Preparation and Evaluation of Crosslinked Polyelectrolyte Complex Membranes

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ABSTRACT: This article discusses a new method to make the crosslinked polyelectrolyte complex (PEC) membranes that has suitable mechanical strength, good solute permeability, hydrophilicity and biocompatibility in dialysis applications. PECs were obtained by mixing two aqueous solutions of water-soluble gelatin and carboxymethyl cellulose (CMC). PEC membranes were prepared under various conditions to determine a suitable mixing ratio of gelatin to CMC, concentration of crosslinking agent and film fablicating conditions, and their properties and permeability of solutes were estimated. Effects of the relative amounts of bound water and free water in the membrane on the solute permeation and mechanical properties were studied. This new PEC membrane not only offers higher solute permeability than the commercial Cuprophane membrane but also shows suitable mechanical strength, non-toxicity and non-pyrogen for dialysis application.
KEY WORDS Carboxymethyl Cellulose / Gelatin / Polyelectrolyte Complex / Crosslinking Agent / Membrane / Solute Permeability / Biocompatibility /

Many polymers widely used for commercially available membranes and biomaterials are hydrophilic polymers, water-swellable polyelectrolyte complexes (PEC) and graft copolymers, such as hydroxylated polypropylene,[1] sulfonated poly(vinyl alcohol) (PVA),[2] acrylic acid-grafted methyl cellulose,[3] sulfonated polyacrylonitrile,[4] and various polyelectrolyte complexes.[5-9] Polyelectrolyte complexes with high water content can easily be made by crosslinking already-formed polymers, or by simultaneously polymerizing and crosslinking various monomers.

Crosslinked PEC membranes provide good mechanical strength in the wet state and good permeability of water, small and midsize solutes depending on fabrication and crosslinking process conditions. The membranes have high water content and suitable water structure (bound and free water composition). The high water content and water structure influence the diffusion coefficient of the permeating soutes. It is generally accepted that polar groups such as –COOH, –NH₂, and –CHO form hydrogen bonds with water but combine with themselves. This effect weakens the coherence of water clusters and causes efficient diffusion of solutes.[10-13] The structure of water in membranes is also very important when diffusion takes place through water-swollen membranes.[14] Solute diffusion does not take place in the bound water region that has strong hydrogen bonds, but in the free water region.

This work reports is the possibility of making novel hydrophilic membranes.[1,2] Since commonly used cellulosic dialysis membranes exhibit low permeability and poor biocompatibility, this article focuses on the development of a modified cellulose membrane with improved permeability, mechanical strength in wet state and biocompatibility. Gelatin and CMC were blended to form a PEC membranes followed by crosslinking, and the performance of the membrane was studied.

EXPERIMENTAL

Materials

CMC (carboxylic acid content: 0.7 meq g⁻¹) was purchased from Kisida Co. and gelatin (pH 5.5—6.0, 1 wt% solution) was obtained from Kyunggi Gelatin Co. Glutaraldehyde (Kokusan Chemical Works) as a crosslinking agent was used as received. Urea and sucrose were obtained from Wako Pure Chemicals. Poly(ethylene glycol) (PEG, 3400 g mol⁻¹) and vitamin B₁₂ were purchased from Polyscience and Rhone-Poulenc, respectively.

Formation of (Gelatin / CMC) PEC

Gelatin (aqueous solution of 0.5 wt%) was mixed with CMC (aqueous solution of 0.5 wt%) at 50 °C with various weights and pH in the presence of acid. Ultraviolet spectrophotometer (Perkin Elmer, Model 559) was used to measure the maximum turbidity point against mixing ratio (R, weight of gelatin / weight of gelatine and CMC) for gelatin / CMC system.

Preparation of Crosslinked (Gelatin / CMC) PEC Membranes

In the preparation of crosslinked PEC membrane, the homogeneous PEC solution (gelatin : CMC = 2.3 : 1) was cast on the glass plate kept at 50 °C and spread with a casting bar equipped with a spacer. The solvent was allowed to preevaporate at 10 °C until the gel film was obtained and completely dried in a freeze dryer. The resulting film was immersed in a bath of acetic acid (pH 3) to form a PEC membrane. The PEC membrane was washed with distilled water and completely dried at 40 °C for 1 day under vacuum. The polyelectrolyte complex(PEC) membrane was crosslinked for 12 h in aqueous solution of glutaraldehyde (1, 4, and 7 mol L⁻¹ for sample A-1, A-2, and A-3, respectively) to improve mechanical strength in the wet state. Change in the proper-

ties of cellulosic substrates resulting from treatments with crosslinking agent under various conditions has been studied. It is generally accepted that crosslinking agent condenses with CMC with loss of water to form intermolecular crosslinks between macromolecular chains and provides improved wet strength. The resulting crosslinked PEC membranes were washed with distilled water and dried before evaluating performance.

