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Food polymer pullulan-κ-carrageenan composite membrane performed smart function both on mass transfer and molecular size recognition

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ABSTRACT

Biopolymer pullulan (P) can be cross-linked easily by glutaraldehyde, and additive κ-carrageenan (κC) completes an attractive combination for increasing tensile strength sufficiently for practical use. In this study, the κ-carrageenan mass fraction (F_C) was defined as $F_C \equiv (\kappa C[g]/(P[g] + \kappa C[g]))$ and was set at 0.33, 0.50, 0.66, and 0.83. Composite membranes were successfully prepared in our experiment F_C ranges by the casting method. The maximum stress and strain, water content, mass-transfer characteristics, and pure water permeability were demonstrated to be a function of the added cross-linker glutaraldehyde. Increasing the mass fraction of κ-carrageenan enhanced the maximum stress and the maximum strain at break, suggesting that pullulan imparts flexibility and the κ-carrageenan imparts stiffness to the composite membranes. The mass-transfer characteristics were analysed based on changes in the effective diffusion coefficient in the composite membranes. Some water-soluble marker components were employed to estimate the size of the mass-transfer channel in the composite membranes. The reference molecular size was from 60 to 826Da, indicating Urea, Methyl Orange, Indigo Carmine, Bordeaux S, and Brilliant Blue. The effective diffusion coefficient was dramatically changed by a factor of 26,000 for F_C 0.66, even though the molecular weight of the reference only changed by a factor of 14. The F_C value significantly controls not only the mechanical strength but also the molecular size recognition of the membrane. A large dependence on the molecular size was achieved by specific polymer frame-works using the excellent combination of pullulan and κ-carrageenan.

Keywords: Pullulan; κ-Carrageenan; Mechanical strength; Effective diffusion coefficient; Composite membrane; Water permeability

1. Introduction

Biomass is a renewable class of materials of growing interest among researchers in the quest to achieve global sustainability. Biopolymers are a representation of the biomass, in particular starch and cellulose. In recent years, studies on edible films have intensified [1–2]. The potential benefits of these films lie in both their bio-compatible characteristics and relatively low cost. Edible films are primarily composed of polysaccharides, proteins, and lipids, alone or in combination. Such films are an effective barrier for preventing unwanted mass transfers in foods (e.g., water vapor and oxygen transmission), thereby improving their quality and extending their shelf life [3–5]. Composing polysaccharides in the form of blends with varying ratios of polymers offers the possibility of creating different films with improved characteristics.

Pullulan (P) is a water-soluble microbial polysaccharide with excellent film-forming properties. Its films are

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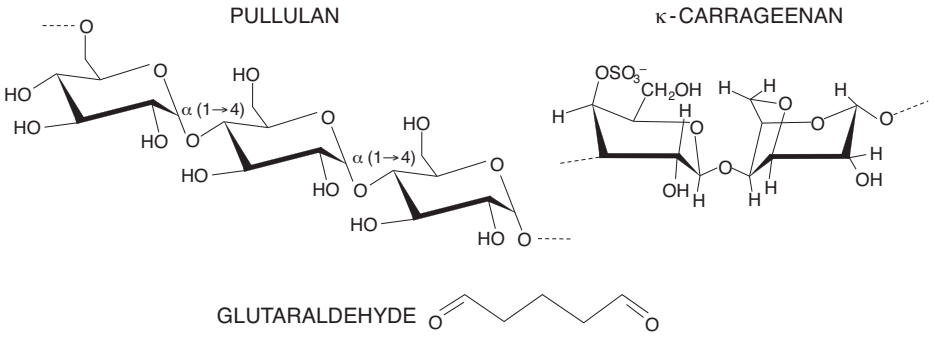


Fig. 1. Molecular structure of pullulan, κ-carrageenan, and glutaraldehyde, as used in this study.

