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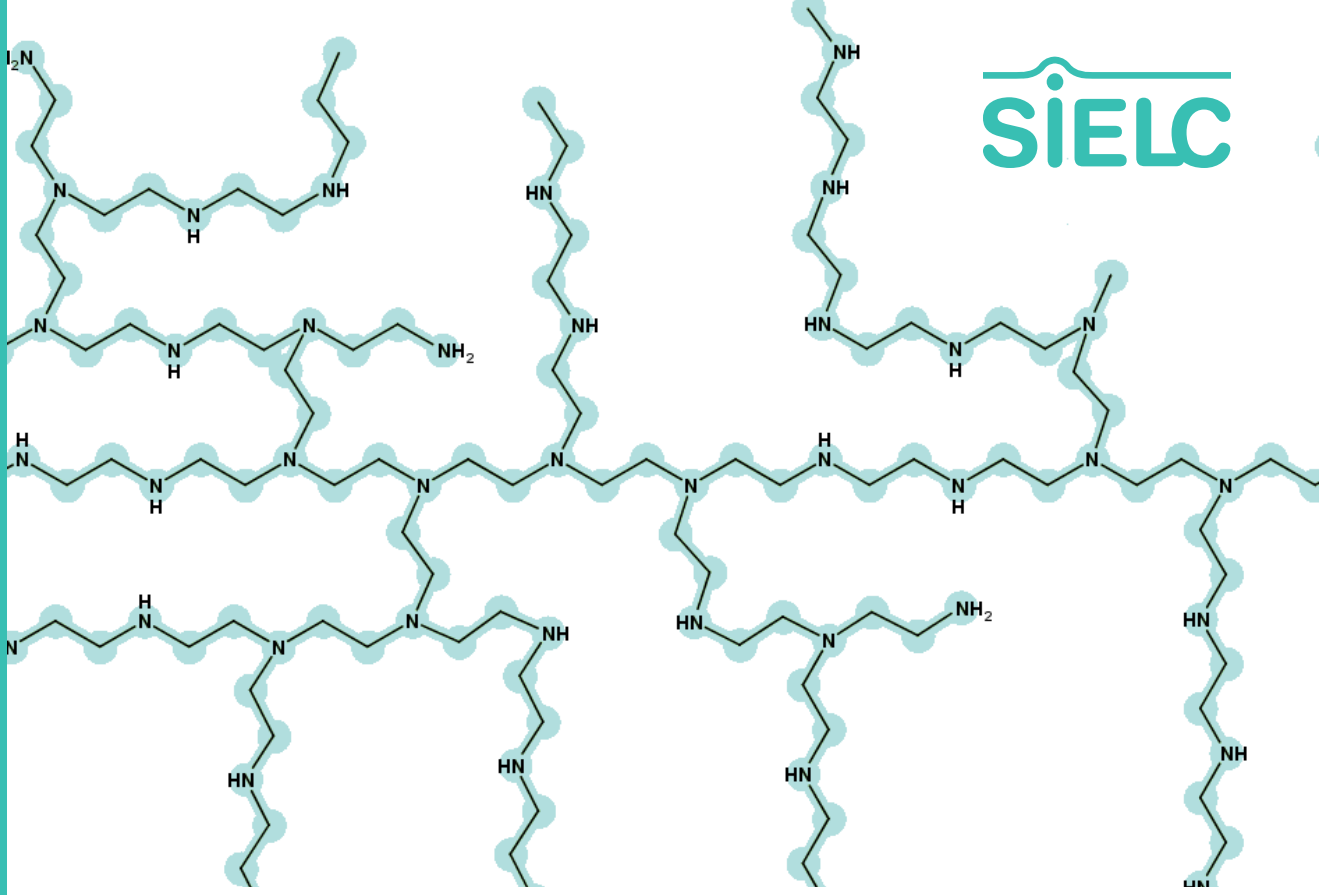

MEASURING PEI

Polyethylenimine in biological samples

PEI Separation problem

Polyethylenimine (PEI) has multiple industrial, medical, biological and research applications. It is a difficult compound to analyze by HPLC. The problem has many degrees of difficulty.

