the flexibility and elasticity of the fibroin membranes are often lost. The key point for dealing with these problems is to improve the molecular orientation and formation of the β-structure without causing a deterioration in physical properties. In our previous studies, it was confirmed that the physical properties of fibroin membranes had been improved by mixing in some polymers, such as chitosan and sodium polyglutamate.[10,11]

Sodium alginate has been widely used in the food, textile, and medical fields. Its molar mass distribution is from 50,000 to 200,000, and it has excellent hydroscopicity. Moreover, it is possible to introduce intermolecular hydrogen bonding between fibroin and sodium alginate. This article reports the influence of sodium alginate on the physical properties of fibroin membranes.

EXPERIMENTAL

Samples

Raw silk was degummed twice with 0.5% NaHCO$_3$ solution at 100°C for 30 min and then washed with distilled water. Degummed silk was dissolved in a solution prepared by dissolving 1 mol CaCl$_2$ into the mixed solvent of 8 mol H$_2$O and 2 mol C$_2$H$_5$OH at 100°C for 5 min. After dialysis against distilled water for 3 days, the solution was purified by spinning in a centrifuge at 4000 rpm for 15 min. The supernatant was collected and was mixed with sodium alginate after determining the concentration of fibroin solution (the mixing rates are shown in Table I). The insoluble materials of the mixture were removed by centrifuging the mixed solution at 4000 rpm for 20 min. For spectral analysis and physical studies, membranes were cast from 5 wt % solution on an acrylic sheet, and were dried for 6 days at 5°C and in air with 80% relative humidity. In ^{13}C-NMR

Table I The Composition of Membranes

Sample Number	Percent $(S/(S + F))$ (wt%)	Fibroin (g)	Sodium Alginate (g)
1	0	2.0	0.0
2	10	1.82	0.18
3	20	1.66	0.33
4	30	1.52	0.46
5	40	1.42	0.57
6	50	1.34	0.67
7	100	0.0	2.0

(S) Sodium Alginate.
(F) Fibroin.

spectral measurements, deuterium oxide was added to the solution at a content of 20 wt %. The acidity of the solutions were adjusted to pH 7.28 with 1 N HCl.

Measurements

Membranes were allowed to soak in air with 65% relative humidity at 25°C for 60 days to achieve swelling equilibria. The strength and elongation of membranes were taken on a Toyo UTM-II tensile tester at a crosshead speed of 4 mm/min, and with a maximum load of 5 kgf. The water content of membranes was calculated by the use of eq. (1).

$$w = \frac{W_1 - W_0}{W_0} \times 100\% \qquad (1)$$

Where w is water content, W_1 and W_0 are weights (mg) of membranes in soaked and dried states, respectively.

Thermogravimetric analysis (TGA) was carried out in air atmosphere, by a Shimadzu DT-30 thermal analyzer at a heating rate of 10°C/min. The decomposition temperatures and weight losses of membranes were evaluated by considering the intersecting point of the tangents of TGA curves.

X-ray diffraction patterns of membranes were obtained with a Rigaku RAD-C wide angle X-ray diffractometer, operated at 40 kV and 30 mA using Cuα radiation. Infrared spectra of membranes were recorded on a Shimadzu IR-435 infrared spectrophotometer at room temperature. ^{13}C-NMR spectra of regenerated fibroin were obtained on a Nihon Denshi JNM-FX200 NMR spectrometer at 25°C,

using hexamethyl–disiloxane (HMDS) as an internal reference.

