TOSOH

Application Note

Characterization of Hydroxyethyl Starch Using Gel Permeation Chromatography

Introduction

Hydroxyethyl starch (HES) is a natural polysaccharide derived 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 capabilities for different cancer treatments. Conclusions from many research works show that HES has great potential to be used in several medicinal treatments in the pharmaceutical market due to its various physicochemical properties.

HES can be obtained with a broad range of molecular weights and polydispersities. HES differs 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 fits a particular application. Due to the increasing potential use of HES, experiments were performed to compare the molecular weights of two grades of HES using gel permeation chromatography. In this study a conventional calibration curve was used to determine the molecular weight of HES samples. Absolute molecular weight of HES can also be determined using a light scattering detector.

Experimental Conditions

Sample analysis was performed on an EcoSEC® GPC System equipped with a RI detector. Separation of 50 µL injections occurred using one each of the following columns in series: TSKgel® G6000PW, TSKgel G5000PW, TSKgel G3000PW, and TSKgel G2000PW, along with 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. Molecular weight averages were determined for each of the HES polymer samples by conventional calibration. A calibration curve for the column series in the experimental conditions was created using PEO/PEG standards. 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*. The HES 2 sample shows a bimodal distribution with an intense peak at the high molecular weight region and a shoulder peak at the low molecular weight region. The HES 1 sample shows a monomodal distribution.

Figure 1. Calibration curve

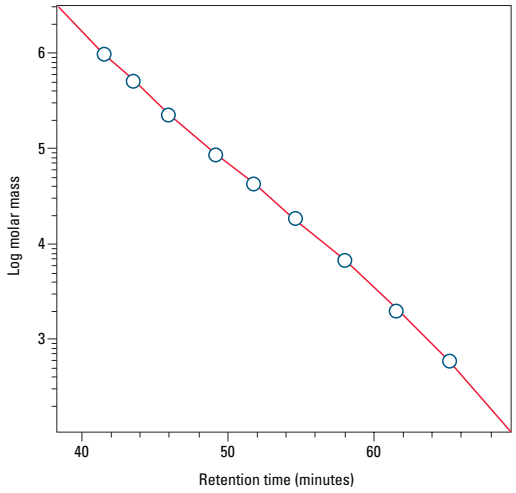
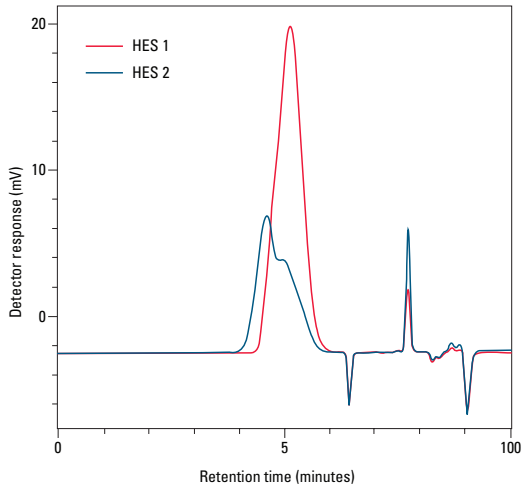


Figure 2. Chromatogram obtained for the HES 1 (red) and HES 2 (blue) sample



Data was processed with the EcoSEC GPC Workstation Software. Molecular weight distribution of the HES samples were determined using the PEO/PEG calibration curve *(Figure 1)*. *Figure 3* shows the differential distribution of the molecular weight of the HES 1 and HES 2 samples. Table 1 lists the calculated average molecular weights.

Figure 3. Differential and integral distribution of molecular weight of HES 1 and HES 2 samples

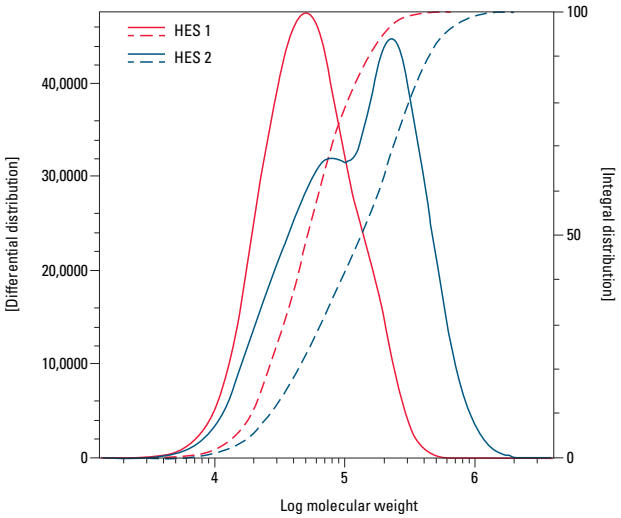


Table 1. Average molecular weights of HES 1 and HES 2

Molecular weight (g/mol)				
Sample	M_n	M_w	M_z	***PDI***
HES 1	28,409	53,548	95,697	1.8
HES 2	47,178	144,625	298,977	3.0

Conclusions

The molecular weight averages and molecular weight distributions of two HES samples, HES 1 and HES 2, were successfully 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 molecular weight distribution and HES 2 has a bimodal molecular weight distribution, which will lead to different properties of the end product.