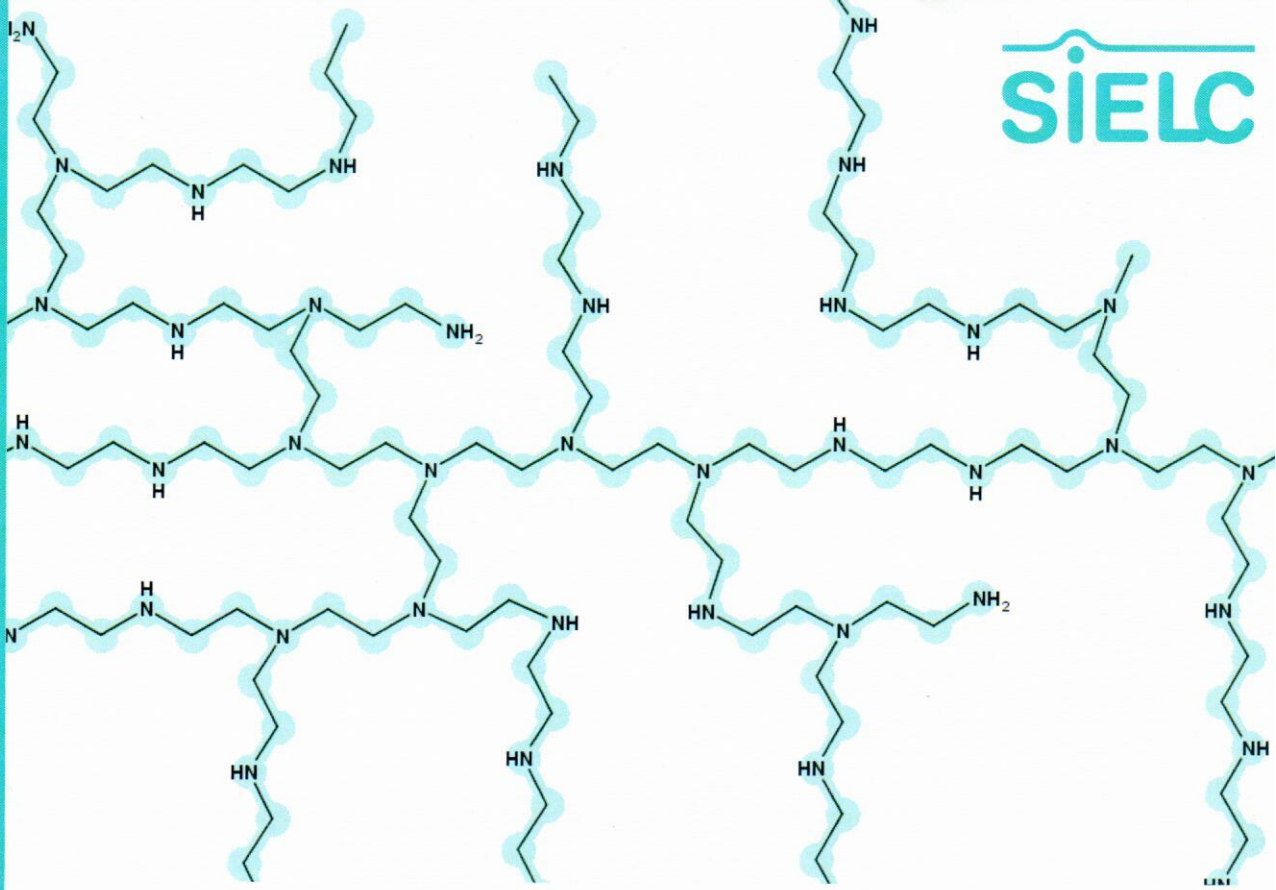


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SIELC



# MEASURING PEI

Polyethylenimine in biological samples



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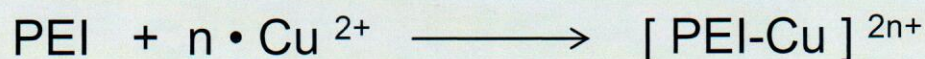


# PEI Separation problem

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).



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).