RESULTS AND DISCUSSION

Intermolecular Hydrogen Bonds

Silk is a well known natural protein and various models are concerned with its amino acid sequence.[12-14] However, all of these models include the alanine–glycine–serine unit. Naturally, the intra- and intermolecular hydrogen bonds might be formed in fibroin.[5] A possible intermolecular interaction between added sodium alginate and fibroin is hydrogen combination, as shown in Scheme 1. Figure 1 shows the IR spectra of membranes. The bands at 1650 cm^{-1} and 1530 cm^{-1} were assigned to the amide I, amide II of random coil, and those at 1630 cm^{-1} and 1522 cm^{-1} were attributed to the same groups of β-form,[15] respectively. As seen from Figure 1, the absorptions assigned to the random coil became weaker and weaker with the increase of sodium alginate, and disappeared when sodium alginate was
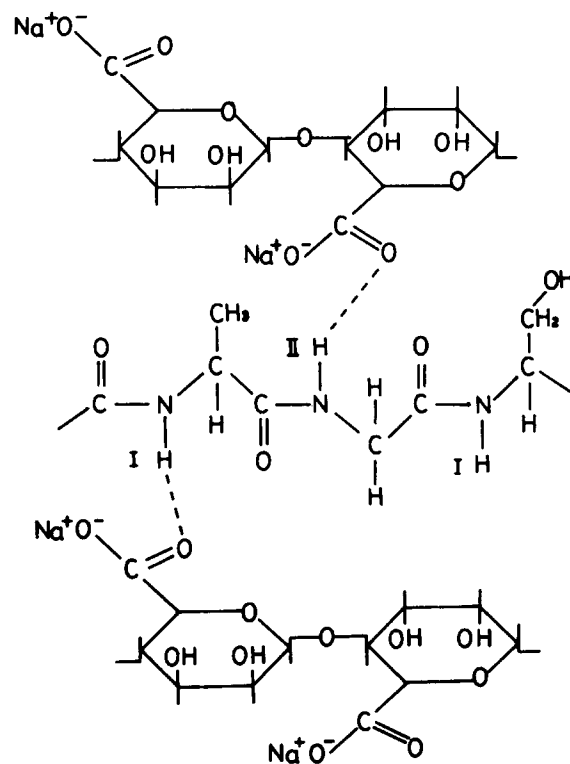
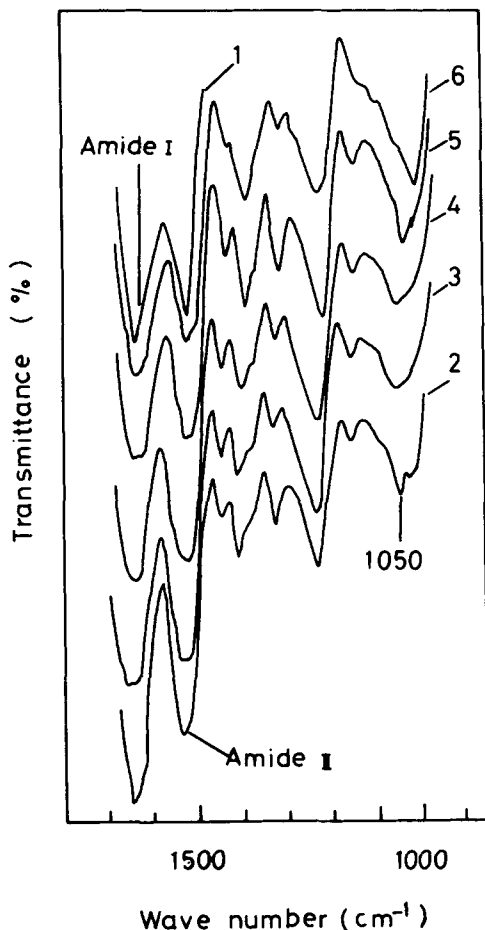**Scheme 1** Hydrogen bonds between fibroin and sodium alginate.

Figure 1 Infrared spectra of membranes. The spectrum number indicates the sample number (Table I).

30 wt %. However, when sodium alginate increased further, absorption bands assigned to random coil reappeared and increased. On the other hand, the β-form absorption bands were strengthened by the increase of sodium alginate, with maximum absorption appearing in Sample 4. Then these bands decreased with further increase of sodium alginate. Furthermore, for the peaks at 1050 cm^{-1} contributed by the CN stretching vibration,[16] those in Samples 3 and 4 were strongest. These results indicated that a part of random coil molecular conformation was converted into β-structure with the addition of sodium alginate. The reason for this is that the intermolecular hydrogen bonds were formed when sodium alginate was mixed with fibroin. This structural transition was most apparently found within 30–40 wt % sodium alginate.

