ELSEVIER

Biochemical Engineering Journal 36 (2007) 43–48

Biochemical Engineering Journal

www.elsevier.com/locate/bej

Water permeability of chitosan membrane involved in deacetylation degree control

Tomoki Takahashi [1], Masanao Imai *, Isao Suzuki

Course in Bioresourse Utilization Sciences, Graduate School of Bioresourse Sciences, Nihon University,
1866 Kameino, Fujisawa, Kanagawa-pref. 252-8510, Japan

Received 13 September 2005; received in revised form 31 May 2006; accepted 12 June 2006

Abstract

Chitosan membrane was prepared by the casting method in combination with *N*-acetylation reaction. Deacetylation degree decreased linearly with increasing added amounts of acetic anhydride. The stoichiometric control of the deacetylation degree to the desired level was successfully performed. The maximum stress and strain were measured and were linearly depended on the deacetylation degree. The chitosan membrane produced by the casting method had an asymmetric cross-section in the thickness direction based on scanning electron microscope. The solubility of the chitosan membrane changed with pH. Ranging over pH 6.6, the chitosan membrane was still stable and had elasticity. The volumetric permeated water flux through the chitosan membrane increased linearly with operational pressure. It was exponentially dependent on the deacetylation degree and was satisfactory at a constant level after repeated use. The deacetylation degree was anticipated as a parameter for production of chitosan membrane for practical applications.

Keywords: Chitosan; Membrane; Deacetylation degree; Water permeability

1. Introduction

Chitin and chitosan are available bioresources, particularly in crab shells or crustacean skin. Both chitin and chitosan have been applied in various fields, including wastewater treatment [1–4], artificial dialysis membranes [5,6], biodegradable plastics [7–9], and diet control [10,11]. In particular, chitosan is known as a membrane material that is capable of antifungal and antibacterial activity [12–14].

Chitin is a straight-chain polysaccharide composed of β-1,4-linked *N*-acetyl-D-glucosamine and is made from crab shells or crustacean skin. It is insoluble in both natural and acid aqueous phases. Chitosan is refined by removing an acetyl group from the chitin and dissolving in a diluted acid phase. *N*-acetyl chitosan (chitin) was produced by reacting with acetic anhydride, and it became gelled due to decreasing the cation charge of the molecular chain (Fig. 1).

This study demonstrates the manufacturing of chitosan membrane by a casting method in combination with *N*-acetylation reaction. This method is anticipated to be a useful means of preparing a desired level of deacetylation degree. The effect of deacetylation degree of chitosan on the physical properties and water permeability were investigated for practical use.

2. Materials and methods

2.1. Materials

A low molecular weight of chitosan powder produced from crab shell was purchased from Aldrich (Milwaukee, WI). The mean molecular weight was determined as 3.89×10^5 in our previous paper [1]. The chitosan was dissolved in an aqueous acetic acid solution, commonly used as a solubilization component for chitosan. The acetic acid, which had an assay of up to 99%, was purchased from Wako Pure Chemical Industries Co. Ltd. (Osaka). The polyethylene glycol (PEG, average

---

Abbreviations: DD, deacetylation degree; M_w, molecular weight; M-x, the membrane code in this paper as “M-x” refers to the solution prepared by adding acetic anhydride x μl into the chitosan solution; PVS-K, potassium polyvinyl sulfate; PEG, polyethylene glycol; SEM, scanning electron microscope

* Corresponding author. Tel.: +81 466 84 3978; fax: +81 466 84 3978.
E-mail address: XLT05104@nifty.ne.jp (M. Imai).

[1] Present address: Department of Industrial Chemistry, Faculty of Engineering Division I, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan.

1369-703X/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bej.2006.06.014

Nomenclature

A	additive volume of acetic anhydride (μl/50 g-chitosan solution (2 wt.%))
DD	deacetylation degree (%)
$\mathrm{d}w/\mathrm{d}t$	water mass flux per unit area and thickness ($\mathrm{kg\,m^{-2}\,m^{-1}\,s^{-1}}$)
J_V	volumetric water flux per unit area and thickness ($\mathrm{m^3\,m^{-2}\,m^{-1}\,s^{-1}}$)
P	operational pressure (MPa)
$\mathrm{p}K_\mathrm{a}$	dissociation constant
t	time (s)
w	mass of permeated water (kg)

Greek letters

λ_max	maximum strain
ν_C	kinematic viscosity of chitosan solution ($\mathrm{m^2\,s^{-1}}$)
ν_0	kinematic viscosity of solvent absent of chitosan ($\mathrm{m^2\,s^{-1}}$)
σ_max	maximum stress ($\mathrm{N\,m^{-2}}$)

$M_\mathrm{w} = 7500$), methanol (99.8%), and acetic anhydride (97.0%) used for preparing the chitosan membrane were also purchased from Wako.