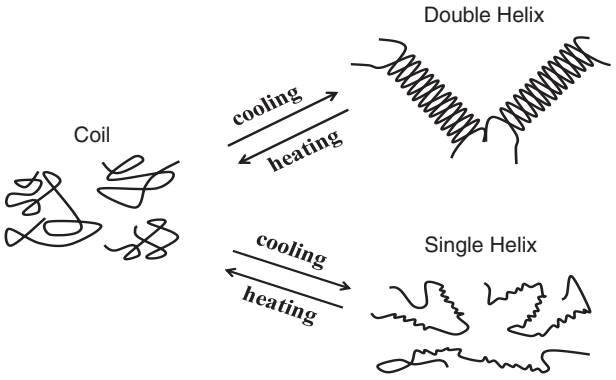


Fig. 2. Two models of the conformational transition of κ-carrageenan.

colorless, tasteless, odorless, transparent, flexible, highly impermeable to oil and oxygen, and heat-sealable. Pullulan is an exocellularhomopolysaccharide produced by the fungus Aureobasidiumpullulans. It is a linear mixture of α-D-glucan consisting mainly of maltotriose repeating units interconnected by α-(1→6) linkages. The regular alternation of α-(1→4) and α-(1→6) bonds results in distinctive structural flexibility and enhanced water-solubility [6–8].

Carrageenan is water-soluble polymers extracted from red algae. They are used in the food and pharmaceutical industries as gelling and stabilizing agents and for microencapsulation and immobilization of drugs and enzymes. Carrageenan consist of alternating copolymers of α-(1→4)-D-galactose and β-(1→4)-3, 6-anhydro-D or L-galactose [9]. Several isomers of carrageenan are known as κ-, λ-, and ι-carrageenan; they differ in the number and positions of ester sulfate groups on the repeating galactose units. κ-Carrageenan (κC) has only one negative charge per disaccharide and tends to form a strong and rigid gel. Carrageenan also undergoes a thermo-reversible ion-induced conformation transition from a disordered (coil) to an ordered (helix), and these transitions are both temperature- and concentration-dependent. At temperatures below its transition temperature, κ-carrageenan assumes a helical configuration between polymer strands, forming a more rigid structure. These transitions from coil to helix have been observed by many researchers. Several investigators favour the coaxial double helix as the fundamental ordered conformation. Upon reducing the temperature or increasing the salt concentration, double-helical stretches will be formed in a thermo-reversible process. Other groups of researchers have presented evidence of the single helix as the fundamental ordered state of carrageenan prior to association and gel formation. The two kinds of molecular structure thought regarding the conformation transition of κ-carrageenan are illustrated in Fig. 2 [10–15]. Whether it is a single or double helix, the structure becomes more rigid in the helical conformation, as seen in the figure.

Water-soluble glutaraldehyde was chosen as the crosslinking agent because of its ability to facilitate the formation of linkages from hydroxyl groups [16–17]. Crosslink of pullulan with glutaraldehyde involves the reaction of the –OH radical of pullulan with the aldehyde groups of the glutaraldehyde. The chemical structures of pullulan, κ-Carrageenan, and glutaraldehide are shown in Fig.1.

This paper describes the preparation and mass-transfer characteristics of a pullulan-κ-carrageenan composite membrane. A composite membrane made from natural polymers was successfully prepared by the casting method. The membrane was clearly recognized to be an attractive combination of sufficiently elevated tensile strength for conventional applications and to possess a molecular-size screening effect.

2. Materials and methods

2.1. Materials

Pullulan (MW 60,000~240,000Da) was provided by Hayashibara Biochemical Laboratory (Okayama, Japan). κ-carrageenan (MW 500,000Da) and glutaraldehyde

(MW 100.12Da) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Urea (MW 60.06Da), Methyl Orange (MW 327.34Da), and Bordeaux S (MW 604.48Da) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Brilliant Blue (MW 826.0Da) was purchased from Sigma Chemical Co. (Perth, WA). Indigo Carmine (MW 466.37Da) was purchased from Kokusan Chemical Works, Ltd. (Tokyo, Japan).

2.2. Preparation of pullulan-κ-carrageenan composite membrane

Pullulan and κ-carrageenan were dissolved in distilled water (70°C) using a magnetic stirrer to prepare film-forming solutions of various blend-weight ratios. All polymer solutions were prepared based on 3 g total polymer weight dissolved in 100 ml of distilled water at 70°C for one hour. In addition, each was stirred for one hour at 70°C with cross-linker glutaraldehyde added. The polymer solutions were then cast onto petri dishes, followed by drying in an electrical blast-drying chest at 65°C for 24 h. The dried membranes (attached to the petri dishes) were immersed in distilled water for one hour. The swollen membrane was spontaneously peeled from the petri dish at 25 ± 1°C for further testing. Membrane samples were tested in triplicate.