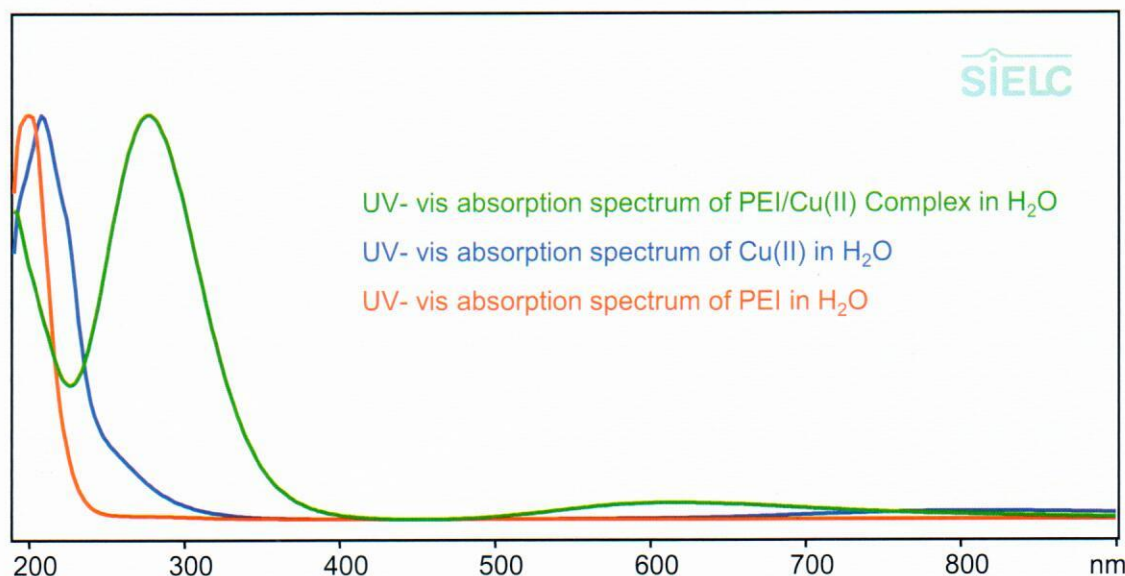


Fig. 1. UV spectra of PEI • Cu<sup>2+</sup> complex in water (a); Cu<sup>2+</sup> spectra in water (b); PEI spectra in water (c)

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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 CuSO<sub>4</sub> 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  $\mu$ L Solution A (or unknown sample), 300  $\mu$ L Solution B, and 600  $\mu$ L of water; place in a plastic HPLC vial for analysis.

### For PEI Mn >2,000 (GPC)

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

HPLC conditions described below (Fig. 2).

