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Clinical dextran purified by fractional ultrafiltration coupled with water washing

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ABSTRACT

Dextran with extremely narrow molecular weight distribution (MWD) is demanded for clinical use. To elucidate the effect of fractional ultrafiltration on the purification of clinical dextran, a range of ultrafiltration membranes with 100, 30, 5 and 1 kDa molecular weight cut-off (MWCO) were applied. High Performance Gel Permeation Chromatography (HPGPC) analysis indicated that the MWD of ultrafiltrated dextran fractions are wide, with polydispersity index (D) between 2.2 and 5.4, suggesting that the MWD of dextran is hard to be strictly controlled by fractional ultrafitration. However, when coupled with water washing during ultrafiltration process, the homogeneity of dextran was greatly improved. An ultrafiltration fraction of 5–30 kDa (Mw ≈ 35 kDa) with narrow MWD (D = 1.2) was obtained after 5 times of water washing. The results show that fractional ultrafiltration coupled with water washing can be used as a simple and effective method to improve the quality of clinical dextran.

1. Introduction

Dextran is a polysaccharide composed of D-glucose units with predominantly α-(1→6) linkages in the main chain and α-(1→2), α-(1→3) and α-(1→4) linkages in the branch (Robyt, Yoon, & Mukerjea, 2008; Vettori, Mukerjea, & Robyt, 2011). It is characterized by the flexible random coil structure and a high solubility in water (Kawakita et al., 2008; Seto, Ohto, & Kawakita, 2011). The flexible and extended properties are conferred by α-(1→6)-linked backbone (Belder, 2003). Several reports suggest that solvent is of considerable importance in solution conformation of dextran (Belder, 2003; Heinze, Liebert, Heublein, & Hornig, 2006; Seto et al., 2011). Dextran exhibits quite compact structure in poor solvents such as methanol and ethylene glycol, but its structure could considerably expand in good solvents including water, formamide, DMSO, DMA/LiCl and glycerol (Belder, 2003; Heinze et al., 2006). Seto et al. (2011) found that the dextran chains, which are produced by enzymatic reaction on the surface of the membrane, exhibit reversible extension and shrinkage in water and methanol–water solution.

Previous investigations have demonstrated great applications of soluble dextran in food, pharmaceutical and fine chemical industries (Gloria Hernández, Livings, Aguilera, & Chiralt, 2011; Janciauskaite, Rakutyte, Miskinis, & Makuska, 2008; Strüber et al., 2001) because of its non-immunity, non-toxicity, biodegradability and biocompatibility (Vollmer et al., 2009). Moreover, different molecular weights of dextrans and their derivatives have shown effective medical functions in clinic, such as plasma substitute, excipient of pharmaceutical tablets (Gil et al., 2008), blood expanders, antithrombotic agents (Jones et al., 2008) and so on. However, the structural diversity and complexity of branches make it difficult to achieve a selective and reliable control of the molecular weight of dextran. As a result of the poor quality of dextran and its derivatives, allergy and other side effects in clinic have been reported (Bircher, Hédin, & Berglund, 1995; Faich & Strobos, 1999).

Extensive work has been done on controlling the molecular weight of dextran. A traditional method for controlled-molecular-weight dextran is acid hydrolysis coupled with organic solvent fractional precipitation (Purama, Goswami, Khan, & Goyal, 2009), which produced molecular weights of dextrans with a wide range of a few thousand to several million Daltons. In addition, this process not only consumes a large number of organic solvents, but also brings losses which would lead to a relatively low yield of dextran. Another method suggested by Mountzouris, GilmourI, and Rastall (2001) is enzymatic hydrolysis of industrial grade dextran (Mw: 5×10^3–40×10^3 kDa) from *Leuconostoc mesenteroides* using an endodextranase in an enzyme membrane reactor, which can produce a relatively low molecular weight (Mw < 10 kDa) dextran. A recent study suggested that dextrans with different molecular weights can be synthesized by selecting the concentrations of dextransucrase, sucrose and temperature (Falconer, Mukerjea, & Robyt, 2011). It has been showed that a combined enzyme system of dextransucrase and dextranase can be used to produce isomaltooligosaccha-

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rides and oligodextrans (Mw < 20 kDa) for prebiotic use, where dextransucrase was for the synthesis of dextrans and dextranase was for the regulation of molecular weights (Goulas, Fisher, Grimble, Grandison, & Rastall, 2004). However, dextran product of these enzymatic methods needs further purification for specific purposes.

Ultrafiltration, a low energy-consuming and easy operating technology, is drawing extensive attention in the application of separation and purification recently. From the research made by Chen et al. (2008), a new method called electric ultrafiltration coupled with solvent crystallization showed great potential of ultrafiltration to control the MWD of clinical dextran.

