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셀룰로스의 분석을 위한 새로운 시료처리 및 크기배제크로마토그래피

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A New Sampling and SEC Method for Analysis of Underivatized Cellulose

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요 약. 셀룰로스시료를 치환체로 만들지 않고 분자량 및 분자량분포를 결정하기 위한 새로운 시료처리 및 크기배제크로마토그래피방법을 확립하였다. 먼저 셀룰로스시료를 N-methylmorpholine N-oxide(NMMO)에 녹인 후 dimethyl sulfoxide(DMSO)에 희석함으로써 50/50(w/w) NMMO/DMSO에 녹은 약 0.1%의 시료용액을 만들었다. 컬럼으로는 글루코오스를 입힌 polydivinylbenzene 겔을 채운 크기배제크로마토그래피용 컬럼을 이용하였으며 용리액으로는 0.05 M의 LiBr와 2.5%의 바탕용액을 포함하는 DMSO를 이용하였다. 유속은 1 mL/min로 일정하게 유지하였고, DMSO의 점도를 낮추기 위해 컬럼을 포함하는 전체 시스템을 80 °C로 유지하였다. 0.05 M의 LiBr를 가함으로써 SEC 바탕선의 표류를 방지할 수 있었고 2.5%의 바탕용액을 가함으로써 시료와 컬럼충진물질 사이의 상호작용을 감소시키는 효과를 얻었다. 좁은 분포를 가지는 pullulan표준물질로부터 얻은 컬럼보정곡선을 이용하여 두 개의 펄프시료와 두 개의 셀룰로스 스펀지 시료의 평균분자량을 결정함으로써 펄프로부터 스펀지를 만드는 서로 다른 두 가지 공정에서의 셀룰로스 분자들의 분해정도를 결정, 비교할 수 있었다.

ABSTRACT. A new sampling and size exclusion chromatography (SEC) method for the analysis of underivatized cellulose are established. In this method, cellulose materials are first dissolved in N-methylmorpholine N-oxide (NMMO) and diluted by adding dimethyl sulfoxide (DMSO) to make the sample solutions of about 0.1% in 50/50 NMMO/DMSO (w/w). Sample solutions are analyzed using a glucose-treated divinylbenzene (DVB) SEC column and DMSO containing 0.05 M LiBr and 2.5% blank as the eluant. The flow rate was constant at 1 mL/min and the whole SEC system including the column was heated at 80 °C to reduce the viscosity of DMSO. Addition of 0.05 M LiBr eliminated SEC baseline drifting, and addition of 2.5% blank seems to reduce the interaction between the sample and the column packing. SEC molecular weights were determined using a calibration curve constructed from a series of narrow pullulan standards, and they were used to measure the degree of degradation during two different pulp-to-sponge processings.

INTRODUCTION

As the most abundant and reproducible polymer of β-D-glucopyranose, cellulose and its derivatives have been found broad industrial applications, such as in paper, fiber, sponge, plastic, membrane, and food, etc.[1] However cellulose has still not reached it's potential utility in many areas of application. One of major reasons for this is that many end-use applications require cellulose be in a different form from that found in nature. In most of these ap-

plications, it is necessary first to dissolve cellulose and then process it from such solutions into the desired products. Unfortunately cellulose is not easily dissolved in solvents of general use, and dissolving steps of most existing cellulose solvent systems are considered to be cumbersome or expensive. A need for more efficient and economical cellulose solvent systems has long been recognized, and many reports have been published covering wide range of approaches.[2-16]

Finding an efficient solvent system for cellulose is also important for determination of molecular weight distribution of such materials. Information on molecular weight and/or its distribution provides a better understanding of processing mechanisms of both existing and potential applications of cellulose materials. Size exclusion chromatography (SEC) separates polymers based on their hydrodynamic size, and is widely used for determination of molecular weight distribution of various polymers. Because of the problem associated with obtaining solutions of cellulose, most of SEC work for cellulose has been done for cellulose derivatives such as the nitro-, acetyl-, carbanyl- and carboxymethyl compounds. Although SEC analysis of these cellulose derivatives is relatively simple, some of drawbacks associated with these derivatization procedure are that they often lead to degradation of cellulose molecules, and are wasteful in terms of high sample consumption, inconvenience and time-consuming, sometimes requiring several days to complete.