Measurements

FT-IR (Nicolet, Model 5DX) was used to determine the composition of PEC. The morphology of the completely dehydrated PEC membrane was photographed using a scanning electron microscope (SEM, Hitachi, S-510). To measure water content, samples were immersed in distilled water for 7 days. Water content at equilibrium was determined according to the following equation, Q_u (%)= $[(W_s - W_d)/W_s] \times 100$, where W_s and W_d denote weight of wet and dry samples, respectively. To evaluate the water structure in the membrane, a DuPont 910 thermal analyzer and cell base were used for all melting measurements. A membrane sample (6—15 mg) equilibrated at 37℃ was sealed in an aluminum pan and cooled with liquid nitrogen to −70℃ in DSC. The cell was slowly heated in a stream of nitrogen gas at a program rate of 5℃ min⁻¹ up to 20℃. Tensile strength and elongation under the dry and wet state were estimated according to ASTM D-638 by an Instron Model 4201 with a crosshead speed of 10 mm min⁻¹ and 5 Kg_f load cell was used.

Permeation Experiment

The permeation cell in this study had two compartments of equal volume (100 mL) as shown in Figure 1. Temperature of the compartment was maintained at 37℃ with a constant stirring at 300 rpm to eleminate any concentration polarization. Concentrations of urea, sucrose, vitamin B_{12}, and PEG 3400 were 200, 200, 50, and 100 mg L⁻¹, respectively. Concentrations in the feed and permeating compartments were detected using ultraviolet spectrophotometer and differential refractometer (Waters, Model R 403). Particularly for the vitamine B_{12} solution, the light was blocked during permeation to obtain precise absorption curves measured at 550 nm.

The solute permeability coefficient P was calculated from the following equation obtained from mass balance equation, i.e.,

$$P = \frac{-d}{A(1/V_1 + 1/V_2)t} \times \ln \left[\left(1 + \frac{V_1}{V_2}\right) \frac{C_t}{C_o} - \frac{V_1}{V_2} \right] \qquad (1)$$

where V_1, V_2, A, d, C_o, and C_t are the volumes of the concentrated and dilute compartment, membrane area (9.62 cm²), thickness, and concentrations of the concentrated compartment at times 0 and t, respectively.

Chemical Toxicity and Pyrogen Test

Chemical toxicity and pyrogen test for biomedical application were performed with the help of Green-Cross Medical Co. according to the USP rabbit test.[15]
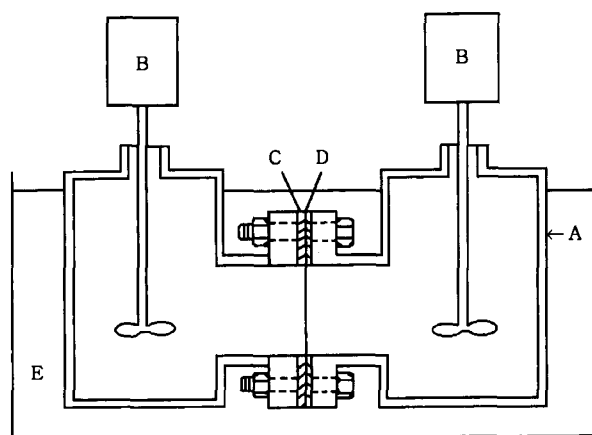

Figure 1. Solute permeability measurement apparatus: A, glass cell; B, mechanical stirrer; C, silicon rubber ring; D, PEC membrane; E, water bath.