The bands at 1630 cm^{-1} and at 1522 cm^{-1} belong to 80% C═O and 60% NH,[15] respectively. If the C═O band of sodium alginate was not participated

in hydrogen bonds, the random coil domain must be increased, and should show stronger random coiled absorptions than for Sample 1. The above results demonstrated that the random coil domain decreased, as shown by the relative bands. Therefore, it seems that the C═O of sodium alginate should participate in intermolecular hydrogen bonding with glycine NH (I) or alanine NH (II).

On the other hand, ^{13}C–NMR chemical shifts of C═O of both glycine and alanine C_α and C_β for Samples 3–6 are substantially shifted to a higher field than those observed in Samples 1 and 2, as shown in the correlation diagrams (Fig. 2 and Table II).[16] Chemical shifts of serine C═O, C_α, and C_β were also found at a high field for Samples 4–6, as a result of the well-known effect of hydrogen bonds.[17] It is reasonable to think that HO groups also participated in hydrogen bonding. ^{13}C–NMR results are consistent with those of IR spectra.
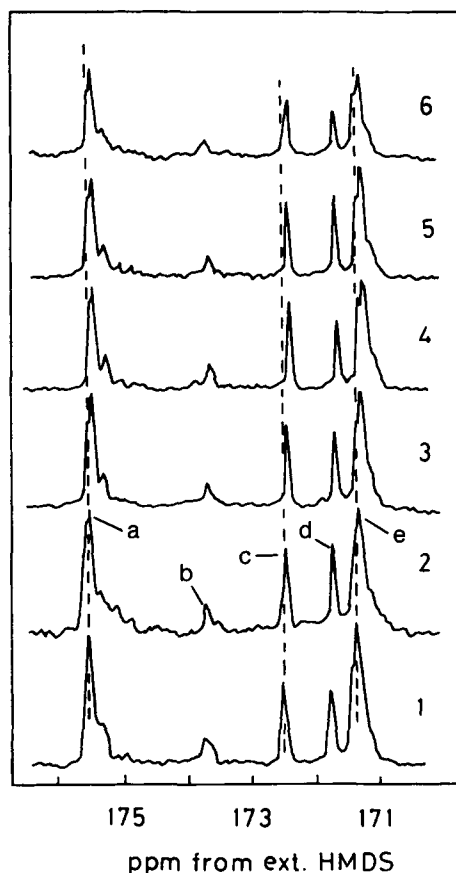

Figure 2 ^{13}C NMR spectra of C═O for three amino acid residues in the aqueous solutions of sodium alginate and fibroin. The number indicates the sample number (Table I). (a) Ala, (b) Tyr, (c) Ser, (d) for the Ala–Gly–Ser sequence, (e) for the Ala–Gly–Ala sequence.

Table II ^{13}C NMR Chemical Shifts of Three Amino Acid Residues in Aqueous Solutions of Sodium Alginate and Fibroin

Amino Acid Residue		Sample Number					
		1	2	3	4	5	6
Gly	C_α	42.66	42.66	42.66	42.66	42.66	42.66
Gly	C=O	171.31[a]	171.31[a]	171.25[a]	171.20[a]	171.25[a]	171.25[a]
Gly	C=O	171.72[b]	171.72[b]	171.66[b]	171.63[b]	171.66[b]	171.66[b]
Ala	C_α	49.99	49.99	49.99	49.93	49.93	49.93
Ala	C_β	16.58	16.58	16.58	16.53	16.53	16.53
Ala	C=O	175.58	175.58	175.58	175.49	175.49	175.49
Ser	C_α	55.86	55.86	55.86	55.80	55.80	55.80
Ser	C_β	61.29	61.29	61.29	61.23	61.23	61.23
Ser	C=O	172.45	172.45	172.45	172.39	172.39	172.39

[a] For the Ala–Gly–Ala sequence.   [b] For the Ala–Gly–Ser sequence.

