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Add the following:

^<312> MOLECULAR WEIGHT DETERMINATION FOR ALGINATES

Average molecular weight and polydispersity are critical properties in understanding and control of excipient composition and variability during formulation development, manufacturing, and testing of polymeric excipients. The following procedures are used to determine average molecular weight and polydispersity for alginates. The procedure is suitable for the following substances:

- Alginate Acid
- Potassium Alginate
- Sodium Alginate

Other alginate materials may be added as future revisions to the chapter. The effect of trace calcium has not yet been evaluated. The methodologies in this general chapter are not part of any monograph specification unless the chapter is referenced in the monograph according to [General Notices, 3.10 Applicability of Standards](#).

METHOD

• APPARENT WEIGHT-AVERAGE MOLECULAR WEIGHT AND POLYDISPERSITY

Mobile phase: 200 mM [sodium chloride](#) in [water](#)

Diluent 1: 200 mM [sodium chloride](#) in water for potassium alginate and sodium alginate

Diluent 2: For Alginate Acid, prepare a 1:1 (v/v) mixture of 200 mM [sodium chloride](#) and 10 mM [sodium hydroxide](#).

System suitability solution: Prepare 1 mg/mL of ethylene glycol solution in *Diluent 1* for Sodium Alginate and Potassium Alginate and in *Diluent 2* for Alginate Acid from [USP Ethylene Glycol RS](#).

Standard solution 1 (for sodium alginate and potassium alginate): Fill 3 vials of 3 different grades of commercially available polyethylene oxide- and polyethylene glycol-based calibration kits¹ with 1.5 mL of *Diluent 1* and let it stand for at least 12 h. Further, dilute this solution 5 times with *Diluent 1* and mix well, to have a final concentration of 0.15 mg/mL for polyethylene oxide with an average molecular weight of 969,000 Da and final concentrations of 0.3 mg/mL for polyethylene glycol and the other polyethylene oxide.

Standard solution 2 (for alginate acid): Fill 3 vials of different grades of commercially available polyethylene oxide- and polyethylene glycol-based calibration kits with 1.5 mL of *Diluent 2*, and let it stand for 12 h. Further, dilute this solution 5 times with *Diluent 2*, to have a final concentration of 0.15 mg/mL for polyethylene oxide with an average molecular weight of 969,000 Da and to have the final concentrations of 0.3 mg/mL for polyethylene glycol and the other polyethylene oxide.

Sample solution 1 (for sodium alginate and potassium alginate): Prepare 0.5 mg/mL of sodium alginate and potassium alginate in *Diluent 1*.

Sample solution 2 (for alginate acid): Prepare 0.5 mg/mL of alginate acid in *Diluent 2*.

Chromatographic system

(See [Chromatography \(621\)](#), [System Suitability](#).)

Mode: LC

Detector: Refractive index

Column: 8.0-mm × 300-mm, 12-μm packing [L38](#)

Temperatures

Column: 50°

Detector: 25°

Flow rate: 0.5 mL/min

Injection volume: 100 μL

Run time: 40 min

System suitability

Samples: *System suitability solution*, *Standard solution 1*, and *Standard solution 2*

Calibration curve: Plot the elution volumes of the peak maximum (V_p) on the x-axis against the $\log M_p$ (M_p represents peak molecular weight) on the y-axis for the peaks from the polyethylene oxide/polyethylene glycol standards to generate a calibration curve using suitable gel permeation chromatography or size exclusion chromatography (GPC/SEC) software. The correlation coefficient for the calibration curve is NLT 0.99.

Suitability requirements

Plate count: NLT 12,000 for the ethylene glycol peak, *System suitability solution*

Resolution: Resolution between all of the standard peaks should be NLT 1.0 in each *Standard solution*

Tailing factor: NMT 2.0 for the ethylene glycol peak, *System suitability solution*

Analysis

Samples: *Standard solution 1, Standard solution 2, Sample solution 1, and Sample solution 2*

Calculation: Compute the data using the same GPC/SEC software and determine the number- and weight-average molecular weights, M_n and M_w , in g/mol (Da), respectively, for the chromatogram of each *Sample solution*.

Calculate the polydispersity for the tested substance:

$$\text{Result} = M_w/M_n$$

M_w = weight-average molecular weight from each *Sample solution* (g/mol)

M_n = number-average molecular weight from each *Sample solution* (g/mol)

ADDITIONAL REQUIREMENTS

- [USP REFERENCE STANDARDS \(11\)](#).

[USP Ethylene Glycol RS](#) ▲ (USP 1-Aug-2024)

¹ A commercially available PEO/PEG Ready Cal kit from PSS (Polymer Standard Service, part number PSS-peokitr 1) was used. The kit includes 10 different standards with the peak molecular weight (M_p) range that covers the region of approximately 23,800–969,000 Da (g/mol). Users need not use the standards with M_p less than 600 Da (g/mol) for the calibration curve. An equivalent standard kit can also be used.

Auxiliary Information - Please [check for your question in the FAQs](#) before contacting USP.

Topic/Question	Contact	Expert Committee
<312> MOLECULAR WEIGHT DETERMINATION FOR ALGINATES	Documentary Standards Support	ETM2020 Excipients Test Methods

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