In our studies, the glutaraldehyde added pullulan-κ-carrageenan solution was dispensed to the petri dishes and then put a part of ones in desiccators to be dried at room temperature (25 ± 1°C) for 1 week, the others were dried in an electrical blast-drying chest at 45°C, 65°C, 85°C respectively for 24 h. As you can see in Fig. 3.

One sample dried at room temperature (25±1°C) for 1 week and relatively-low temperature (45°C) for 24 h with very low crosslinking tend to be weak and flexible, whereas another sample dried at over high temperature with high degree of crosslinking are too hard and rigid to experiment. Therefore, we chose 65°C for drying our composite membrane, and it performed excellent formation of the composite membrane for our studies. Thereafter, we could assume that crosslinking almost be taking place during drying at high temperature. Incidentally, authors cannot observe the phenomenon of gelation when the glutaraldehyde added during membrane preparation at 70°C.

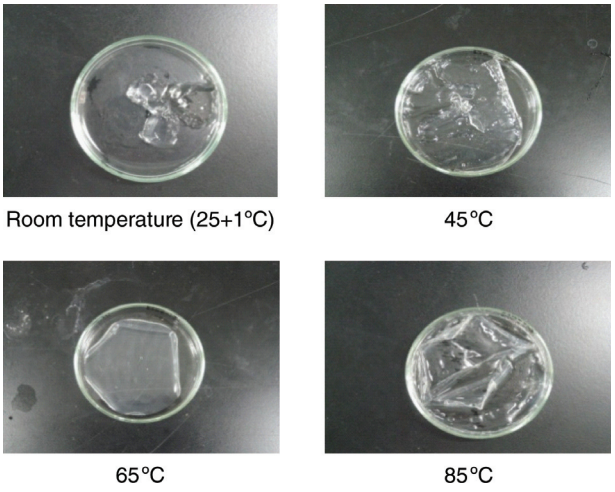


Fig. 3. The crosslinking states of pullulan-κ-carrageenan composite membrane after drying at different temperatures. (GA 70 mM, F_C 0.33).

2.3. Mechanical strength and elastic characteristics

A rheometer (CR-DX500, Sun Scientific Co., Ltd., Tokyo, Japan) was used to determine the tensile strength and the percentage elongation at the break. Three rectangular-strip specimens (10 mm wide, 40 mm long) were cut from each membrane for tensile testing. The initial grip separation was set to 20 mm, and the crosshead speed was set to 1 mm/s. The initial membrane thickness was measured using a micrometer (Mitutoyo, Kanagawa, Japan). The average thickness of the membrane strip was used to estimate the initial cross-sectional area of the membrane sample.

2.4. Measurement of mass-transfer flux

The concentration of the solution transported through the membrane in regular time is required to estimate the mass-transfer characteristics of the membrane. Diffusion is a fundamental phenomenon in several physical and chemical processes, representing the natural movement of neutral or charged species in solution. The diffusion coefficient in liquid is an important parameter in understanding the complex processes of mass transfer. There are several empirical methods for estimating the diffusion coefficient in aqueous phase that consider infinite dilution and are based on molecular-size indicators. This paper introduces one of these methods as follows:

$$\text{Wilke \& chang, } D_w = \frac{1.86 \times 10^{-18} (\varphi_B M_w)^{0.5}}{\mu_w v_{A^{0.6}}} T \qquad (1)$$

where D_w is the diffusion coefficient of the solute in water[$m^2 \cdot s^{-1}$], μ_w is the viscosity of water [Pa s], and [K] is the association factor for solvent B at the required temperature T (for water, φ_B=2.6). M_W is the molar mass of water [$g \cdot mol^{-1}$], and v_A is the molar volume of solute A at the normal boiling point [$m^3 \cdot mol^{-1}$].

