Purification of Collagen by Thermal Sensitive Polyurethane Membrane

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Abstract: A novel thermal-sensitive polyurethane (TSPU) membrane with a thermal switch was prepared via wet phase inversion technique and used for separation of NaCl, glycin from collagen solution. From Differential Scanning Calorimetry and X-Ray Diffraction, the TSPU showed segmented structure (i.e. the hard segment and soft segment) and independent phase transition temperatures (the phase transition temperature of the soft segment defined as switch temperature, Ts). Scanning Electron Microscope and Atom Force Microscope were employed to study the morphology structures of membranes, the results indicated that the membrane had relative dense surfaces (or skin) and porous cross sections, which controlled their selectivity and permeability. When the temperature varied ±10℃ around the Ts, the porosity of membranes increased from 51.7% to 73.3%, showing a significant improvement to thermal stimuli. When this TSPU membrane was used for the separation of NaCl, glycin from collagen solution, we found the ions of Na^+ and Cl^- could permeate the TSPU membrane at any temperature, and higher temperature resulted in higher penetration rate; While the penetration of glycin relied on the temperature, that is, a barrier effect at lower temperature ($T < Ts$) and higher permeation fluxes at higher temperature were observed. Typically, when the temperature exceeded the Ts, the permeation flux of glycin increased markedly, showing sensitivity to thermal stimuli. Collagen, due to its large molecule size, could not permeate the TSPU membrane in all temperature range. As a result, NaCl, glycin and collagen with different sizes could be selectively separated by TSPU membrane driven by the temperature.

Key words: thermal sensitive polyurethane; membrane; purification; collagen

1 Introduction

In recent years, porous polymeric membranes are finding extensive applications in separation technology. [1-5] Phase inversion method, including dry or wet phase inversion processes, [6-9] are the traditional methods for making porous polymeric membrane. By means of these phase inversion technologies, various porous membranes which are widely used for gas separation, reverse osmosis, ultrafiltration, and microfiltration were successfully developed from a variety of materials, such as cellulose derivatives, polyamides, polyimides, and polysulphones. [10-12] However, these porous membranes are hardly relevant to environment stimuli, that is, it has no ability to response to the external stimuli [13] and shows no intelligent characteristics. As already known, the size and shape of free-volume (FV) holes available in polymer membrane materials control the rate of molecules diffusion and its permeability, so how to control the size scale of holes available in polymer is the key issue to realize the controllable separation. [14-15] In our previous work, we prepared a thermal sensitive polyurethane (TSPU) membrane with a thermal switch by a dry film-formation method and employed it for water vapor permeation. [16] The results indicated that the FV hole size and WVP of this TSPU membranes show sensitivity to temperature stimuli, especially, when the temperature varied from T_s-10℃ to T_s+10℃, the FV hole size of this TSPU membrane increased from 0.23 nm to 0.467 nm, and the water vapor permeability increased from 4.30 kg/m^2·24h to 8.58 kg/m^2·24h, showing an significant improvement by 114%.[16] But this TSPU membrane

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is a type of dense/non-pore membranes, the lower permeability at low temperatures and worse selectivity at higher temperatures limits its wide application for controllable separation. As a continuous work, in this study, we prepared a porous TSPU membrane by a wet phase inversion process and used it for selective separation for NaCl, L-glycin and glutin from water solution. In comparison with non-porous TSPU membranes prepared by dry film-forming method, the later prepared by wet phase inversion process possess suitable pores size itself which permits some molecules with small size permeation at lower temperature. On the other hand, temperature changes can further increase the size scale of pores, especially, when the temperature rises from T_s-10℃ to T_s+10℃, phase transition often accompanies a significant increase in pore sizes, which will result in another molecules with larger size scale permeation, as a result, molecules with different size scale can be selective separated by TSPU membrane driven by temperature. So, the main purpose of this work is to investigate how the different micro-porous structure of TSPU membranes and temperature influence on the selectivity of separation, finally to develop new type polymeric membranes with thermal-sensitivity and selectivity for membrane separation fields.

