Regenerated Cellulose Capsules for Controlled Drug Delivery, Part 2: Modulating Membrane Permeability by Incorporation of Depolymerized Cellulose and Altering Membrane Thickness

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ABSTRACT: For application of regenerated cellulose (RC) membranes in capsule dosage forms, the methods to modify drug release from these membranes are described. Membranes were fabricated by blending native and depolymerized celluloses dissolved in dimethyl sulfoxide and paraformaldehyde solvent system, prior to casting on molds, precipitation in water, and thermal annealing. The effect of laminating layers of RC to fabricate membranes with increasing thickness was also investigated. Solute diffusion studies using ionic and hydrophobic solutes, as well as large protein molecules, were conducted in side-by-side diffusion cells. Microscopic as well as physiological evaluation of these membranes indicated that pore size, porosity, and water uptake decreased as the fraction of depolymerized cellulose increased in the membranes. Permeability analysis of small ionic and hydrophobic solutes indicated that the solute transport across the hydrated membrane occurs through diffusion in the water-filled pores that are formed *in situ*. The apparent path for solute diffusion increases as the fraction of depolymerized cellulose increases. Permeability analysis of large protein molecules indicated that the pore sizes and distribution in these membranes is heterogeneous. Increasing the membrane thickness by lamination of RC does not influence porosity but causes formation of dead-end pores because of blocking by subsequent laminate layers.

Keywords: cellulose capsule; regenerated cellulose; oral drug delivery; depolymerized cellulose; polymeric drug delivery system; passive diffusion; controlled release; permeability; transport

INTRODUCTION

Chemically modified celluloses are routinely utilized in oral controlled release systems. Cellulose ethers, such as ethyl cellulose, methyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, and salts of carboxymethyl cellulose, are well known matrix formers.[1,2] Cellulose esters, such as cellulose acetate, cellulose acetate butyrate, cellulose acetate phthalate, and so on, are predominantly utilized in the formulation of drug release barriers on solid dosage forms.[3,4] However, unmodified cellulose, the parent polymer from which the aforementioned polymers are manufactured, has not found its way into controlled release systems owing to the difficulty associated with processing it in conventional solvents and melt techniques.[5]

For any polymeric coating's applicability in controlled release system, the ability to modify drug permeability through it is critical for providing targeted bioavailability in the gastrointestinal tract. For porous coating systems, the utility of pore-forming agents[6] and plasticizers[7] is common approaches utilized for controlling drug permeability through barrier coatings. Porous membranes are typically formed by incorporating excipients in the barriers that are leeched out *in situ*,[6] utilizing a nonsolvent in membrane coatings that promotes pore formation during phase separation,[8] and employing the drug molecule in the polymeric coating itself.[9] Cellulose derivatives utilized in pharmaceutical coatings routinely utilize pore formers because of their hydrophobic nature, in order to facilitate diffusion of water from the external medium to the encapsulated core and release of solute solution.[10]

In our laboratory, we demonstrated a method of fabricating two-piece hard shell capsules using unmodified cellulose from cotton linters as starting source.[11] In the previous article, we described the membrane formation process and characteristics of RC membranes prepared by dip coating methylolcellulose (MC) solution on cylindrical test tubes, precipitating cellulose on them in excess nonsolvent bath, and then thermally treating the precipitated membrane to remove the nonsolvent. Regenerated cellulose (RC) membranes obtained were monolithic when thermally annealed and porous when exposed to aqueous environment. This unique self-pore-forming capability of RC membranes was determined to be a critical aspect to solute permeability. It was postulated that the self-pore forming capability was associated with the formation weakly hydrogen-bonded domains in the annealed membranes that swell and form water swollen voids when they are exposed to aqueous environment.[12]

Regenerated cellulose membranes are often utilized in dialysis and filtration applications because of their inert nature and the incapability to interact with solute molecules traversing through them.[13] Therefore, controlling porosity, pore size distribution, and hydration is a critical attribute that researchers focus on to improve solute rejection rates of these membranes as well as improving solvent flow through them.[14] Chemical modification of cellulose's hydroxyl groups,[15] radiation treatments,[16] incorporation of hydrophobic additives,[17] applying membrane

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substrates,[18,19] or grafts,[20] and so on are some of the alternative methods recently developed to modulate permeability characteristics of RC membrane. Although effective, these techniques involve utilization of chemicals and equipment that would require toxicological assessment and regulatory clearances prior to utilization in conventional pharmaceutical unit operations.

On the basis of the understanding of membrane formation process observed in our prior study, we hypothesized that the solute permeability characteristics of RC membranes fabricated from the dimethyl sulfoxide-paraformaldehyde (DMSO-PF) solvent system could be altered by modulating the degree of polymerization of the cellulose starting source. The objective of this study was to hence evaluate the use of untreated and depolymerized cellulose starting material on the permeability characteristics of the resulting RC membranes. The modulation capability of this approach was investigated by blending solutions of native and depolymerized cellulose, prior to precipitation. As the utility of these membranes is geared toward pharmaceutical applications, the solute diffusivity through RC membranes was investigated in terms of solute hydrophobicity and size in order to map the internal configuration of pores and pore sizes in these membranes. Furthermore, the lamination approach to increase membrane thickness in fabrication of hard-shell capsule dosage form was also investigated for its impact on solute diffusion through them. The simplistic approach in this study would not only be useful in designing cellulose barrier coatings for targeting drug delivery in pharmaceutical applications, but also for modulation of membrane permeability in dialysis and filtration systems employing RC.