The mass-transfer setup in our experiment is illustrated in Fig. 4. The membrane was sandwiched between two equal-volume compartments. The compartments

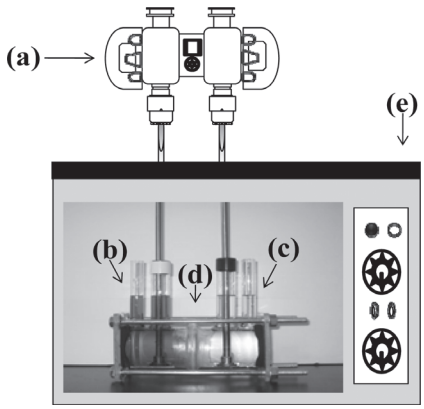


Fig. 4. Schematic diagram of the mass-transfer setup in our experiment. (a) Agitating motor, (b) Mass-transfer cell (feed side), (c) Mass-transfer cell (stripping side), (e) Membrane, (d) Constant temperature water tank (303 K).

had a volume of 200 ml and an effective membrane area of 0.24 m^2. The feed compartment was filled with water-soluble marker components (Urea, Methyl Orange, Indigo Carmine, Bordeaux S, Brilliant Blue) in solution (190 ml), and the stripping compartment was filled with distilled water (190 ml). During the experiment, the two compartments of solutions were stirred at a constant speed (850 min^{-1}) in order to minimize the mass-transfer resistance of the soluble boundary layer above the membrane. Samples were taken from the two compartments at regular time intervals of 30 to 60 min. The sample concentration of the samples was analysed with a spectrophotometer (UV Mini 1240, Shimadzu). It is supposed that since the concentrations in the two compartments were uniform, the mass-transfer flux was so small that the diffusion process can be regarded as a quasi-steady state. Accordingly, we can use Eqs. (2) and (3) to calculate the effective diffusion coefficients.

$$\ln\left(1-\frac{2C_s}{C_{fi}}\right) = -2\frac{A}{V}K_{OL}t \tag{2}$$

$$K_{OL}^{-1} = k_{L1}^{-1} + k_m^{-1} + k_{L2}^{-1} \tag{3}$$

The film mass-transfer resistances $k_{L1}{}^{-1}$ and $k_{L2}{}^{-1}$ in the overall mass transfer resistance $K_{OL}{}^{-1}$ can be neglected because of the sufficiently turbulent conditions in the two compartments during the experiment. $K_{OL}{}^{-1}$ did not depend on the stirring rate, therefore it directly indicates the membrane mass-transfer coefficient ($k_m = Deff \cdot l^{-1}$). The effective diffusion coefficient in the membrane (*Deff*) was evaluated from k_m. The initial membrane thickness l in the swollen state was measured with a micrometer.

2.5. Water content

Swelling of the cross-linked membranes was measured using distilled water as the swelling medium in order to determine the degree of cross-linking achieved with the glutaraldehyde concentration. In general, a lower degree of swelling has been attributed to a higher degree of cross-linking.

Membranes were immersed in distilled water for 24 h at 25±1°C. The swollen membranes were then placed between two dry filter papers to remove residual water from the membrane surface and weighed. After being dried at 65°C for 24 h, the dried membrane was re-weighed. The water content of the membrane was calculated using Eq. (4):

$$\text{Water Conter}\,(W_t\%) = \frac{w_s - w_d}{w_s} \tag{4}$$

Where w_s is the mass of the swollen membrane, and w_d is that of the dried membrane.

2.6. Pure-water permeability

The pure-water permeability of the membranes was measured under steady-state conditions. Prior to the experiments, the membrane was immersed in pure water for 12 h and was then installed into the pure-water permeability set-up.

The pure-water permeability experiment used an ultra-filtration cell with a volume of 200 ml and an effective filtration area of 0.21 m^2. A pressure regulator valve was installed between the filtration cell and the pump in order to monitor the variation in applied pressure during filtration. A magnetic stirring bar was installed at the upper surface of the membranes but not attached to the membrane. Operational pressure was adjusted by a nitrogen gas. A petri dish was set in electronic balance to collect the permeated water from the ultra filtration cell. The mass of permeated water with aging variation was accurately measured based on the indication of the electronic balance.

A schematic representation of the module and setup is presented in Fig. 5. The pure-water permeability experiment was conducted at different pressures using the following equation:

$$J_V = \frac{V_p}{A\Delta t} \tag{5}$$

where J_V is the water flux [$m^3_{water} \cdot m^{-2}_{memb.} \cdot s^{-1}$], V_p is the volumetric amount of permeated water [m^3], A is the membrane area [m^2], l is the membrane thickness [m], and is the sampling time [s]. J_V is the water-permeated flux per unit membrane thickness, defined as ($J_V\ l^{-1}$) [$m^3_{water} \cdot m^{-2}_{memb.} \cdot m^{-1}_{thickness} \cdot s^{-1}$].