- It is not a single compound; but a mixture of different molecules with different lengths and branching structures
- It has multiple charges in acidic and neutral pH, which is most common in HPLC
- PEI molecules have no UV chromophores and can not be measured by UV-Vis detector, the most common detector in analytical laboratories. Instead this analysis requires MS, CAD, ELSD with their own limitations of the mobile phase composition
- It irreversibly binds to silica-based columns, limiting the type of adsorbents that can be used for analysis
- If composition of PEI with proteins or peptides needs to be analyzed then the peptide/protein signal can interfere with PEI peak

SIELC developed a new methodology and a corresponding HPLC column to address these difficulties and offer a simple and reliable method for PEI quantitation in any liquid samples. The method is based on forming a complex of PEI with Cu (II) which has strong UV and visible light adsorption maximums (Fig. 1).

$$PEI + n \cdot Cu^{2+} \longrightarrow [\,PEI\text{-}Cu\,]^{2n+}$$

This complex can be measured by UV-Vis detector and can be separated from Cu (II) signal and other Cu complexes using specially designed PEI specific HPLC column (Fig. 2).

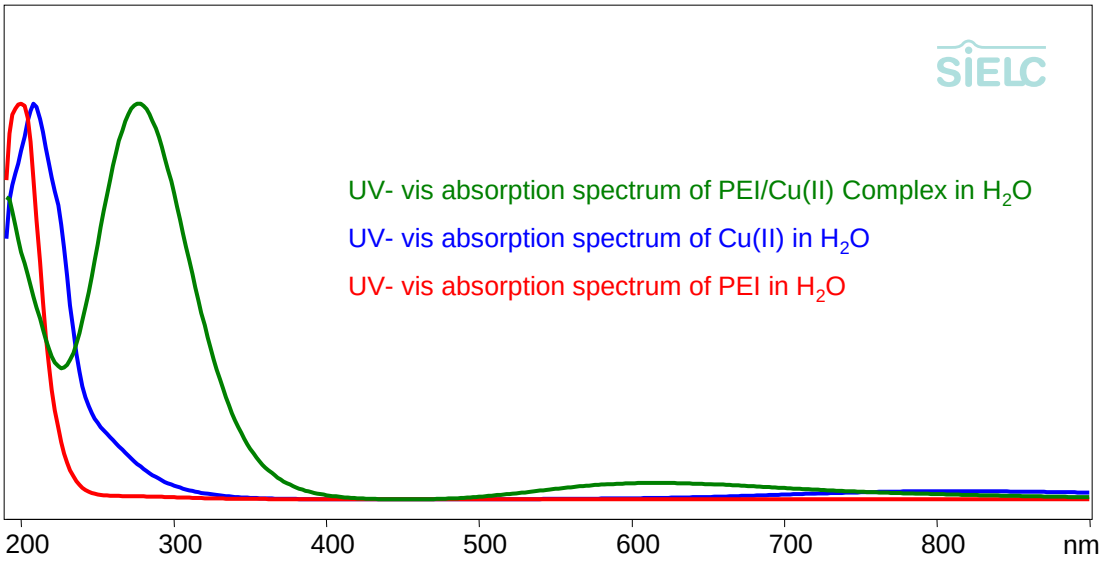

Fig. 1. UV spectra of PEI • Cu^{2+} complex in water (a); Cu^{2+} spectra in water (b); PEI spectra in water (c)

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PEI Analysis

PEI Standards Solution A

For the preparation of the PEI standard solution, 50 mg of PEI was accurately weighed and transferred into a 5 mL volumetric flask and dissolved in water with sonication. The PEI stock solution (10 mg/mL) should be stored in a cold dark place and can be used for a week to prepare standards of required concentration.