2 Experiment

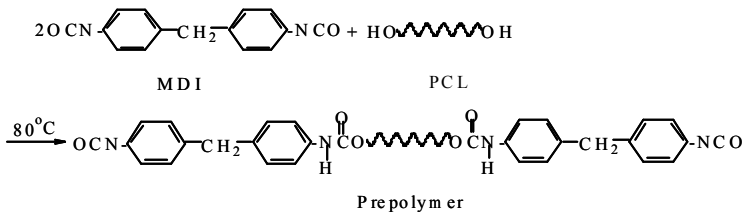
2.1 Materials

Polycaprolactone diols (PCL, M_w=4000 g/mol, Daicel) were dried at 100-120℃ under high vacuums (0.5 mmHg) for 12 h before use. Extra pure grade of *4,4'*-diphenylmethane diisocyanate (MDI, Aldrich Chemical), and *1,4*-butanediol (BDO, Aldrich Chemical) were used with PCL to prepare TSPU. Dimethylformamide (DMF, Kelong, Co., China) was used as solvent with further purification to remove trace water. Biochemical grade L-Glycin (M_W=74, Shanghai Kangda Amino-Acid Co) and glutin (M_W=50000-70000, Shanghai Kangda Amino-Acid Co) were used to prepare the aqueous solution. The analytical grade Ninhydrin (Shanghai Reagent Co) and Folin-phenol (Kelong, Co., China) was used as colorimetric reagent.

2.2 Preparation of TSPU

A 500 ml round-bottom, four-necked separable flask equipped with a mechanical stirrer, nitrogen inlet, thermometer and condenser with a drying tube was used as a reactor to prepare the polyurethane. Dried PCL (0.02 mol) and MDI (0.04 mol), which would provide the switch temperature, were charged into the dried flask. The soft-segment formation reaction was carried out in DMF at 80℃ for 2.0 h, followed by hard-segment formation reaction with MDI (0.04 mol), and BDO (0.06 mol) at 75℃ for 2.0 h. Finally, a viscous, transparent polymer solution was obtained. The reaction and structure of TSPU was showed in Fig.1.

The first step:



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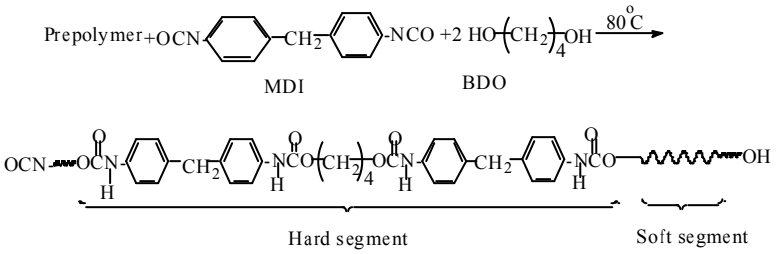


Fig. 1 The reaction of TSPU

2.3 Preparation of TSPU membrane via wet phase inversion technique

The TSPU membranes were prepared via wet phase inversion technique: TSPU solution (15wt%) was first stirred by a magnetic stirrer for 1.0 h. Membranes were prepared by casting TSPU solution on a cellophane paper and immersed in distilled water at 25℃ for 1h. During this wet phase inversion film-formation process, solvent (DMF) was replaced by water and the TSPU precipitated and formed porous membrane. Finally, the TSPU membranes were taken out from DMF/water solution and washed with distilled water to remove solvent, then dried at 40℃ under vacuum condition (1 mmHg) for the next 24h. The thickness of membrane was measured in dried state and controlled at 0.1±0.01mm.