In the present study, fractional ultrafiltration was applied to investigate its feasibility on controlling the molecular weight of clinical dextran. In addition, repeated water washing of the ultrafiltrated dextran fraction was carried out to confirm the extension of dexran coils resulting from the flexibility of random coil structure. It is expected that clinical dextran will be further purified with our proposed method, which is simple, rapid and economical.

2. Experimental

2.1. Materials

Dextran-40 (dex-40) was purchased from Guilin Pharmaceutical Co. (Guilin, China). Dextran standards were obtained from Polymer Laboratories Ltd. with a molecular weight range of 5900, 11,800, 22,800, 47,300, 112,000, 210,000, 404,000 Da. Hollow fiber ultrafiltration system with a series of membranes with MWCO of 100, 30, 5 and 1 kDa were obtained from General Electric of the US.

2.2. Methods

2.2.1. Separation of dextran by fractional ultrafiltration

Dex-40 was dissolved and diluted to 1 g/L of solution with deionized water. The solution was firstly pumped to the 100 kDa membrane at a transmembrane pressure of 0.2 MPa, and the permeate was collected as feed for the 30 kDa membrane. The same operations were performed to the 1 kDa membrane.

Permeate flux (F) of each membrane in L/(m^2 h) (LMH) during fractional ultrafiltration was calculated using the following equation:

$$F = \frac{V}{S \cdot t} \quad (1)$$

where V is the volume of permeate (L); S is the total membrane surface (m^2) and t is the ultrafiltration time (min).

Retentates of these three membranes and permeate of 1 kDa membrane were collected respectively using rotary evaporation and vacuum dehydration. Thus, dex-40 was divided into four fractions: >100 kDa, 30–100 kDa, 1–30 kDa and <1 kDa in terms of different MWCO of ultrafiltration membranes.

2.2.2. Process of fractional ultrafiltration coupled with water washing

Hollow fiber membranes with MWCO of 30 kDa and 5 kDa were employed in this section in order to obtain high membrane flux, and deionized water was used in water washing process. 5 g/L of dex-40 was firstly pumped through the 30 kDa membrane at a transmembrane pressure of 0.2 MPa. The permeate was collected as feed for the 5 kDa membrane. During washing process, retentate of 5 kDa membrane was sent back to the feed tank (the volume of the feed tank is 1 L). When the volume of solution in the feed tank dropped to 200 mL, 200 mL deioned water was added. The same treatment to the solution in the feed tank was repeated for six times and each washing sample was dried to constant weight.

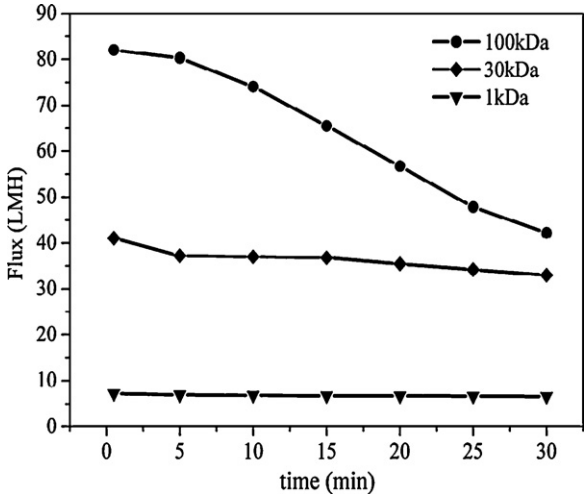

Fig. 1. Temporal variation of flux for different ultrafitration membranes.

2.2.3. High Performance Gel Permeation Chromatography (HPGPC) determination

The MWD of dex-40 and dextran fractions obtained from ultrafitration were measured by Agilent 1100 series GPC system (Agilent, USA), equipped with Agilent G1362A differential refraction detector. TSK-Guard SW (7.5 mm × 75 mm) guard column, TSK 4000 PW (7.5 mm × 300 mm) and TSK 3000 PW (7.5 mm × 300 mm) gel columns were connected in series at 23.0 ± 0.1 °C; ultrapure water was used as the mobile phase at a flow rate of 1 mL/min. All samples were diluted to 10 mg/mL solutions and filtered using 0.45 μm membranes (Membrana, Germany); 20 μL of solution was analyzed in each run. The MWD information such as weight-average molar mass (Mw), number-average molecular weight (Mn) and D value (Mw/Mn) that indicates the polydispersity of dextran were given by the Agilent GPC Data Analysis Software, based on the dextran calibration curve generated by dextran standards. The relative percentages of each ultrafiltration fraction in different molecular weight divisions were obtained using peak area normalization method.