Crystallinity of the Membranes

The X-ray diffraction patterns of membranes indicate that the crystallinity of these membranes depend on the mixing rate of sodium alginate (Fig. 3). Sample 7 did not show any characteristic crystalline peak, indicating that the sodium alginate is an amorphous material. Sample 1 showed very weak and broad, unoriented crystalline peaks. To the contrary, Samples 2–6 show characteristic β-form crystalline diffraction peaks.[2] These results indicate that the β-form crystal was increased with the increase of sodium alginate. In other words, intermolecular hydrogen bonding between sodium alginate and fibroin was introduced. As a result, the molecular orientation proceeds to higher levels.

However, the β-form crystal decreased when the mixing rate of sodium alginate was more than 40 wt %, because the excess sodium ionic polymer formed amorphous domains itself. According to Fig. 3, the crystallinity of membranes increased in following order:

$$4 > 3 > 2 > 5 > 6 > 7$$

Thermal Stability

Samples 2–6 showed two steps of active weight loss with elevated temperature, as seen in Figure 4. The greatest weight loss was found at an active step in the temperature range of 250–400°C, which is attributed to disintegration of the intermolecular side chains.[18] The secondary weight loss, at 440–550°C, was believed to be caused by some type of chemical reaction, such as main chain disintegration or rearrangement of some carbon atoms. Decomposition

temperature and residual weight (Table III) increased in the sequence of $4 > 3 > 2 > 5 > 6 > 7$. This order is the same as that of their crystallinities,
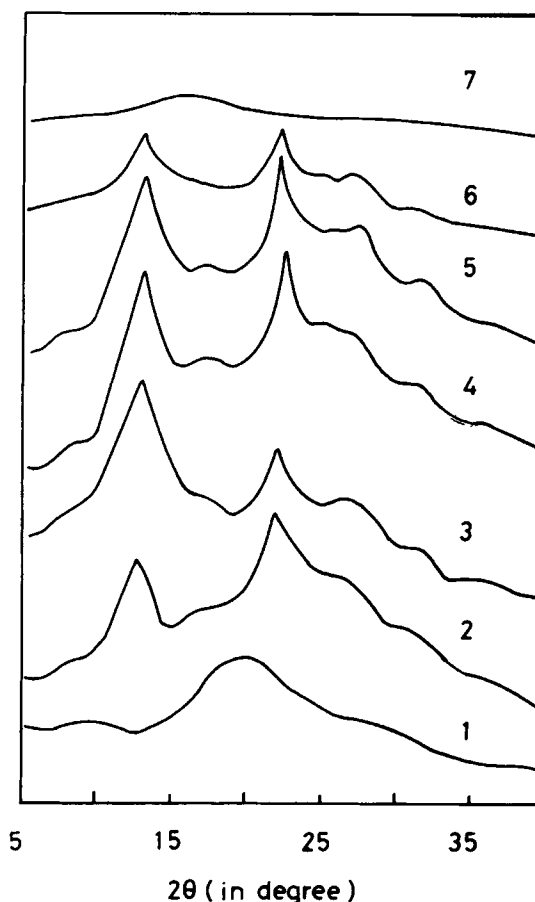

Figure 3 X-ray diffraction patterns of membranes. The number indicates the sample number (Table I).
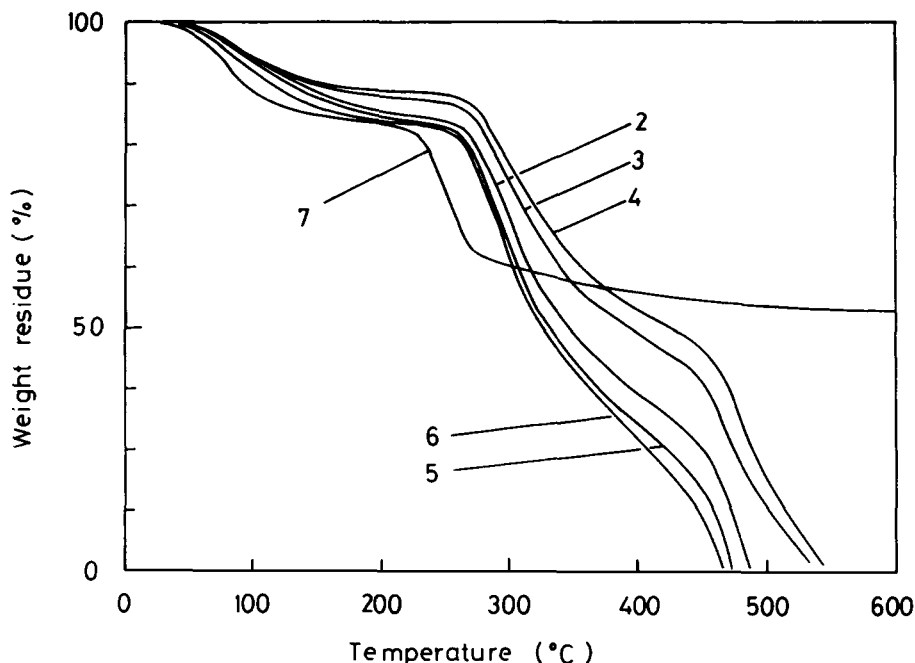
Figure 4   TGA thermograms of membranes. The number indicates the sample number (Table I).