Copper Sulfate Solution B

The standard stock solution of copper(II) sulfate (10 mg/ml) was prepared in water. 50 mg of CuSO4 was accurately weighed and transferred into a 5 mL volumetric flask and dissolved in water and sonicated if needed.

General procedure for PEI copper (II) complex analysis

For PEI Mn 400-2,000 (GPC)

Mix 100 µL Solution A (or unknown sample), 300 µL Solution B, and 600 µL of water; place in a plastic HPLC vial for analysis.

For PEI Mn >2,000 (GPC)

Mix 100 µL Solution A (or unknown sample), 100 µL Solution B, and 800 µL of water; place in a plastic HPLC vial for analysis.

HPLC conditions described below (Fig. 2).

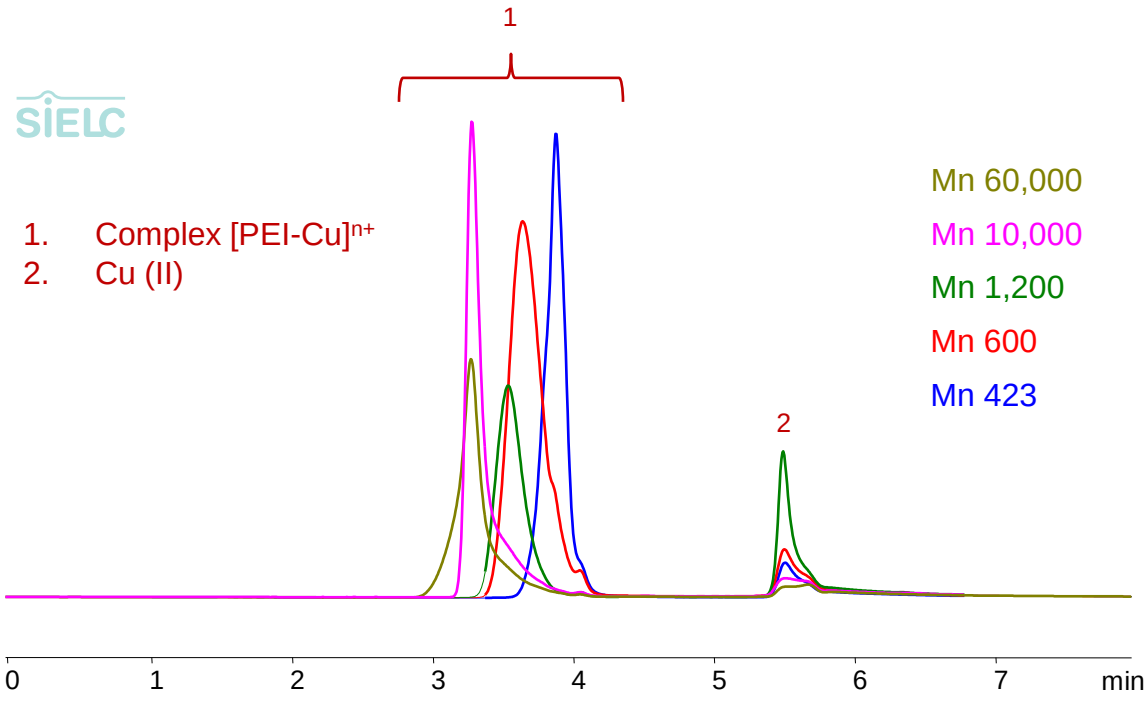

Fig. 2. Chromatograms of PEI complex with Cu(II). Different molecular weight PEI materials were used supplied by Sigma-Aldrich. Analytical column: PEI 4.6 x 250 mm, 5 µm. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: UV 285 nm. Injection: 10 µL of PEI standard with $CuSO_4$