2.4 Instrumental analysis

The phase transition temperature of the TSPU was measured by Netzsch 200PC thermal analyzer with a DSC module, which was purged with nitrogen gas and quenched with liquid nitrogen. The TSPU samples (5-10 mg) were heated in sealed aluminum pans and scanned from −50℃ to 200℃ with a heating rate of 10℃/min. In order to erase earlier thermal history of the sample, the results of DSC analysis relied on the second heating run instead of the first run. SEM (Hitachi Model S-520, Japan) was employed to observe cross-section of the membranes. The membranes were cryogenically fractured in liquid nitrogen and then sputtered with platinum. The SEM micrographs were taken at certain magnifications. The morphological structure of the membranes was tested by using Philips Analytical X-Ray (Philips X'pert XRD System) at voltage of 40V, 40mA current and a radiation of wavelength 1.542×10^{-10}m (1.542 A°). Spectra were obtained in the range of Bragg's angle 2θ= 5°-40°. The scanning speed was 0.03 s per step. The spectrophotometer (Lambda 25, Perkin Elemer) was used to measure the concentration of the amino acid and protein.

2.5 Selective separation for NaCl,L- glycin, glutin with TSPU membrane

The experimental apparatus is schematically illustrated in Fig. 2. The test cell for membrane dialysis of glycin was shook by the oscillator (HSL, Donglian Co., China), the shaking rate was 120r/min. The original solution of NaCl (4g/L, 50ml), L-glycin (5g/L, 50ml) and glutin (5g/L, 50ml) were put in the container enclosed with TSPU membrane and the purified water (400ml) was filled in the flask. The test TSPU membrane with 8.0 cm^2 surface area was immersed into the oscillator at the different temperatures of 30, 40, 50 and 60℃. In addition, the concentrations of the L-glycin, glutin in water solution in the flask were tested by the Ninhydrin colorimetric method and by the Folin-Lowry method, respectively, the test time interval is 1h, 2h, 3h, 4h, 5h, 6h, 7h, 8h, 9h and 10h. An average of three parallel samples was used for each measurement.

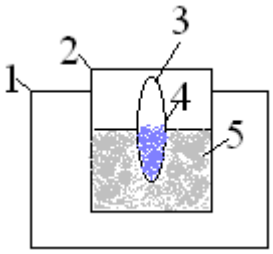


Fig. 2 Apparatus diagram for permeation experiment

1: oscillator, 2: flask, 3: a container enclosed with TSPU membrane, 4: L-glycin (NaCl, glutin) water solution, 5: the purified water.

3 Results and Discussion

3.1 The segmented structure and the switch temperature of TSPU

TSPU has often been observed with a block or segmented structure and consists of two incompatible phases, i.e. a thermally soft segment and a hard segment. In many cases, the thermally soft segment shows a phase transition temperature (crystalline melting transition or glass transition temperature), which can be used as a switch temperature (T_s). The segmented structure and the T_s of TSPU can be characterized by DSC analysis. On DSC curve (Fig. 3), two peaks at 50.6℃ and 226.4℃, corresponding respectively to the phase transition of the soft segment and the hard segment are found, which reveals that the sample is the phase-separated into soft segment and hard segment, and the incompatible soft and hard segments show obviously independent phases and different phase transition temperatures. This conclusion can be further determined by XRD analysis.