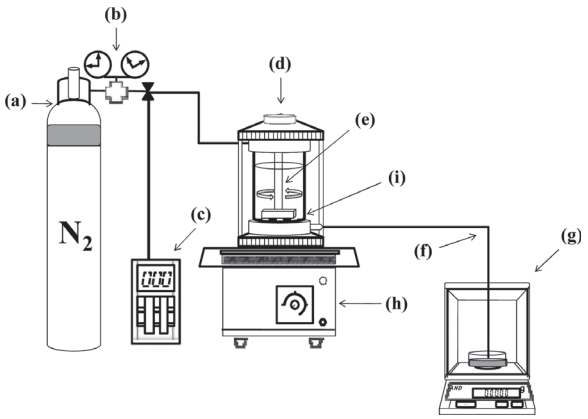


Fig. 5. Schematic diagram of filtration cell used to measure steady pure-water permeability through the membranes. (a) N_2 gas, (b) Valve, (c) Transducer, (d) Filtration cell, (e) Magnetic stirring bar, (f) Pipe, (g) Electronic balance, (h) Magnetic stirrer, (i) Membrane.

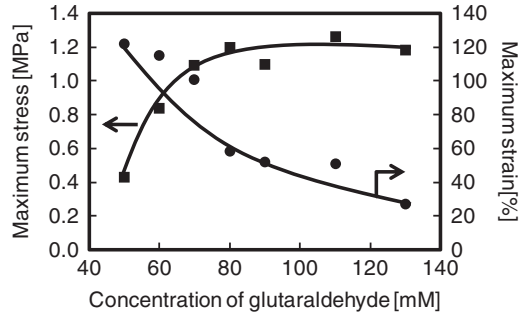


Fig. 6. Effect of additive glutaraldehyde concentration on the maximum stress and strain of prepared pullulan-κ-carrageenan composite membrane. F_C was set at 0.33.

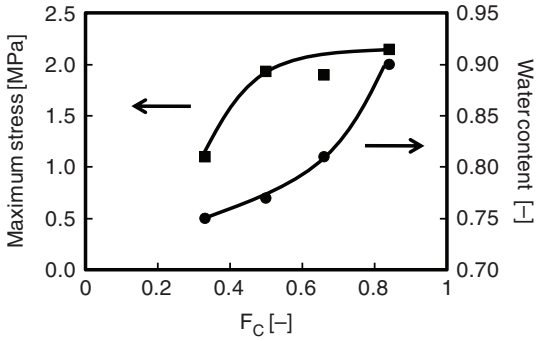


Fig. 7. Effect of the fraction of κ-carrageenan (F_C) on the maximum stress and water content on a pullulan-κ-carrageenan membrane (GA 90 mM).

3. Results and discussion

3.1. Mechanical strength and elastic characteristics

It can be seen in Fig. 6 that the mechanical characteristics of the composite membranes in our experiment exhibited increasing strength (max. stress) and decreasing elongation (max. strain) as the glutaraldehyde level increased. The polymeric framework of the pullulan-κ-carrageenan composite membrane became more densely populated with increasing glutaraldehyde concentration, and its mechanical strength was elevated. The mechanical stress increased with increasing glutaraldehyde concentration and then levelled off over 70 mM. In contrast, the mechanical strain gradually decayed with glutaraldehyde concentration.

Fig. 7 demonstrates that the increase in the mass fraction of κ-carrageenan increased the maximum stress and water content under the same cross-linking conditions. The mass fraction (F_C) was defined as $F_C \equiv (\kappa C[g]/(P[g] + \kappa C[g])$, depending on the addition of κ-carrageenan, the membrane strength continued to increase regardless of the water content. Moreover, the frame network of the composite membrane depends on both the concentration of the cross-linker and the mass fraction of the co-polymer. It is likely that swelling of the composite membrane increases the permeability.