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Summary

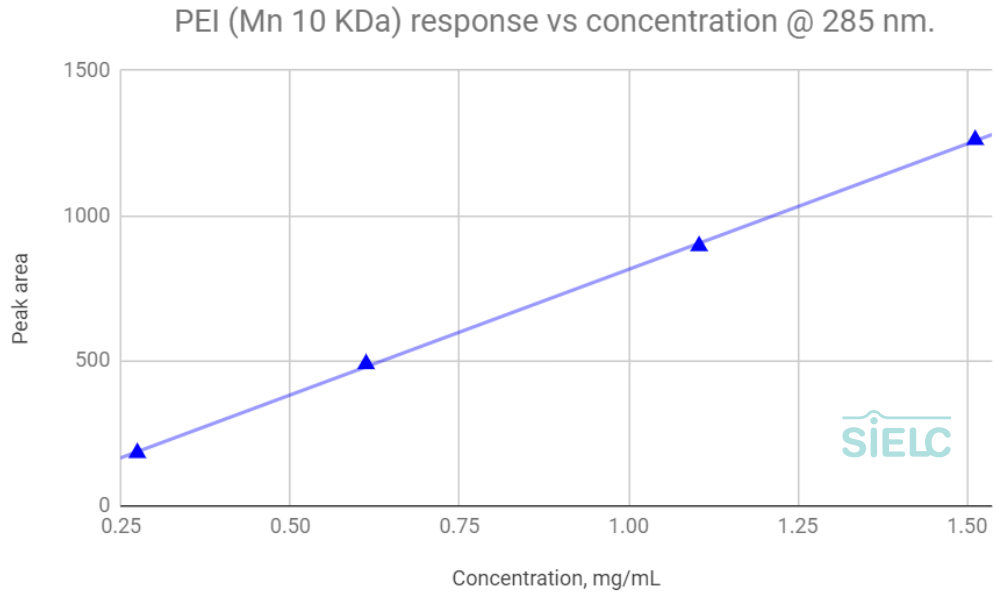

Fig. 3. Linearity study of the PEI analysis quantitation method. Analytical column: PEI 4.6 x 250 mm, 5 µm. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: UV 285 nm. Injection: 10 µL of PEI standards with $CuSO_4$

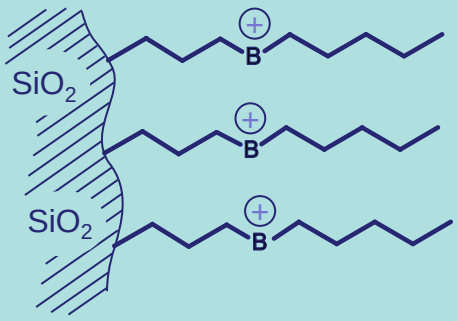

Fig. 4. Schematic structure of PEI columns surface chemistry

PEI specific column was designed to combine ion-exclusion and size-exclusion phenomena to allow for separation of PEI polymers from most other higher- and lower-molecular weight compounds and excess of Cu (II) ions.

PEI elutes from the column as a complex with Cu(II) ions and can be easily detected at 285 nm UV or at 630 nm in visible spectra. The last wavelength is less sensitive, but is very characteristic for this complex.

Simple sample preparation includes mixing the unknown with Cu(II) stock solution followed by HPLC separation. Sensitivity (LOQ) down to 10 ppm of PEI in samples routinely achieved.

Column part number

PEI-250.46.0510 or

To order a column or ask a question send your message to **sales@sielc.com** or call us at **+1 (847) 229-2629**

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Other Detection Modes & Carryover

Some applications may call for Evaporative Detection modes such as Evaporative Light Scattering Detection (ELSD), Charged Aerosol Detection (CAD), and Electrospray Ionization (ESI) for Mass Spectrometry (MS). This method can be easily adapted for evaporative detection as shown below in Fig. 4. The Cu(II) complex is not needed to enable evaporative detection, so unadulterated PEI samples can be injected directly on to the column for analysis.

