JSB is an authorised partner of Agilent Technologies Value Added Reseller

#401

Analysis of cationic polymers by Size Exclusion Chromatography with TSK-GEL® PWXL-CP

Regina Roemling[1], Kuniyuki Tokunaga[2] and Hiroyuki Moriyama[2]
[1]Tosoh Bioscience GmbH, Stuttgart, Germany
[2]Tosoh Corporation, Tokyo, Japan

In recent years water soluble cationic polymers are gaining more and more interest in different applications ranging from hair conditioning to gene transfection and drug delivery. The molecular weight distribution of polymers is usually characterized by SEC coupled with refractive index, viscometric or laser light scattering detection. A challenge in aqueous SEC analysis of cationic polymers is the interaction between sample molecules and the packing material, which disturbs separation efficiency and reproducibility[1, 2]. Interactions can be suppressed with high salt eluents but at the risk of precipitation and poor recovery of the sample. We describe the separation of cationic polymers with a new generation of SEC columns, developed for the analysis of cationic polymers at low salt conditions.

CATIONIC POLYMERS

Water soluble cationic polymers can be designed with performance profiles for a broad range of different application fields. They are used in detergents and cleaners, as coagulants and flocculating agents in the paper industry and waste water purification as well as in many technical applications. Another application field is the cosmetics industry, where cationic polymers are used in hair shampoos as conditioning agents[3].
Cationic polymers based on ethyleneimine (PEI) exhibit a strong and permanent binding to surfaces, they are exceptional adhesives. As a result they can be used in a variety of applications such as coatings, as primers for polymer compounds in film formation, and for surface modification in dyeing of plastics. Polyallylamine (PAA) and other polyamine copolymers are used in the coating of textiles to prevent colour fading[4]. Cationic dextranes, polyethyleneimines and polylysines are used for transfection of cells or for drug delivery because they form complexes with nucleic acids like DNA or siRNA[5]. The behaviour and efficiency of cationic polymers for a certain application is based upon the size and characteristics of the individual polymer.

AQUEOUS SIZE EXCLUSION CHROMATOGRAPHY

SEC combined with multi angle laser light scattering (MALLS), refractive index or viscometric detection is a common mean for the characterization of polymers. The challenge in SEC analysis of cationic polymers is the potential interaction of the highly adhesive polymers with the column packing. This impairs peak shape and molecular weight determination[6]. With most SEC columns the undesired interactions are suppressed by using a mobile phase with a high salt content, bearing the risk of precipitation and low recovery.
A new type of SEC column was developed to overcome the need of high salt eluents in SEC determination of water-soluble cationic polymers. TSK-GEL PWXL-CP columns are based on the well known PW-type of polymeric resins for aqueous SEC. The surface of the polymethacrylate base material was modified by incorporating a cationic functionality. This modification results in high recovery of cationic polymers and enables elution under low salt conditions. The columns show high theoretical plate numbers, linear calibration curves and high durability. They are produced with three different pore sizes resulting in different separation ranges.

REPRODUCIBILITY OF SEC ANALYSIS OF POLYALLYLAMINE (PAA)

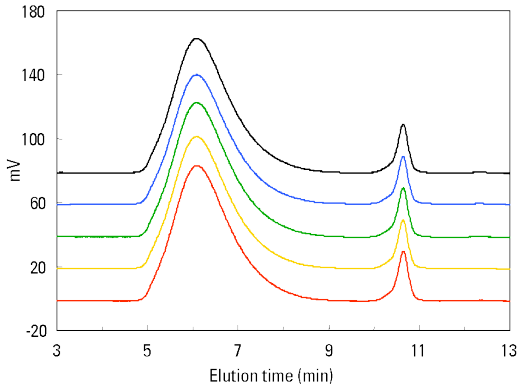

Figure 1

Column:	TSKgel G5000PWXL-CP, 7.8 mm ID x 30 cm L
Eluent:	0.1 mol/l $NaNO_3$
Flow rate:	1.0 ml/min
Detection:	RI
Temperature:	25°C
Sample:	polyallyamine-HCl (PAA)
Sample load:	3 g/l, 100 µl

TOSOH

LOW SALT SEC ANALYSIS WITH TSK-GEL PWXL-CP COLUMNS

The high reproducibility of SEC separations of cationic polymers on TSK-GEL PWXL-CP columns was demonstrated by repeated injection of a high molecular weight polyallylamine (PAA). PAA (MW 438 000) was injected onto a TSKgel G5000PWXL-CP column and analysed at 25°C using a mobile phase containing 0.1 M NaNO3. Figure 1 shows the high reproducibility of the SEC analysis without any adsorption of the polymer. The recoveries of the PAA polymer were measured as well. High recoveries of more than 97% were obtained with all three types of TSK-GEL PWXL-CP columns.

The analysis of different low molecular weight polymers of allylamine (PAA) and ethyleneimine (PEI) can be performed by combining two of the TSKgel G3000PWXL-CP columns in series (Figure 2). The TSKgel G3000PWXL-CP column has the smallest pore size in the series of SEC columns for cationic polymers. The use of two columns in series provides a very good separation of the three PEI polymers with molecular weights of 3000, 8000, and 21000 respectively. PEI is of special interest as a powerful non viral gene-transfection agent, which condense nucleic acids by ionic interactions.