Many solvent systems have been employed to dissolve underivatized cellulose. However, most of them are limited because of their poor compatibility with packing materials of currently available SEC columns. Therefore common solvent systems are not suitable as a solvent for SEC analysis of cellulose materials. Only a couple of reports on analysis of underivatized cellulose by SEC were reported.[17,18] One is the use of Cadoxen, an exotic metal chelate complexing agent.[17] Cadoxen is an ethylene diamine complex of cadmium, $[Cd(en)_3(OH)_2]$, which possesses a high solvent power for cellulose, producing solutions of reasonable stability and has good optical properties as a SEC eluant. The sample is dissolved in Cadoxen, and then analyzed on low pressure, moderate efficiency SEC columns which must be packed by the operator. However, Cadoxen solutions of cellulose have low thermal stability and high viscosity, and the elution time changes due to the reduction in column efficiency.

Dimethyl acetamide (DMAc) containing a small amount of LiCl has also been used as an eluant/solvent for SEC analysis of underivatized cellulose fibers.[18] The solution was prepared by first swelling the cellulose fibers and activating their pores in water and then replacing the water with dry DMAc. The cellulose was then dissolved in DMAc containing 6% LiCl. The solution was diluted by adding more DMAc to make the sample solution of 0.1% cellulose in DMAc containing 0.5% LiCl. The advantage of this method over the previously described Cadoxen method is that it allows the use of high efficiency, high performance SEC columns (HPSEC). In this method, the retention of polystyrene standards increases as the column temperature is lowered, which implies the standards are adsorbing onto the polystyrene gel column, and this effect increases as the column temperature decreases. It has also been pointed out that this method lacks reproducibility. Despite much effort and need to find an efficient and reliable SEC method, no single method stands out as the method for SEC analysis of cellulose.

The goal of this study is to develop an efficient and reliable HPSEC method for analysis of cellulose without derivatizing it. The first essential step is to find a solvent for cellulose that is suitable for SEC. It has been reported that tertiary amine oxides might be suitable solvents for underivatized cellulose.[13] Among the amine oxide family, N-methylmorpholine N-oxide (NMMO) has been found to be the best solvent for underivatized cellulose.[14-16] NMMO has several advantages as a solvent for SEC analysis of cellulose. NMMO can dissolve underivatized cellulose, and is non-toxic, fully recoverable and therefore non-polluting. More importantly, NMMO solution of

cellulose is miscible with dimethyl sulfoxide (DMSO) which is being widely used as a SEC carrier/solvent.

EXPERIMENTAL

Size exclusion chromatography (SEC). The Waters 150C High Temperature SEC instrument was used for SEC experiments. SEC column was 1× Jordi Gel DVB Linear Mixed Bed GBR column (10×500 mm) obtained from Jordi Associate (Bellingham, MA, USA). The column was calibrated using eight narrow Pullulan standards purchased from the Waters Associate (Milford, MA, U.S.A.), and reported molecular weights are not absolute but pullulan-equivalent molecular weights. SEC eluant was DMSO containing small amount of LiBr (0.05 or 0.1 M) and the blank (2.5 or 5%). Flow rate was kept constant at 1 mL/min throughout this study. Sample solutions were filtered through a 0.2 micron Nylon disposable syringe filter before the injection. The injection volume was 200 microliters. Detector was a refractive index detector that is housed inside of the Waters 150C instrument. The whole system was heated at 80 °C to reduce the viscosity of the eluant. SEC data were collected and processed using Polymer Labs' PL Caliber software.