3.2. Measurement of mass-transfer flux

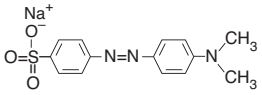
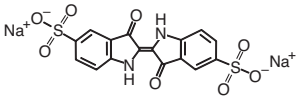
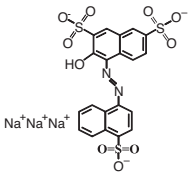
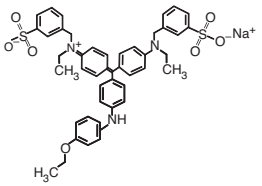
The mass-transfer characteristics were evaluated from the effective diffusion coefficient estimated by measuring the mass-transfer rate in the composite membrane. Water-soluble marker components were employed to determine the size of the transfer channel in the membrane. The reference molecular size was from 60 to 826Da indicating Urea, Methyl Orange, Indigo Carmine, Bordeaux S, and Brilliant Blue (Table 1).

The diffusion coefficient (D_W) in bulk aqueous phase was estimated by Wilke & Chang's correlation (Eq. (1)). The effective diffusion coefficient in the membrane (*Deff*) was lower than D_W, due to diffusion channels in the composite membranes (Fig. 8). The effective diffusion coefficient in the membrane *Deff* dramatically changed, ranging within molecular weight by 26000-fold in a case where molecular weight only changed by 14-fold.

The effective diffusion coefficients of the components of lower molecular weight strongly depended on the κ-carrageenan fraction F_C. As it appears, *Deff* evidently decayed under lower F_C conditions. The large dependence of *Deff* on the F_C value suggests that the polymer frame-works become denser under lower F_C conditions. In the case of Bordeaux S, the change of effective diffusion coefficient with F_C was different from other tested chemicals'. The detail evaluation on mass transfer of Bordeaux S was necessary in future.

In addition, the steep change of the effective diffusion coefficient between Indigo Carmine and Bordeaux S in the each types of composite membrane was appeared. Authors therefore assumed that the size of the mass

Table 1
Water-soluble model components

Model Components		MW [Da]	MS [Å]
A: Urea		60	6.0
B: Methyle Orange		327	10.6
C: Indigo Carmine		466	11.9
D: Bordeaux S		604	13.0
E: Brilliant Blue		826	14.4

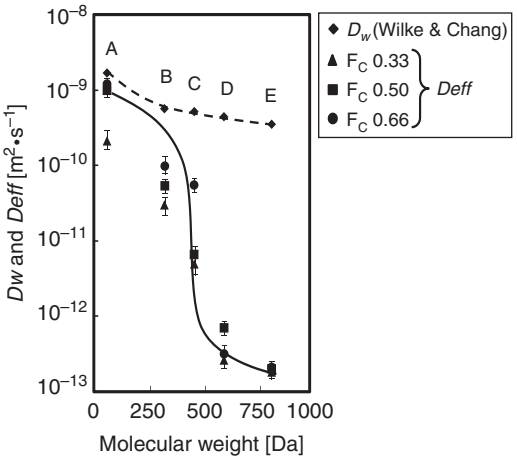


Fig. 8. Effect of molecular weight on the effective diffusion coefficient of a pullulan-κ-carrageenan composite membrane. A: Urea (60Da), B: Methyl Orange (327Da), C: Indigo Carmine (466Da), D: Bordeaux S (604Da), E: Brilliant Blue (826Da).

transfer channel was almost equivalent to molecular size (approx.12) of Indigo Carmine, and it was suggested that mass transfer channel size was monodispersity.

3.3. Pure water permeability

The pure-water flux as a function of applied pressure was measured to investigate the stability and hydraulic properties of biopolymer composite membranes. In

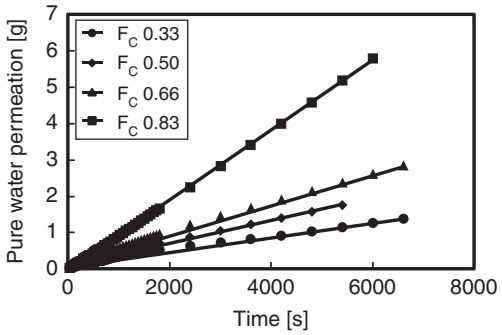


Fig. 9. Permeability of pure-water on pullulan-κ-carrageenan composite membrane (GA 70 mM) over time. (298 K, 100 kPa).

Fig. 9, time course experiments were performed on four tested membranes (F_C 0.33, F_C 0.50, F_C 0.66 and F_C 0.83) at a constant concentration of the cross-linker glutaraldehyde (70 mM). The water flux of the composite membrane was calculated from the experimental curve. The volumetric water flux was recalculated using the density of the permeated water.