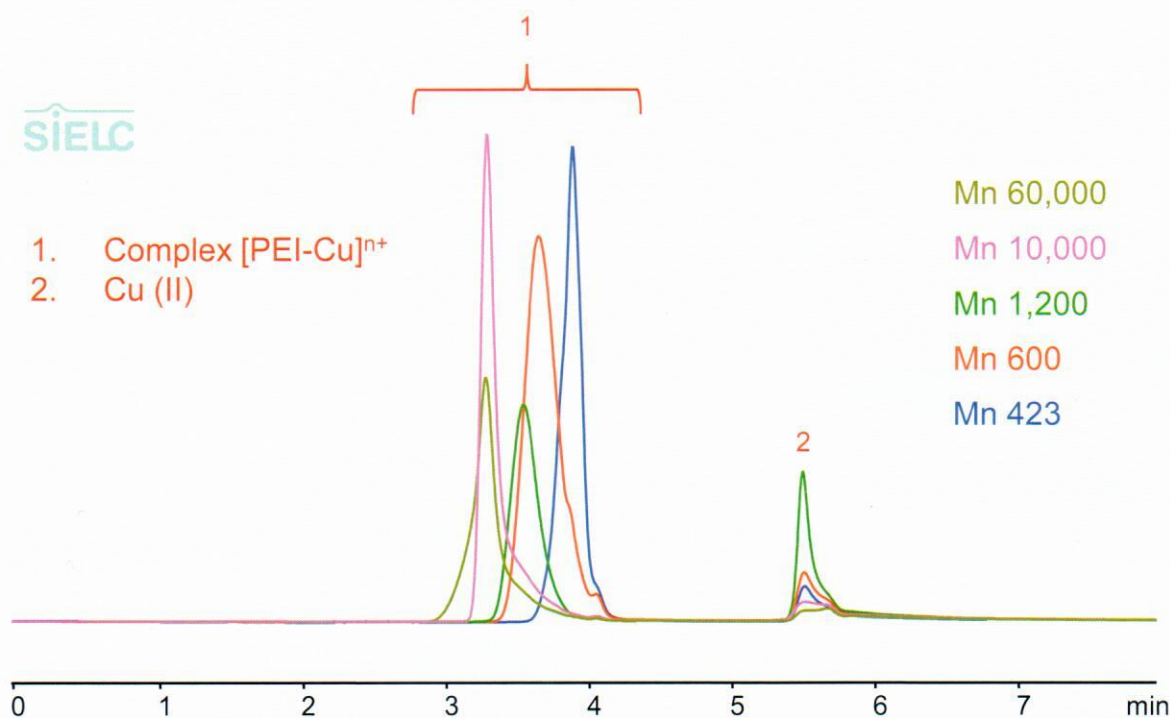


Fig. 2. Chromatograms of PEI complex with Cu(II). Different molecular weight PEI materials were used supplied by Sigma-Aldrich. Analytical column: PEI 4.6 x 250 mm, 5  $\mu$ m. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: UV 285 nm. Injection: 10  $\mu$ L of PEI standard with CuSO<sub>4</sub>

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# Summary

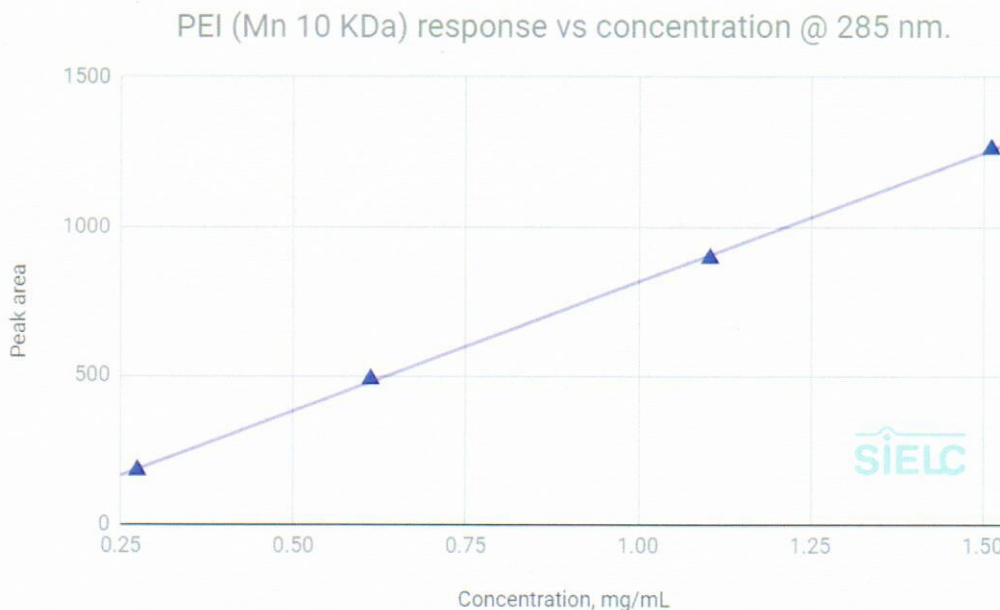


Fig. 3. Linearity study of the PEI analysis quantitation method. Analytical column: PEI 4.6 x 250 mm, 5  $\mu$ m. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: UV 285 nm. Injection: 10  $\mu$ L of PEI standards with CuSO<sub>4</sub>

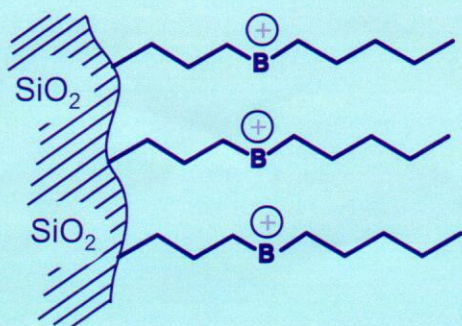


Fig. 4. Schematic structure of PEI columns surface chemistry

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-46.250.0510

se

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