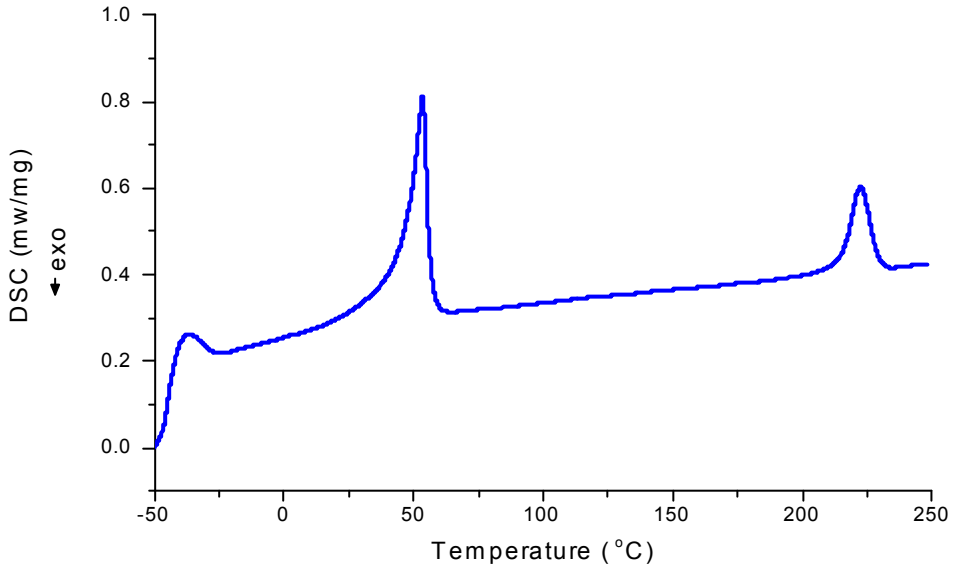


Fig. 3 DSC curve of TSPU

Fig. 4 is the XRD patterns of TSPU. When an X-ray beam is incident on a crystalline membrane, diffractions occur due to the interaction between the incident beam and the periodically aligned atoms. Peak position and peak intensity are the important parameters for a diffraction peak. [17] For the TSPU, the peak position relies on the morphological structure of polymer, and the peak intensity is proportional to

the crystallinity of the soft segment or hard segment.

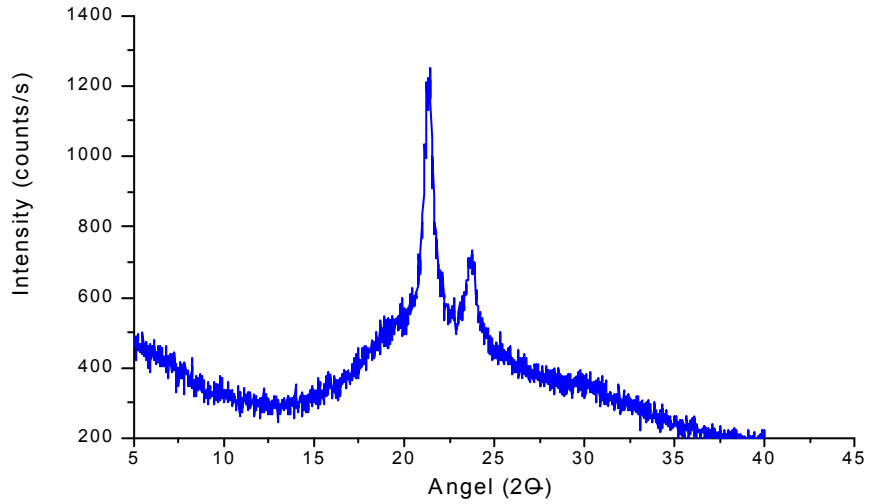
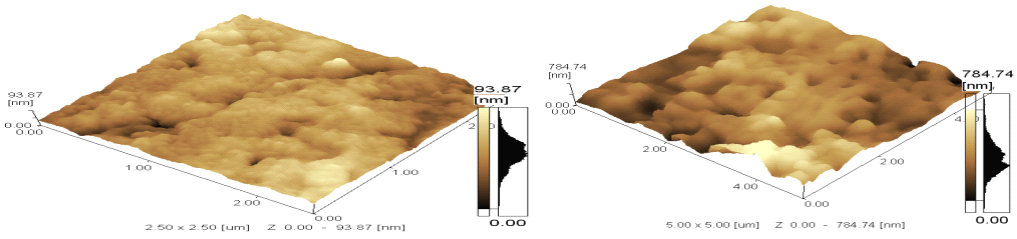


Fig. 4 XRD graphs of TSPU membrane at 25℃

In XRD pattern, two dominant characteristic peaks at 2θ of 21.4°, 23.7° corresponding to the crystalline peaks of the soft and hard segment are found, which also implies that the TSPU membrane is the intrinsic phase-separated structure. This conclusion keeps good consistent with DSC analysis.