Materials. Two pulp and two regenerated cellulose sponge products were used for the cellulose sample. The cellulose sponge samples are two different dish-cleaning sponge products that are currently available in the market, and are named as "sponge-1" and "sponge-2" respectively for the sake of discussion. Two pulp samples are those from which the "sponge-1" and "sponge-2" products are made, and are named as "pulp-1" and "pulp-2" (the "sponge-1" is made from the "pulp-1" and the "sponge-2" from the "pulp-2", respectively).

Sample preparation. Dry cellulose (pulp or sponge) samples were grounded down into small pieces in a food blender, and transferred into a rotary vacuum evaporator. 50/50 NMMO/Water is then added into the rotary evaporator, and is slowly heated in a water bath. As temperature increases, water evaporates out and NMMO precipitates out. At further increase in temperature, NMMO is slowly melted, and at the same time, the ground cellulose pieces are slowly dissolved into the molten NMMO to become a clear brown solution. DMSO is then added into the evaporator to dilute the solution down to make the sample solution of about 0.1% cellulose in 50/50 (w/w) NMMO/DMSO.

RESULTS AND DISCUSSIONS

Among SEC columns that are known to be compatible with DMSO, a Jordi Gel DVB Linear Mixed Bed GBR column is chosen for cellulose analysis. This column is packed with polydivinylbenzene (DVB) gel whose surface is modified with glucose, and is expected to work well with relatively hydrophilic solvents such as water or DMSO. Because of the large number of aromatic rings inherent in the packing's structure, samples containing aromatic rings or atoms such as oxygen or nitrogen with unshared electron pairs may be retained strongly on this column causing a peak tailing. Sometimes the addition of a small amount of competing electron-rich solvent such as acetonitrile or a salt such as LiBr into the mobile phase reduces the interaction between the sample and the column packing.

Fig. 1 shows SEC chromatograms of the blank obtained with various carrier compositions. The blank was prepared by the same procedure as the sample preparation method described earlier in the experimental section except that the cellulose was absent. The carriers for *Fig.* 1-A, B, C, D, and E are pure DMSO, DMSO containing 0.05 M LiBr, DMSO containing 0.1 M LiBr, DMSO containing 0.1 M LiBr and 2.5% blank, and DMSO containing 0.1 M LiBr and 5% blank, respectively. The flow rate was kept constant at 1 mL/min, and the whole system including the column was held at 80 °C to reduce the viscosity of DMSO. Use of pure DMSO as the SEC eluant resulted in the baseline drifting as seen in *Fig.* 1-A. The baseline starts drifting up at around 13 min, and goes down

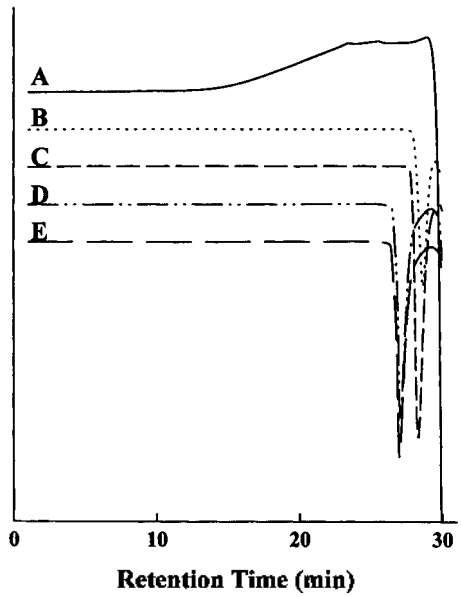

Fig. 1. SEC traces of blank (50/50 NMMO/DMSO) obtained with various eluant compositions. Eluant for A= pure DMSO, B=DMSO containing 0.05 M LiBr, C= DMSO containing 0.1 M LiBr, D=DMSO containing 0.1 M LiBr and 2.5% blank, and E=DMSO containing 0.1 M LiBr and 5% blank, respectively. SEC column is 1× Jordi Gel DVB Linear Mixed Bed GBR column (10× 500 mm), and the flow rate=1 mL/min. The system was heated at 80 °C.