RESULTS AND DISCUSSION

Formation of (Gelatin-CMC) PEC

In PEC systems composed of synthetic polymers, actual complexation reactions cannot be accurately predicted. Very simple theories based on colloidal chemistry were proposed by Oosawa[16] and Nakajima,[17] who consider very simple and specific models and assume a homogeneous distribution of molar mass with no contribution from interaction forces other than electrostatic interactions.
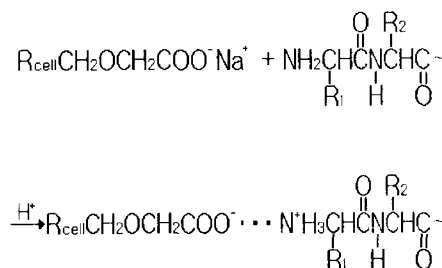
(Gelatin-CMC) PEC

Optimum conditions for the PEC formation were confirmed by complex phase separation curves influenced by effects of mixing ratio of gelatin and CMC at various pH. Figure 2 shows that PEC was formed well at all mixing ratio of gelatin and CMC in the range of pH 2.4—5.2. As the amount of gelatin increased, PEC was formed well up to pH 5. From the turbidity test for various mixtures of gelatin and CMC at pH 2 and 3, minimum transmittance at 575 nm was 70 and 72% in the mixing ratio of about 0.63 and 0.70, respectively as shown in Figure 3. The ideal mixing ratio of gelatin to CMC for the maximum complex phase separation was approximately 1.7 : 1 at pH 2 and 2.3 : 1 at pH 3.

Non-crosslinked PEC film was identified by FT-IR and corresponding features are shown in Figure 4. In the spectrum of PEC the amide I band and amide II band of
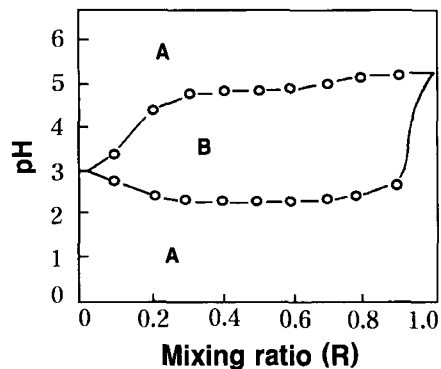
717

Figure 2. Complex phase separation influenced by pH and mixing ratio of gelatin and CMC: A, homogeneous phase; B, separation phase.
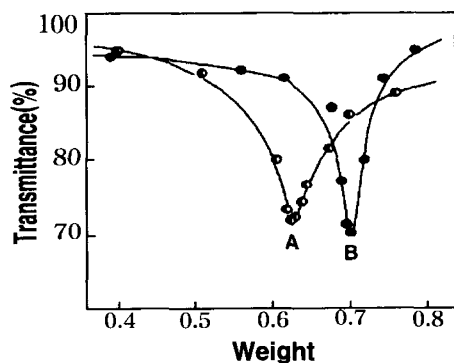

Figure 4. Infrared spectra of gelatin, CMC and polyelectrolyte complex: a, gelatin; b, CMC; c, polyelectrolyte complex.

Figure 3. Turbidity curves plotted against pH for PEC formation of (gelatin and CMC) at 575 nm: A, pH 2; B, pH 3.

Crosslinked (Gelatin-CMC)PEC

the gelatin appear at $1655 \, \text{cm}^{-1}$, $1535 \, \text{cm}^{-1}$, respectively, at $1447 \, \text{cm}^{-1}$ as for the ionized carboxylate band of CMC. In addition to FT-IR results, water insolubility of the non-crosslinked PEC film is indirect evidence for PEC formation.

Properties and Morphology of Crosslinked (Gelatin-CMC) PEC Membrane

The concentration of crosslinker more than $7 \, \text{mol} \, \text{L}^{-1}$ did not greatly affect the improvement of physical properties, morphology and permeability characteristics. Therefore, concentration of the crosslinking agent for prepering sample were varied and the optimal value was selected for the membrane.