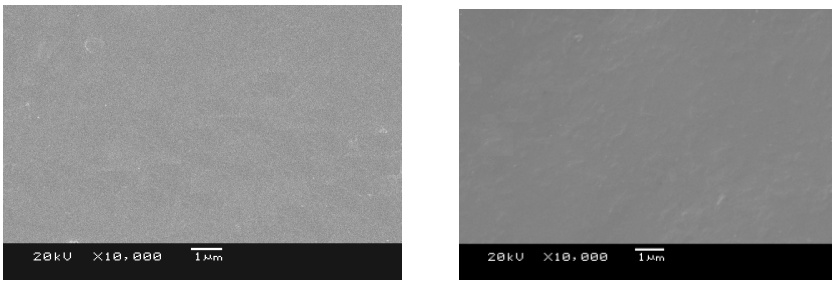
3.2 The morphological structure of TSPU membranes

AFM and SEM were employed to observe the surface and the cross-section features of the polyurethane materials. From Fig. 5 and Fig. 6, it can be seen that the topography of double surface of the TSPU membrane has a few of small pores with size scale lower than 20 nm, whilst there are many pores on the cross-section of the TSPU membrane (Fig. 7) and the pore size is approx 1μm. Moreover, the path of the pores is flexural and asymmetric. Therefore, this TSPU membrane prepared by the wet phase inversion has its particular and porous structure in the solidification process. This difference in pores structure both on surface and in inner in wet phase inversion film-formation process has also been observed by many researchers[18-20]. Although this phenomenon is far from being understood, the importance for its permeability and selectivity in membrane separation is well recognized. [21] Because this dense surface with small pores only permits small size molecules permeation, which provides the possibility of selective separation for TSPU membranes.



A B

Fig. 5 AFM photograph of the upper (A) and bottom surface (B) of TSPU membrane



A B

Fig. 6 SEM photograph of the upper (A) and bottom surface (B) of TSPU membrane

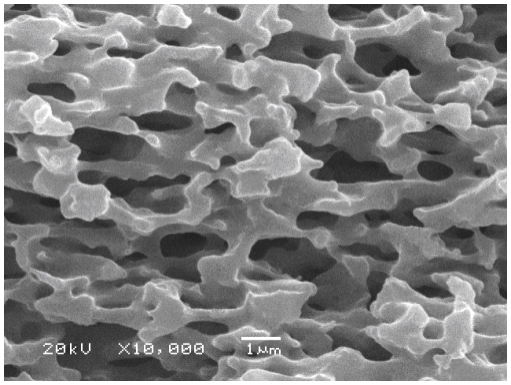


Fig. 7 SEM photograph of the cross-section of TSPU membrane

3.3 TSPU membrane for selected separation for NaCl, glycin and glutin

From SEM and AFM analysis, we found the TSPU membrane prepared by the wet inversion process has a dense surface and an asymmetric and large porous cross section, which plays an important role in its selective separation for molecules with different size scale, such as NaCl, L-glycin and glutin. Tab. 1 shows the permeation of NaCl, L-glycin and glutin as a function of the temperature in 1h.

Tab. 1 The permeation of NaCl, L-glycin and glutin as a function of the temperature in 1h*

Temperature (℃)	30	40	50	60
NaCl	√	√(data)	√	√
L-glycin	×	√	√	√
glutin	×	×	×	×

* the "√" means the permeation of the tested chemicals; the "×" means no permeation of the tested chemicals.

According to the Tab. 1, it can be seen that the Na^+ and Cl^- can permeate the TSPU membrane even at 30℃, while the molecules of L-glycin and glutin show no permeation at this point because of their large molecule size, which means that NaCl can be firstly separated from the mixture solution at low temperature. Continuously, when the temperature was raised to 40℃, the L-glycin began to permeate the TSPU membrane and the higher the temperature, the higher the permeation flux. No matter how the temperature changes, the glutin can not permeate the TSPU membrane in any case.