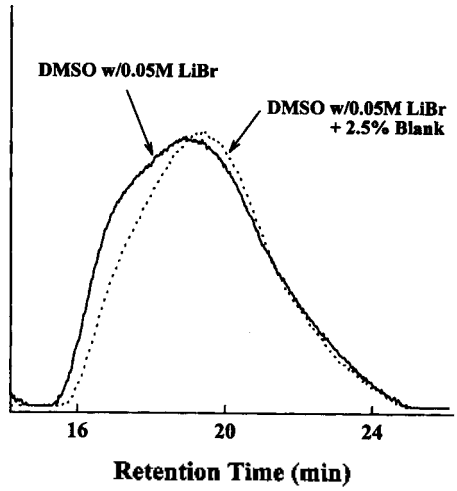

Fig. 2. SEC chromatograms of "sponge-1" sample obtained in two different eluant compositions. Flow rate and temperature are the same as those in *Fig.* 1.

quickly at about 30 min, yielding a very high negative peak. The high negative peak appearing at around 30 min is probably due to the adsorption of NMMO onto the column. When 0.05 M of LiBr was added into DMSO, the baseline became flat and stable as seen in *Fig.* 1-B. The negative NMMO adsorption peak became smaller and eluted earlier, indicating reduction of adsorption. Addition of LiBr at higher concentration (0.1 M) did not make a significant difference in SEC baseline (*Fig.* 1-C). It is known that the addition of a small amount of the blank into the eluant may reduce the interaction between the sample and the column packing. Addition of 2.5% of blank into DMSO/0.05 M LiBr did not remove the NMMO adsorption peak, but made the adsorption peak elute earlier, indicating further reduction of the adsorption (*Fig.* 1-C). Addition of higher concentration of blank at 5% (*Fig.* 1-E) did not make a significant difference.

To investigate the effect of the carrier composition on SEC peak area and on the molecular weight results in more detail, the "sponge-1" sample was run in 4 different carrier compositions (DMSO containing 0.05 M LiBr, DMSO containing 0.1 M LiBr, DMSO containing 0.05 M LiBr and 2.5% blank, and DMSO containing 0.05 M LiBr and 5% blank) at the same flow rate and the system temperature as in *Fig.* 1. *Fig.* 2 shows two SEC chromatograms of the "sponge-1" sample obtained in two different carrier compositions (DMSO containing 0.05 M LiBr and DMSO containing 0.05 M LiBr and 2.5% blank). The chromatograms obtained in DMSO containing 0.1 M LiBr and in DMSO containing 0.05 M LiBr and 5% blank are not shown as they are almost identical as those obtained in DMSO containing 0.05 M LiBr and in DMSO containing 0.05 M LiBr and 2.5% blank, respectively. Apparently the addition of LiBr at 0.05 M or 0.1 M does not make a significant difference, and nor does the addition of the blank at 2.5% or 5%.

The peak areas and the average molecular weights calculated for chromatograms obtained in four different carrier compositions are summarized in *Table* 1 and 2. In *Table* 1, no significant diff-

Table 1. SEC peak areas of "sponge-1" sample obtained with various eluant compositions

Carrier Composition	Peak Area
DMSO w/0.05 M LiBr	2.8×10^4
DMSO w/0.1 M LiBr	2.8×10^4
DMSO w/0.05 M LiBr and 2.5% blank	3.0×10^4
DMSO w/0.05 M LiBr and 5% blank	3.1×10^4

erence was observed in the peak area between peaks obtained with 0.05 M and 0.1 M LiBr, and between peaks obtained with 2.5% and 5% blank as expected from *Fig.* 2. Addition of the blank into DMSO however resulted in slightly higher peak area, indicating a reduction in the interaction between sample and the column packing.