and indicates that the thermal stabilities of membranes were improved by their crystalline domains.

Water Absorption

Figure 5 shows the relationship between water contents of membranes and sodium alginate. At the beginning, the absorbability of membranes was improved greatly by the increase of the sodium alginate content, due to the strong hydroscopicity of the ionic polymer. However, this behavior was not apparent when the content of sodium alginate was in the scale of 10–30 wt %, because the high crystallinity of the membranes partly prevented the combination of water and hydrophilic amino acid residues or sodium ions. Finally, very high sodium alginate content (> 40 wt %) resulted in lowering the crystallinities

of the polymer blends, which supported a more rapid increase in absorption of water (Fig. 5).

Mechanical Properties

The rupture strengths of the membranes were also affected by sodium alginate. The experimental results are plotted in Figure 6. At first, rupture strength increased linearly vs. the content of sodium alginate, to a maximum point at 30 wt %. Then, rupture strength decreased to a constant. This trend agrees well with the order of their crystallinities. β-sheet structure obstructed the slipping of molecular chains, which improved the rupture strengths of the polymer blends. However, the increase of random coil and water content may decrease the rupture strengths of the membranes. It must be pointed out

Table III   Thermal Properties of Membranes

Sample Number	2	3	4	5	6	7
I Temperature (°C)	264	266	268	261	258	228
I Weight Loss (%)	18.9	17.2	11.5	16.4	15.8	17.5
II Temperature (°C)	450	452	462	446	442	272
II Weight Loss (%)	67.5	58.5	54.2	75.4	69.3	38.4

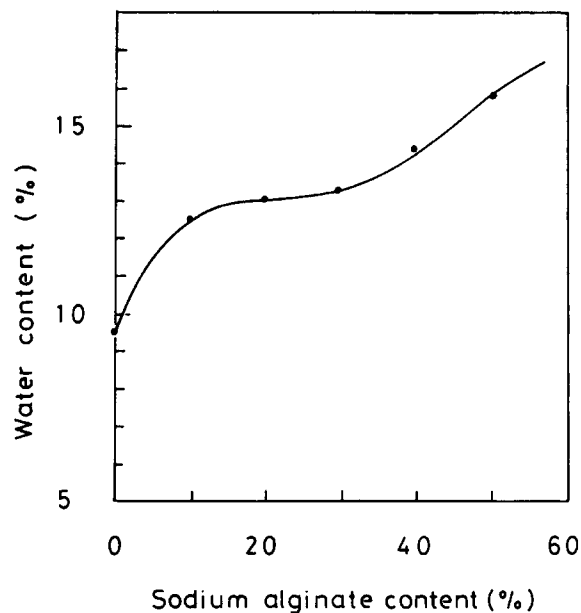(I) Primary decomposition.   (II) Secondary decomposition.

Figure 5   Relation between the water content and sodium alginate content of membranes.

that, when the sodium alginate content was greater than 50 wt %, the rupture strength of the membranes was mainly due to the ionic polymer.