Fig. 8 shows the concentration changes of L-glycin as a function of time and temperature (in the flask). Just as mentioned above, when the temperature is at low temperature (say, 30℃), the L-glycin

molecules could not permeate the TSPU membrane. Nevertheless, in the next temperature range from 40℃ to 50℃, it is not the case. Although most of the main chain of TSPU are still in a glassy state in this temperature range, some of the frozen preliminary segments are released, which increases the size scale of holes on surface, being beneficial for permeation of L-glycin and leading the concentration of the L-glycin to increase from 0 mg/L to 360mg/L. This increase in pores size is very slim and the glycin molecules may later crammed the holes in the membrane surface, so the equilibrium value of the concentration was just about 360mg/L. However, when the temperature was up to 60℃, the permeation flux of L-glycin shown a rapid increase and the equilibrium value of the concentration reached 960mg/L, showing an improvement by 167%. This is because that when the temperature exceed the T_s, TSPU membrane undergone a phase transition from the glassy state to the rubbery state, a large increase of free-volume (FV) holes sizes and an enhanced micro-Brownian motion lead the L-glycin permeate the TSPU membrane quickly, showing sensitivity to temperature stimuli permeation of L-glycin, and it can be used to separate the amino acid smartly by manipulating the temperature.

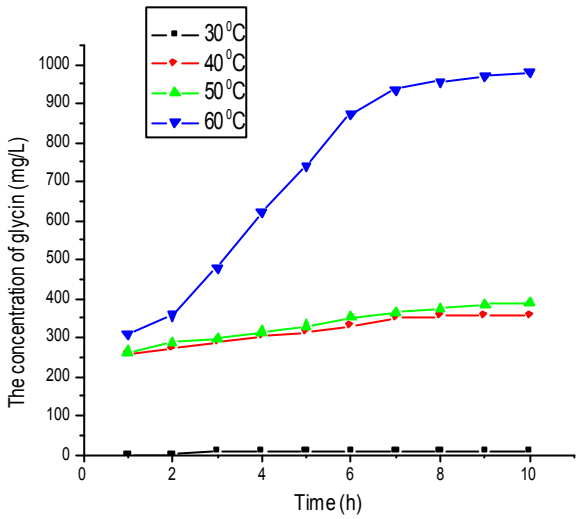


Fig. 8 The concentration of glycin changed with the temperature and time

Just as mentioned above, glutin, because of large molecules size, can not permeate the TSPU membrane in all temperature range. As a result, Molecules of NaCl, glycin and glutin with different size can be selective separated with the TSPU membrane driven by temperature. The ions of Na^+ and Cl^- can permeate the TSPU membrane easily at low temperature, while the glycin can be separated from glutin by rising the temperature to above 40℃, and glutin with large molecule size still remains in the residual solution.

The porous size scale of the TSPU membrane prepared with the wet inversion process can be controlled by temperature and show sensitivity to temperature stimuli, provides a wide application in selective separation for molecules with different size, such as separation of gas or amino acids, purification of dyestuffs, desalt of protein and so on.

4 Conclusions

Through the analysis and discussion above, this paper intends to provide the following tentative conclusions:

(1) The TSPU membrane has a phase-separated structure, and the soft segment and hard segment has

the respective crystalline phase, moreover, the T_s of the TSPU membrane was 50.6℃.

(2) The TSPU membrane prepared with the wet inversion process has dense surface and porous cross-section structure, when the temperature varied from T_s-10℃ to T_s+10℃, the porous hole size of this TSPU membrane increased rapidly, and shown sensitivity to temperature stimuli.

The double surface topography of the TSPU membrane has just a few of small pores with size scale lower 20 nm, and the path of the pores on cross-section is flexural and asymmetric, and the pore size value is about 1μm.

(3) Molecules with different sizes can be selective separated with the TSPU membrane driven by temperature. The ions of Na^+ and Cl^- can permeate the TSPU membrane easily at low temperature, while the glycin can be separated from the mixture solution by rising the temperature to above 40℃, and glutin with large molecule size, can not permeate the TSPU membrane in all temperature range.

Acknowledgements

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