MATERIAL AND METHODS

Materials

Cotton linter sheets obtained from Southern Cellulose Products Inc. (Grade R270; Chattanooga, Tennessee), paraformaldehyde, methanol (99.9%), 1-propanol (99.9%), 1-butanol (99.9%), acetone (99.7%), potassium chloride, and hydrochloric acid (12.1 N) was obtained from Fisher Chemicals (Fair Lawn, New Jersey). DMSO was purchased from Sigma–Aldrich (St. Louis, Missouri), methyl *p*-aminobenzoate from Aldrich Chemical Company Inc. (Milwaukee, Wisconsin), ethyl *p*-aminobenzoate from ICN Biomedicals Inc. (Aurora, Ohio), propyl-*p*-aminobenzoate from Pfaltz and Bauer Inc. (Waterbury, Connecticut), and butyl-*p*-aminobenzoate was obtained from Sigma Life Sciences (St. Louis, Missouri). Ethanol (200 proof) was obtained from Decan Labs (King of Prussia, Pennsylvania), glacial acetic acid (A.C.S grade, 99.7%) from Research Products International Corporation (Mt. Prospect, Illinois), and triethylamine from Mallinckrodt (St. Louis, Missouri). Bovine serum albumin (BSA) and lysozyme chloride (LZY) were purchased from Sigma Life Sciences. The protein assay kit (Micro BCA kit) for analysis of BSA and LZY was purchased from Thermo Scientific (Rockford, Illinois). All materials and chemicals were stored and utilized as received except for BSA and LZY that were initially stored at 4°C and −20°C, respectively, prior to their use.

Methods

Depolymerization of Cotton Linter

Cellulose from cotton linter sheets was depolymerized according to the mineral acid hydrolysis method.[21] Cotton linter sheets, shredded into 1×1 cm^2 pieces and weighing approximately 50 g were placed in a 1-L Erlenmeyer flask filled with 500 mL of 2 N hydrochloric acid and agitated using a magnetic stir bar at 40°C in a thermostatic water bath for up to 72 h (RM 6 Lauda; Lauda Brinkmann L.L.C., Delran, New Jersey). After 72 h, the cellulose slurry was rinsed in running water for 2 h, followed by washing in acetone and finally placed in an oven at 105°C for 24 h. The reaction was carried out in three separate flasks to minimize variability with end-point determination.

Preparation of MC Solution and Fabrication of Membranes

Methylolcellulose solution using native cotton linter and depolymerized cotton linter (MC-DP) with 4.4% (w/w) cellulose were prepared as per the method described by Seymour and Johnson.[22] RC membranes from MC and MC-DP solutions were prepared according to the method described in our previous work.[12] Briefly, glass test tubes (20 mm diameter) were dipped in MC and MC-DP solutions and rotated at 70–100 rpm for approximately 10 min. After application of an even coating of MC on the test tube surface, they were placed in 5 L water bath at room temperature for 3 days to precipitate cellulose on them. These test tubes were then placed in a thermostatic oven at 105°C for 24 h. Annealed membranes were peeled from the glass tubes using forceps and blades and placed in 20 mL glass scintillation vials, sealed with screw caps, and stored in a vacuum desiccator prior to further analysis. Membranes prepared from MC and MC-DP solutions are herein referred to as RC-W and RC-DP membranes.

Blended membranes were prepared by mixing MC and MC-DP solutions in 50 mL beakers in the following ratios: MC–MC-DP: 4:1 (RC-4/1), 3:2 (RC-3/2), 2:3 (RC-2/3), and 1:4 (RC-1/4). The combined solutions were heated up to 50°C and blended using a magnetic stirrer operating at 200 rpm for 30 min. Membranes were then fabricated from these solutions as per the aforementioned procedure. The combination membranes will herein be collectively referred to as blended RC membranes.

Membranes with multiple applications of MC solution were prepared by dip coating glass test tube molds in MC, followed by 10 min precipitation in acetone, 10 min air drying, and then repeating the process until desired number of coatings were applied. After final application of MC solution, the mold pins were immersed in excess water for cellulose precipitation and then dried in a thermostatic oven for 24 h. Membranes with 2, 3, 4 and 5 applications of MC solution are herein referred to as RC-W2, RC-W3, RC-W4, and RC-W5 membranes, respectively.

Determination of Degree of Polymerization

The degree of polymerization of cellulose during depolymerization/hydrolysis was determined by viscometric method at 25 ± 0.5°C using a Cannon–Fenske capillary viscometer (size 100; Fisher Scientific, Fair Lawn, New Jersey). Approximately 0.15–0.7 g/dL solutions of cellulose and of RC membranes were prepared by dissolving them in 0.5 M copper(II)-ethylenediamine (cuen) solution. This solution was thoroughly mixed using a magnetic stirrer and equilibrated to 25°C in a water bath, under nitrogen purge. Approximately 6–7 mL of this solution was filled into the lower bulb of the viscometer (immersed in a water bath at 25°C) and pulled to the upper bulb using capillary vacuum bulb. The solution was then allowed to flow from the upper to the lower markings on the viscometer and the time for efflux recorded. Efflux time of pure 0.5 M