In the formation of (gelatin-CMC) PEC, condensation of charge by electric interaction may give the multiplx structure first proposed by Eisenberg,[18] and results in the formation of strong ionic crosslinking. Therefore, remaining reactive species(-OH) of CMC may react with the crosslinking agent in the preparation of crosslinked (gelatin-CMC) PEC membrane.
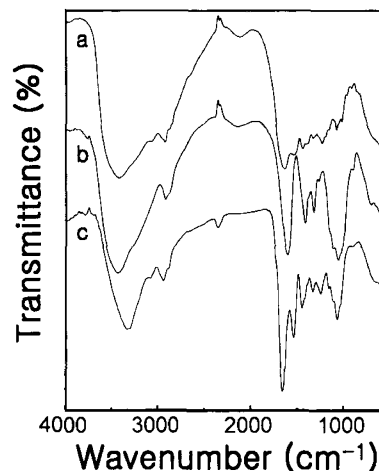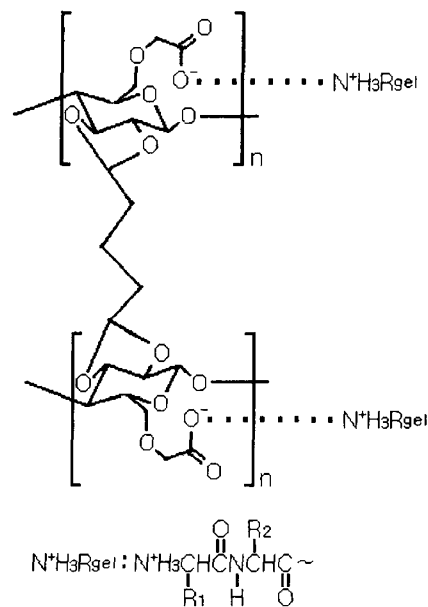
Table I lists the characteristics of all the resulting membranes. The concentration of crosslinking agent influenced the physical properties of the membrane. Water content of samples decreased with growing crosslinking density and was higher than that of commercially available Cuprophane membrane (about 50%). Tensile strength of samples in dry state is regarded as excellent. However, in wet state, all samples have comparable tensile strength (0.28—0.35 kg mm^{-2}) to that of commercial Cuprophane membrane (0.30 kg mm^{-2}).[19] Tensile strength changed depending on the concentration of crosslinking agent and morphology of the membrane. This may be explained by cross-sectional views of the A-1, A-2, and A-3 membranes in Figure 5, where the membrane becomes more dense as the concentration of crosslinking agent in a crosslinking process increases. As the crosslinking density increased, elongation in the wet state decreased greatly at 127—31%. All samples

Table I. Summary of preparation and physical properties of crosslinked PEC membranes

Sample	Preparation conditions			Physical properties			
	Mixing ratio		Concentration of crosslinker	Degree of swelling %	Tensile strength kg mm^{-2}		Elongation %
	Gelatin[a]	CMC[a]	mol L^{-1}	In water	Dry	Wet	Wet
A-1	2.3	1	1	69.0	3.17	0.28	127
A-2	2.3	1	4	64.7	3.42	0.31	95
A-3	2.3	1	7	53.5	4.10	0.35	31

[a] Aqueous solution (0.5 wt%).

Table II. Permeability coefficients for aqueous solutions of various solutes and calculated bound and free water in crosslinked PEC membranes

Sample	Permeability × 10^7 cm^3 cm cm^{-2} s^{-1}				W_t, Water content %	W_b, Fraction of bound water	W_f, Fraction of free water
	Urea	Sucrose	Vitamin B$_{12}$	PEG 3400			
A-1	12.7	2.5	0.63	0.27	69.0	0.34	0.66
A-2	10.3	2.0	0.58	0.21	64.7	0.39	0.61
A-3	8.7	1.8	0.45	0.13	51.8	0.47	0.53
Cuprophane[a]	4.8	2.0	0.57	—	50.0	—	—

[a] Data taken from ref 20.
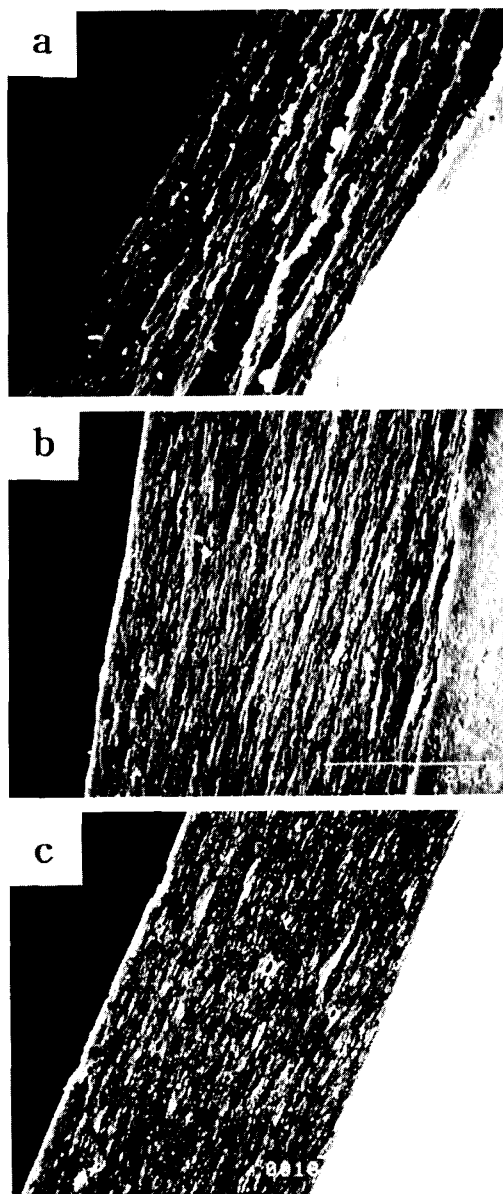
Figure 5. Scanning electron micrographs of crosslinked PEC membranes (×1750): a, A-1; b, A-2; c, A-3.