In *Table* 2, no significant difference was observed between the average molecular weights and polydispersities obtained with DMSO w/ 0.05 M and 0.1 M LiBr as was in the case of the peak area. The polydispersity index (P), determined by M_w/M_n, is a measure of the distribution's broadness which indicates the broadness of a distribution increases. Addition of the blank yielded somewhat higher average molecular weight and lower polydispersity index. The lower polydispersity means the distribution is narrower, and it indicates the addition of blank improves the chromatogram by reducing the interaction between the sample and the column packing materials. Based on the results shown in *Table* 1 and 2, DMSO containing 0.05 M LiBr and 2.5% blank seems to be the best SEC eluant system for cellulose, and was thus chosen in this study.

It is well known that SEC column(s) need to be calibrated for the determination of molecular weight of a sample. Narrow pullulan standards were employed in this study to construct a calibration curve. Pullulan has merits as a calibration standard for SEC study of cellulose in DMSO. Pullulan is a kind of polysaccharide having a similar chemical structure as cellulose, very soluble in DMSO, and is easily available commercially. Eight pullulan standards were divided into two groups to make two mixtures of standards, and the two mixtures were run separately with the carrier chosen above (DMSO containing 0.05 M LiBr and 2.5% blank) at the same flow rate and the system temperature as in *Fig.* 1.

A set of mixture consisting of four standards having nominal molecular weights of 1.6×10^6, 2.1×10^5, 4.8×10^4, 1.2×10^4 and the other set 3.8×10^5, 1.0×10^5, 2.4×10^4, 5.8×10^3, respectively. *Fig.* 3 shows a calibration curve constructed from those eight pullulan standards. The open circles are the experimental data points and the solid line is the result of a curve-fitting using a third order polynomial.

Fig. 4 shows SEC chromatograms of all four samples obtained in DMSO containing 0.05 M LiBr and 2.5% blank at the same flow rate and the system temperature as shown in *Fig.* 1. As is known, SEC elution time increases as the hydrodynamic size (the molecular weight) of the sample decreases, and thus a SEC elution profile directly reveals the molecular weight distribution of the sample. The molecular weight distributions of the "pulp-1" and "pulp-2" shift toward higher retention time (or toward lower molecular weight) as they are processed into the "sponge-1" and "sponge-2", which indicates both processings accompany degradation of cellulose molecules. The

Table 2. SEC molecular weights of Sponge-1 obtained at various eluant compositions

Carrier Composition	M_n[a]	M_w[b]	M_z[c]	P[d]
DMSO w/0.05 M LiBr	6.7×10^4	3.1×10^5	8.1×10^5	4.6
DMSO w/0.1 M LiBr	6.6×10^4	3.0×10^5	8.0×10^5	4.5
DMSO w/0.05 M LiBr and 2.5% blank	7.6×10^4	3.3×10^5	1.1×10^6	4.3
DMSO w/0.05 M LiBr and 5% blank	7.7×10^4	3.2×10^5	1.1×10^6	4.2

[a]M_n=number-average molecular weight; [b]M_w=weight-average molecular weight; [c]M_z=z-average molecular weight; [d]P=polydispersity index ($=M_w/M_n$)

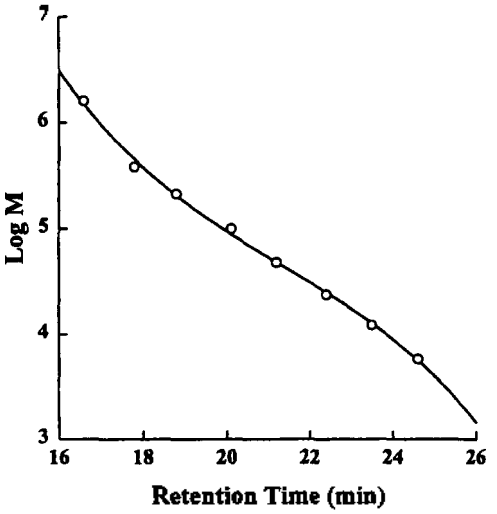

Fig. 3. A calibration curve constructed with eight pullulan standards having nominal molecular weights of 1.6×10^6, 3.8×10^5, 2.1×10^5, 1.0×10^5, 4.8×10^4, 2.4×10^4, 1.2×10^4, and 5.8×10^3. Flow rate and temperature are the same as those in *Fig.* 1.

