TOSOH

APPLICATION NOTE

CHARACTERIZATION OF HYDROXYETHYL STARCH USING GEL PERMEATION CHROMATOGRAPHY

INTRODUCTION

Hydroxyethyl starch (HES) is a natural polysaccharide evolved from amylopectin that has a wide range of applications in human health care such as a blood volume expander, plasma substitute, an anti-shock component in surgeries and anti-tumor for different cancer treatments. Conclusions from many research works show that, HES with various physiochemical properties have a great potential to be used in several medicinal treatments and different drug developments in pharmaceutical industries.

HES are polydisperse solutions of molecules with a broad range of molecular weights. HES differ in physicochemical properties depending on the average molecular weight and molecular weight distribution. Thus, the molecular weight of HES has to be carefully determined accurately so that it finds the right application. Due to the increasing potential of HES, we performed experiments to compare the molecular weights of two grades of HES using gel permeation chromatography. A conventional calibration curve was used to determine the molecular weight of HES samples in the current study. However, absolute molecular weight of HES can be determined by using additional light scattering detector.

EXPERIMENTAL CONDITIONS

Sample analysis was performed on an EcoSEC Ambient Temperature GPC System equipped with RI detector. Separation of 50 µL injections occurred over a bank of one TSKgel G6000PW, TSKgel G5000PW, TSKgel G3000PW, TSKgel G2000PW and a corresponding guard column. The mobile phase and solvent were sodium acetate trihydrate (0.04 mol/L)/ acetic acid at a flow rate of 0.6 mL/min.

The final sample concentrations were approximately 7.0 mg/mL. Data was processed with the EcoSEC GPC Workstation software. Molar mass averages were determined for each polymer sample using a calibration curve. A calibration curve for the column according to the experimental conditions was created using PEO/PEG standards (Figure 1). Calibration curve data was fitted with a cubic function and error values were less than 5%.

RESULTS AND DISCUSSION

The chromatograms obtained for the HES samples are presented in Figure 2. From Figure 2, HES 2 samples show a bimodal distribution with an intense peak at the high molecular weight region and a shoulder peak at the low molar mass region. However, HES 1 shows a mono modal distribution.

CALIBRATION CURVE

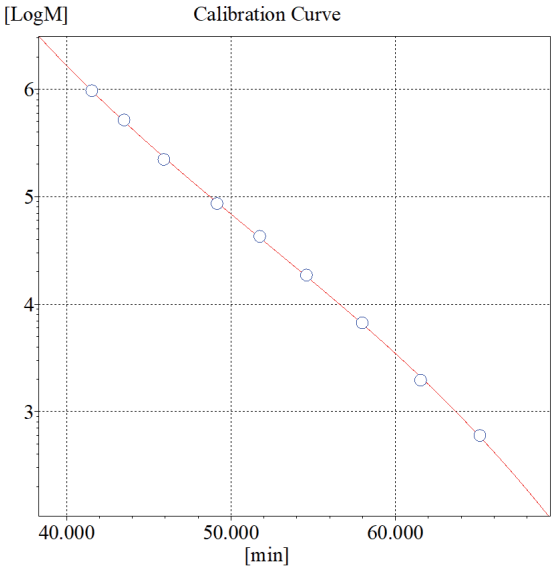

Figure 1

CHROMATOGRAM OBTAINED FOR THE HES 1 (RED) AND HES 2 (BLUE) SAMPLE

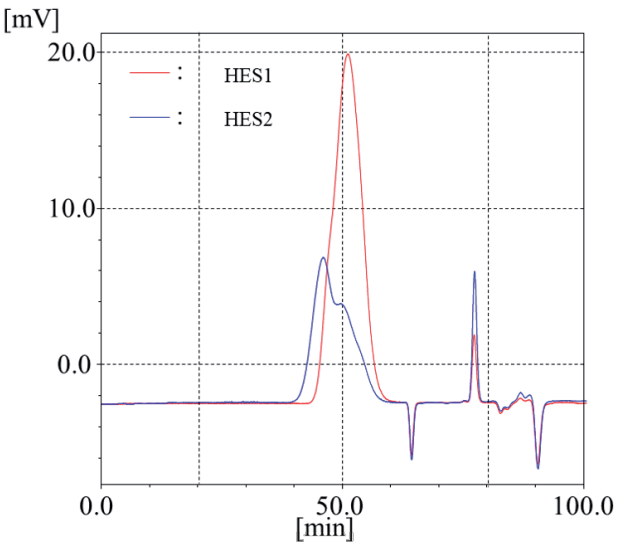

Figure 2

EcoSEC workstation was used for the calculations. The dedicated data analysis tab was used for the determination of MMD and the average molecular weights. Molar mass distribution of HES samples were determined using the PEO/PEG calibration curve (Figure 1). Figure 3 shows the differential distribution of the molar masses of HES 1 and HES 2 sample. Average molecular weights were calculated (Table 1).

DIFFERENTIAL AND INTEGRAL DISTRIBUTION OF MOLAR MASSES OF HES 1 AND HES 2 SAMPLE

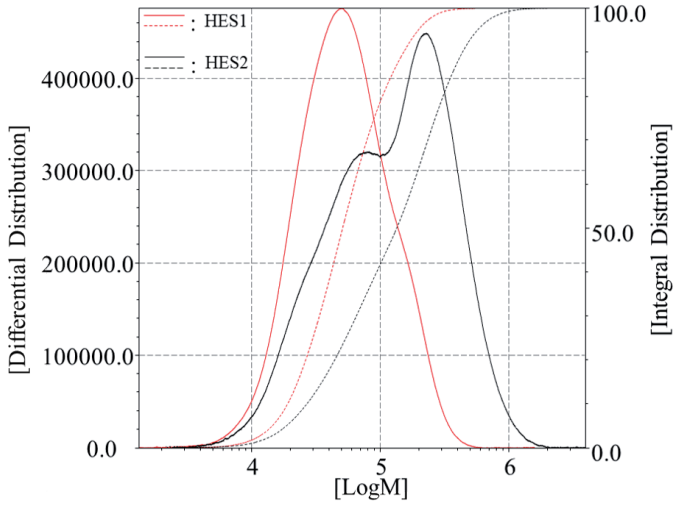

Figure 3

AVERAGE MOLECULAR WEIGHTS OF HES 1 AND HES 2

	Molecular weight (g/mol)			
Sample	Mn	Mw	Mz	PDI
HES 1	28409	53548	95697	1.8
HES 2	47178	144625	298977	3.0

Table 1

CONCLUSION

The molar mass averages and molar mass distributions of two HES samples: HES 1 and HES 2, were determined via a dual flow RI detector using the EcoSEC GPC System and a set of TSKgel PW GPC columns. The GPC analysis shows that HES 1 has a monomodal molar mass distribution and HES 2 has a bimodal molar mass distribution.