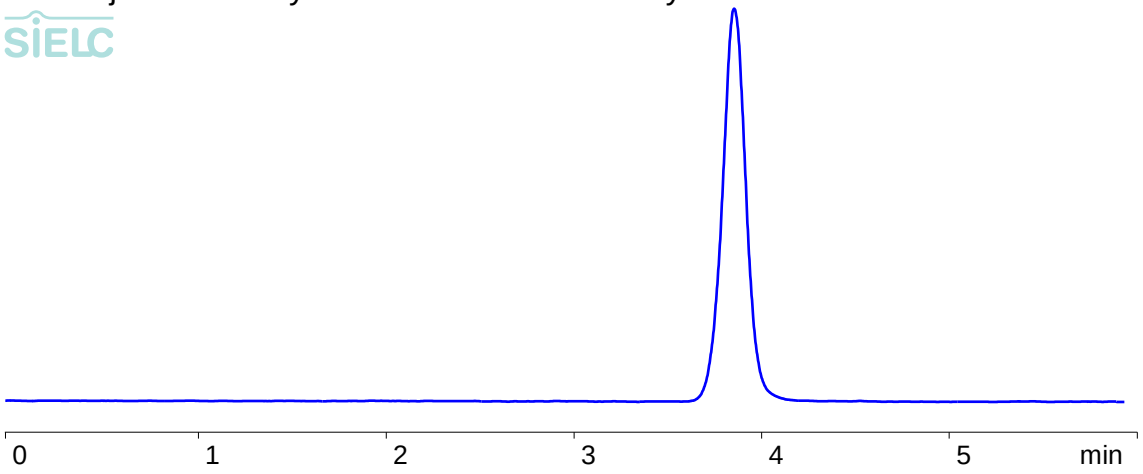

Fig. 5 – Retention of copperless PEI with M_n = 423 detected with ELSD. Analytical column: PEI 4.6 x 250 mm, 5 µm. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: ELSD. Injection: 10 µL of PEI, M_n = 423 .

PEI easily sticks to many of the injection components, including the needle, needle port, etc. This can cause sample carryover over the course of repeated injections. To eliminate this issue, an injection of 2% $HClO_4$ can be added between PEI injections to significantly remove PEI residue left in the needle and its surrounding components from the previous injection. In Fig. 6 below, we show how this 2% $HClO_4$ injection helps remove PEI carryover. The first injection in both experiments is a 1 mg/mL sample of PEI copper (II) complex, M_n = 10,000. Injection 2 is either water (dark blue) or 2% $HClO_4$ (light blue). Injections 3 thru 5 are pure water.

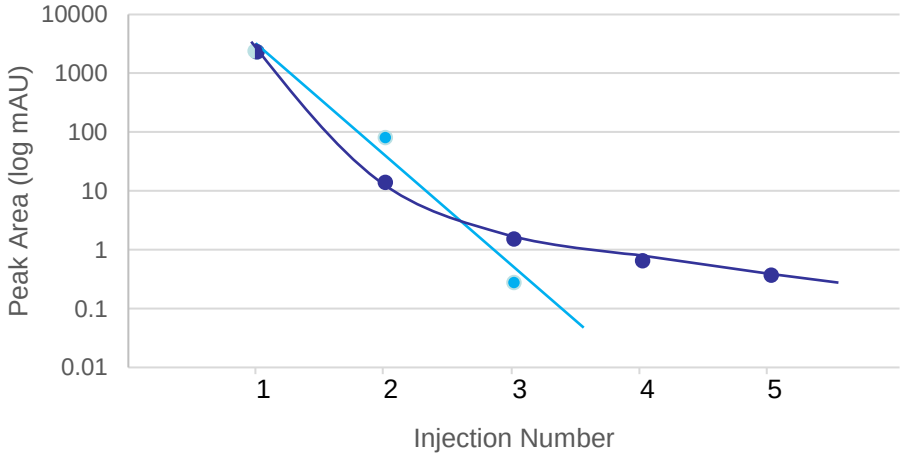

Fig. 6 – Carryover analysis of PEI copper (II) complex with M_n = 10,000 comparing injection of 2% $HClO_4$ with one of 100% water after PEI copper (II) complex injection. Analytical column: PEI 4.6 x 250 mm, 5 µm. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: ELSD. Injection: 10 µL of PEI, M_n = 423 .