SEC ANALYSIS OF LOW MOLECULAR WEIGHT CATIONIC POLYMERS

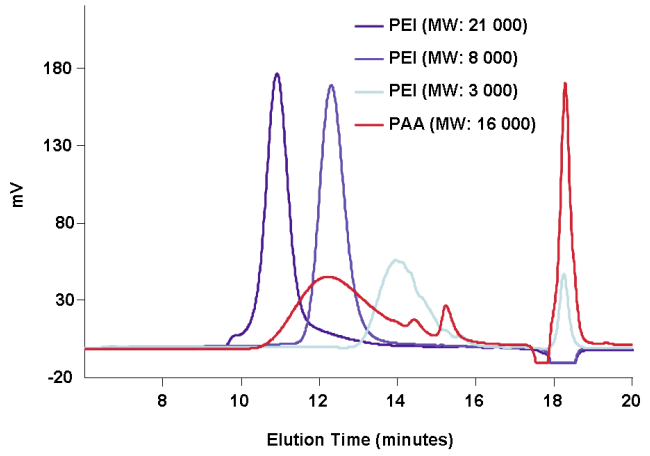

Figure 2

Column:	TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID X 30 cm L
	TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID X 30 cm L
	TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID X 30 cm L
Eluent:	0.1 mol/l $NaNO_3$
Flow rate:	1.0 ml/min
Detection:	RI
Temperature:	25°C
Sample load:	3 g/l, 100 µl

Figure 3 shows the elution profiles of a variety of cationic polymers with different functional groups. For this application the three types of TSK-GEL PWXL-CP were connected in series. The polymers elute in the expected order of their molecular weight demonstrating that there was no influence of the type of functional group. There was no ionic interaction observed which was interfering with the size exclusion mode.

SEC ANALYSIS OF VARIOUS CATIONIC POLYMERS WITH DIFFERENT FUNCTIONAL GROUPS

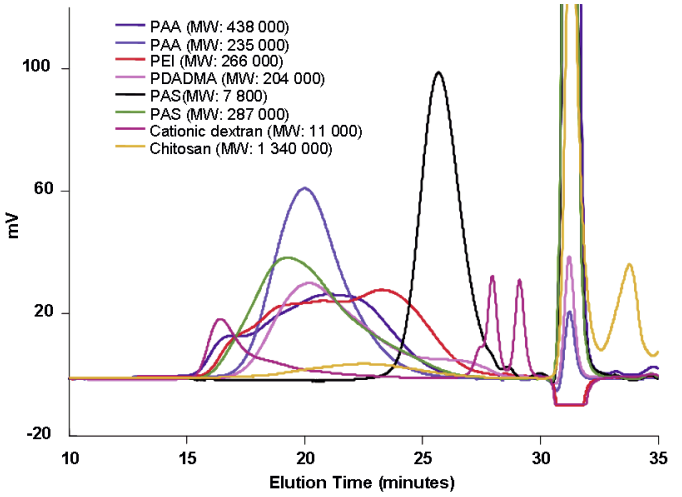

Figure 3

Column:	TSKgel G3000PWXL-CP, 7 µm, 7.8 mm ID X 30 cm L
	TSKgel G5000PWXL-CP, 10 µm, 7.8 mm ID X 30 cm L
	TSKgel G6000PWXL-CP, 13 µm, 7.8 mm ID X 30 cm L
Eluent:	0.1 mol/l $NaNO_3$
Flow rate:	1.0 ml/min
Detection:	RI
Temperature:	25°C
Sample load:	3 g/l, 100 µl

The data presented in this application note shows that the new type of SEC columns can be utilized for the analysis of a variety of aqueous cationic polymers. The recoveries of the cationic polymers were excellent on each grade of the new columns, thus demonstrating that these columns will be valuable to both QC and polymer research laboratories. They provide an improved tool for researchers in the different application fields of cationic polymers ranging from applied chemistry to drug delivery and genetic engineering.

TOSOH

1. M. Liu et al., J. Chromatogr. A, 1104 (1-2), 145-153 (2006)
2. X. Jiang et al., Pharmaceutical Research, 23 (3), 595-603 (2006)
3. C. U. Patel, Int. J. of Cosmetic Science, 5 (5), 181-188 (1983)
4. S. Rosunee et al., J. of Material Science, 38 (10), 2179-2185 (2003)#
5. Th. Merdan et al., Advanced Drug Delivery Reviews, 54 (5), 715-758 (2002)
6. B. Wittgren et al. J.Chromatogr. A, 1002, 101-109 (2003)

With courtesy of TOSOH

Headquarters
JSB International
Tramstraat 15
5611 CM Eindhoven
T +31 (0) 40 251 47 53
F +31 (0) 40 251 47 58

Zoex Europe
Tramstraat 15
5611 CM Eindhoven
T +31 (0) 40 257 39 72
F +31 (0) 40 251 47 58

Sales and Service

Netherlands
Apolloweg 2B
8239 DA Lelystad
T +31 (0) 320 87 00 18
F +31 (0) 320 87 00 19

Belgium
Grensstraat 7
Box 3 1831 Diegem
T +32 (0) 2 721 92 11
F +32 (0) 2 720 76 22

Germany
Max-Planck-Strasse 4
D-47475 Kamp-Lintfort
T +49 (0) 28 42 9280 799
F +49 (0) 28 42 9732 638

UK & Ireland
Cedar Court,
Grove Park Business Est.
White Waltham, Maidenhead
Berks, SL6 3LW
T +44 (0) 16 288 220 48
F +44 (0) 70 394 006 78

info@go-jsb.com
www.go-jsb.com

jsb