Fig. 10 depicts the relationship between the volumetric water flux and the operational acting on the membrane. The water flux increased almost linearly with increasing operational pressure. The permeability of the membranes increases in the following sequence: F_C 0.83> F_C 0.66> F_C 0.50> F_C 0.33. Fig. 10 also illustrates that the water flux of the composite membrane was in primary proportion to the operational pressure, and it was suggested that the water flux of the composite membrane was obeyed by Hagen-Poiseullie flow.

It indicates that a higher mass fraction of κ-carrageenan in the polymer concentration would lead to high water permeability. Based on the pure-water permeability results, we note that the higher mass fraction of κ-carrageenan exhibits significant water flux compared to

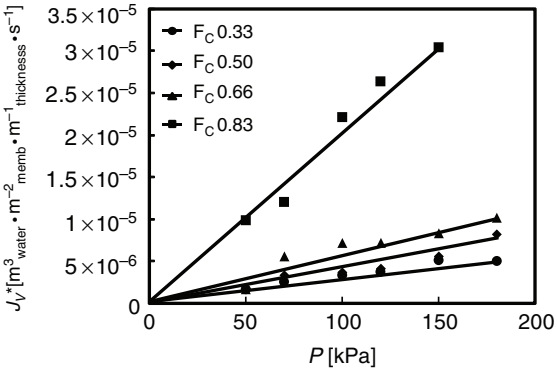


Fig. 10. Pure-water permeability of pullulan-κ-carrageenan composite membrane (GA 70 mM) prepared from different mass fractions of κ-carrageenan (F_C) by applying different pressures.

the higher mass fraction of pullulan. The amount of the κ-carrageenan in the composite membrane performed noticeably high the water flux on the same pressure.

4. Conclusions

Food polymer pullulan-κ-carrageenan composite membrane was successfully prepared by the casting method. It has sufficient mechanical strength enough for a practical use and excellent mass transfer character especially on molecular size screening. The relationship between mass transfer character and the mass fraction of κ-carrageenan in the composite membrane was formulated based on the experiments of mass-transfer flux and pure water flux. The results provided a novel and simple method of preparation membrane and the size of mass-transfer channel based on molecular-size indicators, and suggested that different F_C values significantly affect the mass-transfer permeability. The water permeation flux as a function of applied pressure provided valuable technical information for investigating the stability and hydraulic properties of the composite membranes. It was concluded that pullulan-κ-carrageenan composite membrane possessing a cross-linked hydrophilic structure performed high selectivity and high water flux. Thus, the mechanism of mass-transfer investigations is very useful and informative for the study and analysis of composite membrane.

Acknowledgement

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Symbols

A	—	Area of membrane [m^2]
C_{fi}	—	Initial concentration of feed solution [M]
C_s	—	Concentration of stripping solution [M]
D_w	—	Diffusion coefficient in bulk aqueous phase [$m^2 \cdot s^{-1}$]
$Deff$	—	Effective diffusion coefficient [$m^2 \cdot s^{-1}$]
J_V	—	Water-permeated flux [$m^3_{water} \cdot m^{-2}_{memb.} \cdot s^{-1}$]
J_V*	—	Defined as ($J_V^* = J_V \cdot l^{-1}$) [$m^3_{water} \cdot m^{-2}_{memb.} \cdot m^{-1}_{thickness} \cdot s^{-1}$]
K_{OL}	—	Overall mass-transfer coefficient [$m \cdot s^{-1}$]
K_{OL}^{-1}	—	Overall mass transfer resistance [$(m \cdot s^{-1})^{-1}$]
k_m	—	Membrane mass-transfer coefficient [$m \cdot s^{-1}$]
k_{L1}	—	Film mass-transfer coefficient in feed phase [$m \cdot s^{-1}$]
k_{L2}	—	Film mass-transfer coefficient in stripping phase [$m \cdot s^{-1}$]
l	—	Membrane thickness [m]
ΔP	—	Operational pressure [kPa]
Δt	—	Time [s]
T	—	Temperature [K], defined by Eq. (1)
V	—	Volume of aqueous solution in the transfer cell presented in Fig. 4 [m^3]
V_p	—	Permeated water through the membrane [m^3]
W_t	—	Total water content ratio [%], defined by Eq. (4)
w_s	—	Initial mass of the swollen membrane [kg]
w_d	—	Mass of dried membrane [kg]

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