Figure 7 shows the relationship between rupture elongations and sodium alginate contents of membranes. The rupture elongation increased in three steps with the increase of sodium alginate. In the

first step ($<$ 30 wt %), the increasing rate of rupture elongation was large. However, in the following step (40–60 wt %), the rupture elongations were almost unchanged. In the third step ($>$ 60 wt %), they increased sharply again. The reason for this behavior is that the good tensibility of sodium alginate, and the affect of adsorbed and free water, developed ductility in the polymer blends. On the other hand, the increase of crystalline domains suppressed the slipping behavior of polymer molecular chains.

CONCLUSIONS

The water absorbability, mechanical properties, and thermal stability of fibroin membranes were improved by blending with sodium alginate. For example, the water content of the membrane containing 50 wt % sodium alginate was 66% higher than that of pure fibroin. The rupture strength of the membrane, prepared by mixing 30 wt % sodium alginate, was found to be the highest (18.7 kgf/mm^2). The rupture elongation also increased greatly with the increase of sodium alginate content. The crystallinity and β-structure of polymer blends were the most important factors that influenced the physical properties of membranes. On the other hand, the formation of the β-form crystal was controlled by the intermolecular hydrogen bonding between fibroin and sodium alginate chains.
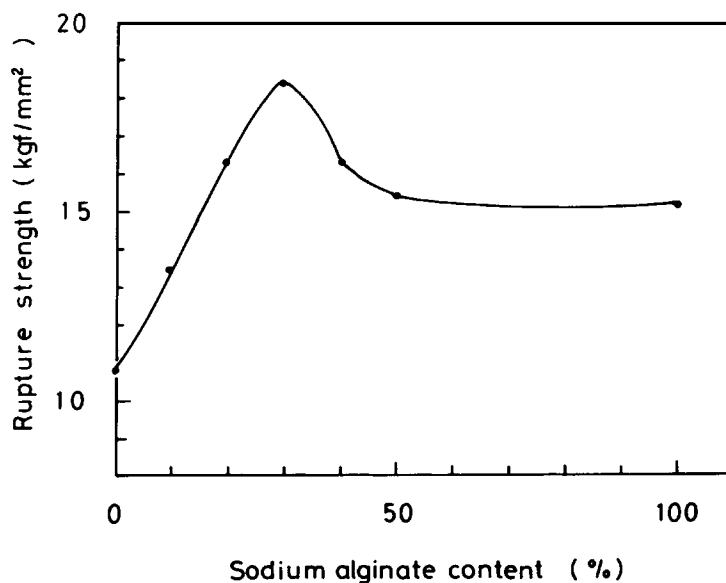

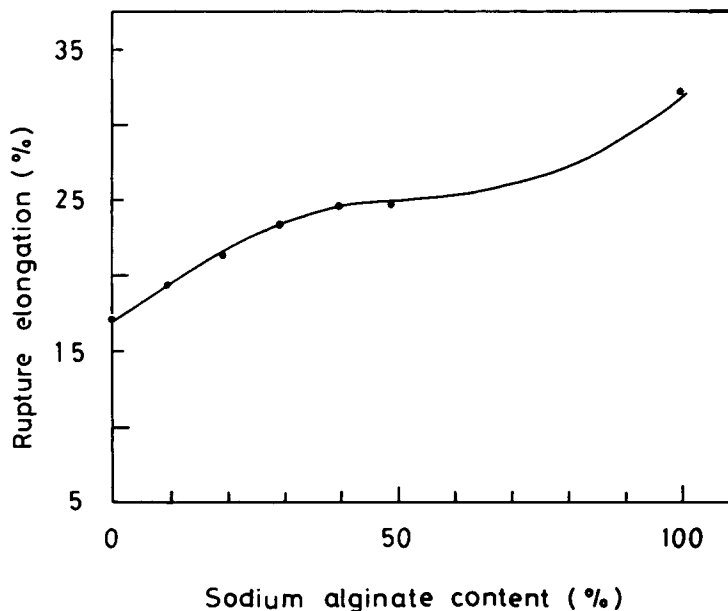**Figure 6**   Relation between the rupture strength and the sodium alginate content of membranes.

Figure 7 Relation between the rupture elongation and the sodium alginate content of membranes.

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