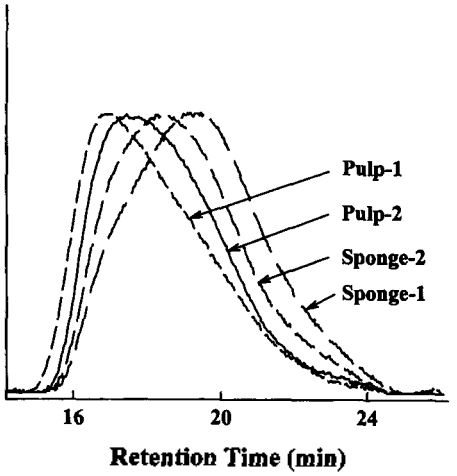

Fig. 4. SEC chromatograms of four cellulose materials obtained in DMSO containing 0.05 M LiBr and 2.5% Blank. Flow rate and temperature are the same as those in *Fig.* 1.

degree of the shift toward higher retention time, and thus the degree of degradation seems higher for the "pulp-1" than for the "pulp-2", meaning more degradation occurs during the "pulp-1"-to-"sponge-1" processing than during the "pulp-2"-to-"sponge-2" processing. The degree of degradation

Table 3. SEC molecular weights of cellulose materials obtained in DMSO containing 0.05 M LiBr and 2.5% blank as the eluant

Sample	M_n	M_w	M_z	P
Pulp-1	1.6×10^5	9.1×10^5	2.8×10^6	5.7
Sponge-1	7.6×10^4	3.3×10^5	1.1×10^6	4.3
%reduction	53	64	61	25
Pulp-2	1.3×10^5	6.0×10^5	1.7×10^6	4.7
Sponge-2	9.7×10^4	4.5×10^5	1.4×10^5	4.6
%reduction	25	25	18	2

can be measured quantitatively by determining average molecular weights of the samples. Average molecular weights determined for the chromatograms shown in *Fig.* 4 are summarized in *Table* 3.

As shown in *Fig.* 4, the molecular weights of the "sponge-1" are lower than those of the "pulp-1", and molecular weights of the "sponge-2" are lower than those of the "pulp-2" due to degradation of cellulose molecules during both processings. It is also shown that the polydispersity index, and thus the broadness of the molecular weight distribution, is also lowered during both sponge-making processings. The % reductions in average molecular weights are much higher for the "pulp-1"-to-"sponge-1" processing (about 60%) than that for the "pulp-2"-to-"sponge-2" processing (about 23%), indicating more degradation in the former than in the latter. The % reduction in the polydispersity index is also much higher for the "pulp-1"-to-"sponge-1" processing (about 25%) than that for the "pulp-2"-to-"sponge-2" processing (about 2%).

CONCLUSION

New methods for sample preparation and for SEC analysis are established for the molecular weight determination of cellulose materials without derivatizing them. In this new SEC method, samples are first dissolved in NMMO and diluted by DMSO to make the sample solutions of about 0.1% cellulose in 50/50 NMMO/DMSO (w/w). Sample solutions are then injected into a glucose-treated

DVB SEC column, and are eluted using DMSO containing 0.05 M LiBr and 2.5% blank as the eluant. The flow rate was kept constant at 1 mL/min and the whole SEC system including the column was heated at 80 °C to reduce the viscosity of DMSO. Addition of LiBr at 0.05 M into DMSO improved the stability of the SEC baseline, and addition of the blank at 2.5% seems to reduce the interaction between sample and the column packing. Addition of LiBr or the blank at higher concentrations did not make any significant differences. SEC molecular weights were determined using a calibration curve constructed from a series of narrow pullulan standards, and the results were used to compare the degree of degradation during two different pulp-to-sponge processings.

Further work is needed for better optimization of this SEC method in terms of the column temperature and flow rate, etc. With the better optimization, this new SEC method can provide accurate molecular weight informations of cellulose materials, which is valuable for understanding of various cellulose applications.

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