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BIST™

In certain applications, such as when polymer and peptide samples may co-elute with PEI, the repulsive modes (SEC and IEC) that govern the separation on the PEI column may not be selective enough to generate meaningful separation. Instead, a new retentive mode developed by SIELC, called Bridge Ion Separation Technology, or BIST™, can be used to separate these multi-charged polymers with high selectivity. This mode can be particularly useful for retaining PEI samples since in most HPLC mobile phases, the pH will generate multiple positive charges on the functional groups of the PEI isomers.

A simple step-gradient method on a positively charged BIST B anion-exchange column allows for the retention of a single peak for each PEI fraction of varying molecular weight, as shown below in Fig. 7. This step-gradient begins with a low aqueous MP and a doubly charged ionic modifier (H_2SO_4), generating the initial BIST™ retention. The switch to a completely aqueous MP with a single-charged ionic modifier ($HClO_4$) ensures the retention occurs in a reasonable amount of time. The step-time for and the concentration of Solvent A can be modified as needed to tune the retention time for each PEI sample.

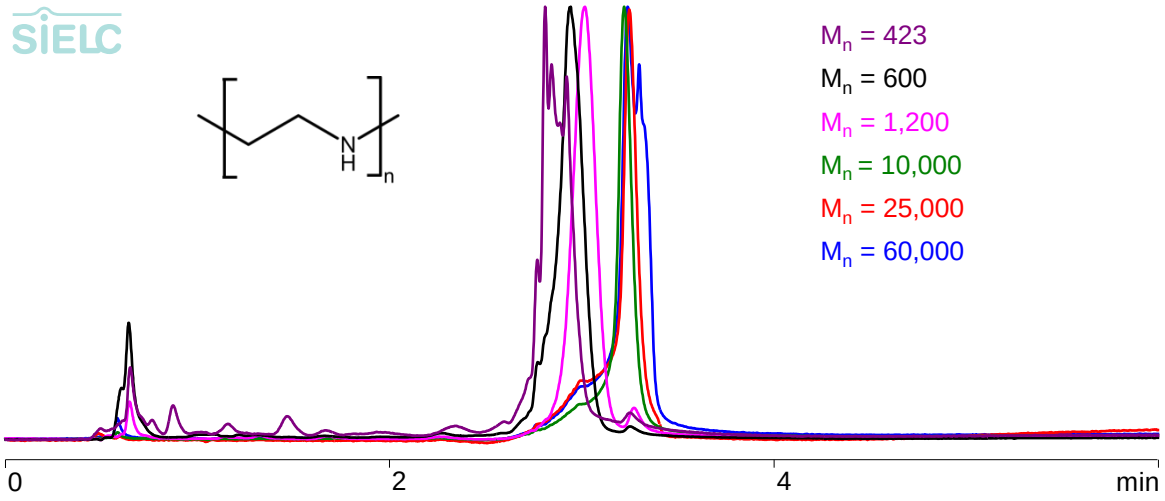

Fig. 7 – Retention of varying M_n samples of PEI with BIST™ using a step-gradient. Analytical column: BIST™ B 4.6 x 50 mm, 5 µm. Flow rate: 1.0 mL/min. Solvent A: MeCN/H2O – 50/50%, H2SO4 – 0.2% for 1 min then Solvent B: MeCN/H2O – 0/100%, HClO4 – 2.0% for 5 min. Detection: UV 210 nm. Injection: 10 µL of PEI standards.

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