were suitable for dialysis.

Solute Permeabilities of (Gelatin-CMC) Membranes

Table II shows the permeability of A-1, A-2, and A-3 membranes for four solutes at 37°C. The concentrations of urea, sucrose, vitamin B$_{12}$, and PEG 3400 were 200, 200, 50, and 100 mg L^{-1} in water, respectively. Only a single membrane was used for each evaluation. Permeability for solutes changes according to affinity between solute and hydrophilic group in the membane material, water structure (weight fraction of free and bound water) in the membane, porosity and morpholoy of membrane. Crosslinked (gelatine-CMC) PEC membranes have higher water content (69.0—51.8%) than (50.0%) Cuprophane, so that solutes pass through the membrane easily. A large molecule has difficulty in diffusing through the membrane compared with a small molecule. Molecular sizes of urea and sucrose are 8.1 Å^2, 27.7 Å^2, respectively. Therefore, high permeability of urea was also due to comparably small molecular size and low molecular weight. All the membranes showed good permeability of solutes, and urea permeability 2-3-fold higher than that of Cuprophane.[20] Solute permeability decreases as molecular weight of solutes increases, and all the membranes show similar molecular weight dependency. Molecular weights of urea, creatinine, vitamin B$_{12}$, and PEG were 60, 113, 1355, and 3500 g mol^{-1}, respectively. It is generally accepted that a large molecule has difficulty in diffusing through the membrane compared with a small molecule. Therefore, compard to small molecular weight of solute, large molecular weight of solute has less permeability through the membrane. With increasing molecular weight of solute, solute permeability decreases almost linearly. Solute permeability also increases with water content in the membrane. This is mainly attributed to the water structure inside the membrane. Water soluble solutes diffuse through the membrane *via* water pockets of clustered water.[21,22] If water content of membrane increase to some extent, water pockets expand largely and solutes diffuse through the membrane easily.

Table III. Chemical toxicity test results of extracts obtained from crosslinked (gelatin-CMC) PEC membranes

Sample	pH deviation [a]	Heavy metals mg L^{-1}	UV absorbance 200—241 nm	UV absorbance 241—350 nm	Total solids mg mL^{-1}	Oxidizable substances [b] mL
Standard	1.5<	<20	<0.08	<0.05	<10	<1.0
Extracts	0.183	Pass	0.021	0.001	0.18	0.2

[a] Standard pH:7 (distilled water). [b] Based on KMnO$_4$ consumption.

Table IV. Pyrogen test results of extracts obtained from crosslinked (gelatin-CMC) PEC membranes

Rabbit no.	Body weight kg	Injection mL	Body temperature/°C Primary	After injection 1	After injection 2	After injection 3 [a]	Raised temp °C	Total raised temp [b] °C
R 1	2.0	20	38.9	38.8	38.8	39.0	0.1	
R 2	2.0	20	38.8	38.8	38.8	38.9	0.1	
R 3	2.0	20	38.7	38.8	38.9	39.2	0.5	(0.7)

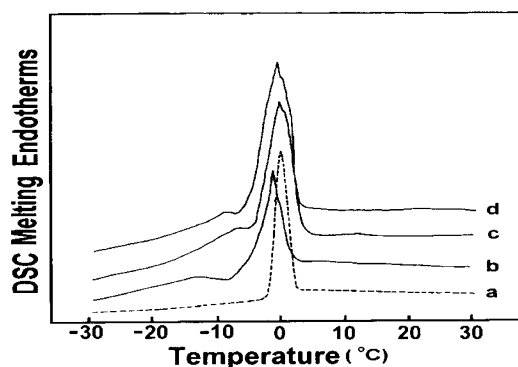[a] Standard< 1.0°C. [b] Elapsed time after injection (h).