2.2. Preparation of chitosan membrane

The desired amount of chitosan powder was added into the PEG 10% acetic acid solution. The mixture was then diluted with methanol, where chitosan concentration was prepared as 2 wt.%. The insoluble matter in the mixing solution was separated by suction filtration; the basic casting solution was then prepared.

Acetic anhydride was conveniently used in preparation from high DD to low DD for desired DD level. Chemical conversion from chitosan to chitin was reasonable way to make a desired level DD. Because of this way was allowed chemical stability. Acetic anhydride ranging from 0 to 400 μl was added to the basic casting solution (50 g) to prepare the desired deacetylation degree of chitosan. The membrane code presented in this paper as “M-x” represents the solution prepared by adding acetic anhydride *x* μl to the chitosan solution (50 g) containing 1 g chitosan; *x* ranged from 0 to 400.

The casting solution was dried in a Petri dish for 12 h at 333 K and then cooled to room temperature (298 K). The residual material was gelled by immersion into a 4% NaOH solution and then formed a chitosan membrane. The membrane was washed with distilled water, and then washed gently in hot water (363 K) to remove PEG.

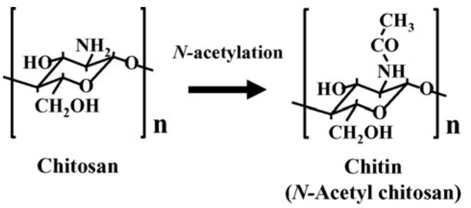

Fig. 1. Chemical structure with *N*-acetylation of chitosan.

2.3. Determination of deacetylation degree

As pretreatment for titration, the sample membrane was immersed into the aqueous ethanolic solutions (20, 40, 60, 80, 100 vol.%) every 15 mim. During the stepwise-serial procedure, the water was gradually removed from the membrane and replaced by ethanol. After this treatment, the sample membrane was then immersed into acetone for 30 min. The membrane was dried at room temperature (298 K). The water in the membrane was completely removed by this series pretreatment. Finally, the membrane was powdered by a powdering machine (Millser IFM-77G, Iwatani, Tokyo). The mean size of powder was prepared in smaller than 200 μm. The powdered membrane was then used as a titration sample.

Determination of deacetylation degree was according to previous papers [1,15,16]. Authors determined the deacetylation degree of chitosan using a potassium polyvinyl sulfate solution (PVS-K) prepared especially for colloidal titration, and we used toluidine blue as the indicator.

2.4. Determination of physical properties

The relationship between the maximum stress and strain with the deacetylation degree was examined as a elastic property of chitosan membrane. It was determined from the critical breaking point of the stretching test pieces. The maximum stress σ_max ($\mathrm{N\,m^{-2}}$) was evaluated as the ratio of the stretching force divided by the cross-sectional area of broken membrane piece. The maximum strain λ_max was measured as the elongation ratio of the initial length of the test piece. These properties were measured using a dumbbell type test piece attached to a Rheometer (SUN SCIENTIFIC Co. Ltd., Tokyo).

2.5. Effect of pH on viscosity as an indicator of the water-solubility of chitosan membrane

The viscosity of the chitosan solution was used as an indicator of the water-solubility of chitosan in the acidic aqueous phase. Dried chitosan membrane (0.2 g) of medium molecular weight was added to 100 ml of the different pH aqueous phases, and the phases were allowed to stand for three days in a cooler box (277 K) to ensure that the chitosan dissolved completely. We used either a phosphate buffer or an acetate buffer to prepare the aqueous phases with pH levels of 4.0–9.0. Insoluble matter was separated by suction filtration. The kinematic viscosity of each solution was measured at 298 K using an Ubbelohde viscometer. The specific viscosity (ν_C/ν_0) was determined as the ratio of kinematic viscosity of the solution (ν_C) to the buffer aqueous phase absent of chitosan (ν_0).

2.6. Morphology of chitosan membrane

The cross-sectional morphology of the chitosan membrane was observed using a scanning electron microscope (SEM, JSM-