3. Results and discussion

3.1. Permeate flux of each ultrafiltration process

The initial flux was computed using the volume of permeate obtained in the first 0.5 min, then permeate was collected every 5 min in order to calculate the flux. Permeate fluxes of three different membranes are shown in Fig. 1. Results indicated a decrease of the average permeate flux of each ultrafiltration fraction with the decreasing MWCO of membranes. A high initial flux of 82.1 L/(m^2 h) was obtained using 100 kDa membrane, but a sharp decline were observed during the 30 min filtration process, which were possibly caused by the concentration polarization and membrane fouling. In contrast, stable fluxes with slightly decrease were obtained for 30 kDa and 1 kDa membranes with average fluxes of 36.4 and 6.8 L/(m^2 h), respectively. However, flux of 1 kDa membrane was so low that it was not suitable for the fractional ultralfiltration process. Therefore, membranes (30 kDa or 5 kDa) with stable and relatively high permeate flux are more favorable for dextran separation.

3.2. Effect of fractional ultrafiltration on the MWD of dextran

HPGPC is an analytical technique used to separate components based on their molecular size in solution, which is useful for rapid

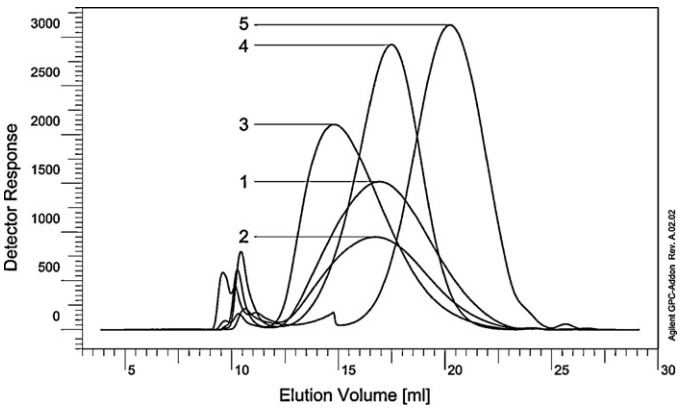

Fig. 2. Elution volume of dex-40 and ultrafiltration fractions: (1) dex-40, (2) >100 kDa fraction, (3) 30–100 kDa fraction, (4) 1–30 kDa fraction, (5) <1 kDa fraction.

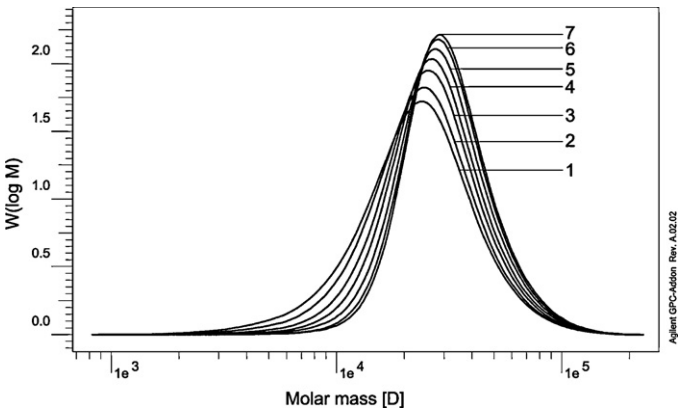

Fig. 3. Effect of washing times on the MWD of the 5–30 kDa fraction: (1) control, (2–7) with 1–6 times washing, respectively.

and reliable characterization of polymer molecular weight and MWD (Alsop & Vlachogiannis, 1982; Nilsoon & Nilsson, 1974). Thus, dext-40 and four fractions (>100 kDa, 30–100 kDa, 1–30 kDa and <1 kDa) separated by fractional ultrafiltration were analyzed using HPGPC to verify the effect of fractional ultrafiltration on the polydispersity of dextran (Fig. 2). The differences of peak area indicated that molecular weights of dextrans can be controlled in a certain extent by fractional ultrafiltration, which made the MWD of fractions more concentrated.

Table 1 shows the relative percentages of dex-40 and ultrafiltrated fractions in different molecular weight divisions. From the view of *D* values, fractions treated with bilateral membranes (30–100 kDa and 1–30 kDa fractions which had *D* values of 2.2 and 2.5 respectively) had a better control in MWD than that with unilateral membrane (>100 kDa and <1 kDa fractions). It was found that the molecular weights of fractions were more concentrated in 10^3–3×10^4 Da and 3×10^4–10^5 Da divisions, indicating the poor effect of fractional ultrafiltrantion on controlling the molecular weights of dextrans. There were no more than 64% of the 1–30 kDa fraction had a molecular weight of 10^3–3×10^4 Da and 59% of the 30–100 kDa fraction had a molecular weight of 3×10^4–10^5 Da. Additionally, none but 16% of the >100 kDa fraction and 6% of the <1 kDa fraction had molecular weights of $>10^5$ Da and $<10^3$ Da, respectively. These results are in agreement with results of Bohrer, Patterson, and Carroll (1984) that dextran diffuses more readily through the membrane. Such a wide difference between the molecular weights of ultrafiltrated dextran fractions and MWCO of ultrafiltration membranes implied that fractional ultrafiltration is not an effective method to achieve a strict control on the molecular weights of dextrans. Applications of dextran fractions could be limited in clinical practice due to the poor polydispersity. Therefore, MWCO of ultrafiltration membranes cannot provide an exact definition to the molecular weights of polysaccharides, which differs from the separation of protein by ultrafiltration.