Figure 6. DSC melting endotherms of free water in PEC membranes and pure water: a, pure water; b, 51.8%; c, 64.7%; d, 69.0%.

Figure 6 shows DSC heating curves of water-swollen (gelatin-CMC) PEC membranes and pure water. With increase in swelling ratio of membrane, an endothermic peak appearing at around 0°C due to the presence of free water grew and separated into two peaks, one close to 0°C and the other shifting toward −4°C. These endothermic peaks confirm two types of water in the membrane,[22] i.e., free water and freezing bound water. The former has a melting temperature comparable with that of bulk water (curve a in Figure 6). The latter shows a lower temperature peak presumably by weak interaction between water and the polymer chain.

Freezing and nonfreezing water in the membranes are listed in Table II for different PEC membranes, calculated from enthalphies given by the areas of peaks appearing at −4 and 0°C. Bound water in the membrane was approximately estimated as,

$$W_b = W_t - (W_f + W_{fb}) = W_t - Q_{endo}/Q_f \qquad (2)$$

where W_b and W_t are weights of bound and total water in the membrane, respectively. W_f and W_{fb} are the weight fractions of free water and freezing bound water, respectively. The sum of W_f and W_{fb} is the ratio of Q_{endo}/Q_f, where Q_{endo} is the observed endothermic heat (cal g^{-1})

originating from bound and freezing bound water from water-swollen membrane and Q_f is the heat fusion of ice (79.8 cal g^{-1}). Sharp endothermic peaks at around 0°C are assigned to free water, and other peaks at lower temperatures to freezing bound water (intermediate water). But all endothermic peaks at lower temperatures are not as remarkable as those for the free water in the membranes. Freezing water in the membranes consists mostly of free water and a very small amount of freezing bound water. Therefore, freezing water in the membranes was regarded as free water in this study. Total water content increases as the membrane becomes less crosslinked and porous. Total water content is equal to sum up of bound water and free water. Therefore, water after calculation of free water from the total water content in the membrane is considered bound water. With increase of crosslinking of PEC, a denser membrane was obtained, and free water decreased. Remaining hydrophilic groups after PEC formation decrease with increase of crosslinking. Water that interacted with hydrophilic groups may be considered free water, and its bonding force is weak compared with that of bound water. The weight fraction of bound water in the resultant membrane thus increases.

Free water increases while the fraction of bound water becomes less as the membranes become crosslinked and porous. The more the increase in total water, the greater the permeability of solutes through the membrane. Free water and freezing bound water may easily form water clusters by hydrogen bonding with each other, but bonding force is obviously weaker than that between bound water. Bound water may be difficult to move because it strongly combines with polymer chains. Therefore, solutes find difficulty in diffusing through this region and may rather pass through the region of free water. Greater free water coupled with porosity in the membrane contributes to the permeation of solutes through the membrane.

Chemical Toxicity and Pyrogen Test

Table III and IV show chemical toxicity and pyrogen test results of extracts obtained from A-1, A-2, A-3 mem-

branes, respectively. The chemical toxicity test on extracts of the membranes satisfied the standard requirements of toxicity level for the biomedical materials. In the pyrogen test for three-rabbit test, raised body temperature after injection of extracts was 0.1—0.5°C and total body temperature (0.7°C) was less than maximum permitted limit (1°C). Materials judged non-pyrogenic in the rabbit test are non-pyrogenic in man. The crosslinked (gelatine-CMC) PEC membranes reported here may be used for dialysis in consideration of physical properties, solute permeability, non-toxicity and non-pyrogen.

CONCLUSION

New crosslinked (gelatin-CMC) PEC membranes were developed, with good physical properties and dialysis performance as well as biocompatibility (non-toxicity and non-pyrogen). Tensile strength in the wet state of membrane ranged from 0.28 to 0.33 kg mm^{-2}, while water content was 53.5—69.0%. The A-1 membrane shows higher permeability of solutes and comparable mechanical properties than the commercial Cuprophane membrane had. The permeability of solutes correlated well with water content or free water in the membrane. With casting–drying–crosslinking, procedures to prepare water swellable membranes with controllable physical properties, morphology and solute permeability were demonstrated.

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