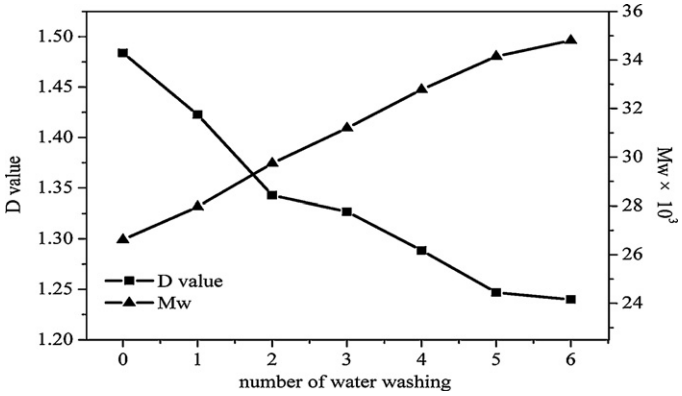

Fig. 4. Effect of washing times on Mw and *D* value of the 5–30 kDa fraction.

3.3. Dextran fraction purified by water washing

Dextran is randomly branched; the main chain and random branches may pucker up and intertwine in the three-dimensional space resulting in a rather compact structure (Vollmer et al., 2009). However, water is a good solvent for the extension of dextran, and data have confirmed the reversible extension and shrinkage of dextran structure in water and methanol–water solution (Seto et al., 2011). Therefore, in the present study, the combination of fractional ultrafiltration and water washing was applied to improve the purification effect of clinical dextran.

The effect of washing times on the MWD of 5–30 kDa dextran fraction is shown in Fig. 3. It can be seen that after water washing, the MWD of the fraction gradually narrowed and the molecular weight was increasing with washing times. Fig. 4 shows Mw and *D* value of the fraction as a function of washing times. The polydispersity of fraction as represented by *D* value was found to decrease from 1.5 to 1.2 and the Mw of fraction increased from 27 kDa to 35 kDa after five times washing, indicating the more homogeneous

Table 1
Molecular weight distributions of dex-40 and ultrafiltration fractions.

Dextrans	Molecular weight distribution (%)				Mw	*D*
	$<10^3$	10^3–3×10^4	3×10^4–10^5	$>10^5$ Da[a]		
dex-40	2	54	39	5	47,818	2.8
>100 kDa fraction	–	48	36	16	116,060	5.4
30–100 kDa fraction	–	27	59	14	73,279	2.2
1–30 kDa fraction	–	64	30	6	54,597	2.5
<1 kDa fraction	6	90	2	2	17,975	3.4

[a] Molecular weights divided into four divisions.

molecular weight of the purified dextran. It is noteworthy that variations of Mw and *D* values are negligible over five times washing.

This observation is of interest, which could be explained by the flexibility of the random coil structure of dextran. Those compact dextrans behave in solution as extendable structures (Belder, 2003) during water washing process, which could contribute to the selectivity of ultrafiltration separation for clinical dextran.

4. Conclusions

Water washing had a positive effect on the polydispersity of dextran during ultrafiltration process, which may be attributed to the extension of branching dextran in the water solution. An ultrafiltration fraction of 5–30 kDa (Mw ≈ 35 kDa) with narrow MWD ($D = 1.2$) was obtained using fractional ultrafiltration coupled with five times of water washing. This method is valuable in the purification of clinical dextran, which can achieve a simple and rapid production of high quality dextran with narrow MWD.

Different from the purification of protein by ultrafiltration, molecular weights of dextran fractions separated by different MWCO ultrafiltration membranes are in poor agreement with the real molecular weights measured by HPGPC. It is considered inappropriate to exactly define the molecular weight of dextran by the MWCO of ultrafiltration membrane. Therefore, much attention should be paid to the separation of dextran using fractional ultrafiltration, especially for the separation of water-soluble branching macromolecules. For the high concentration of ultrafiltration solutions, water washing could improve the selective performance of fractional ultrafiltration.

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