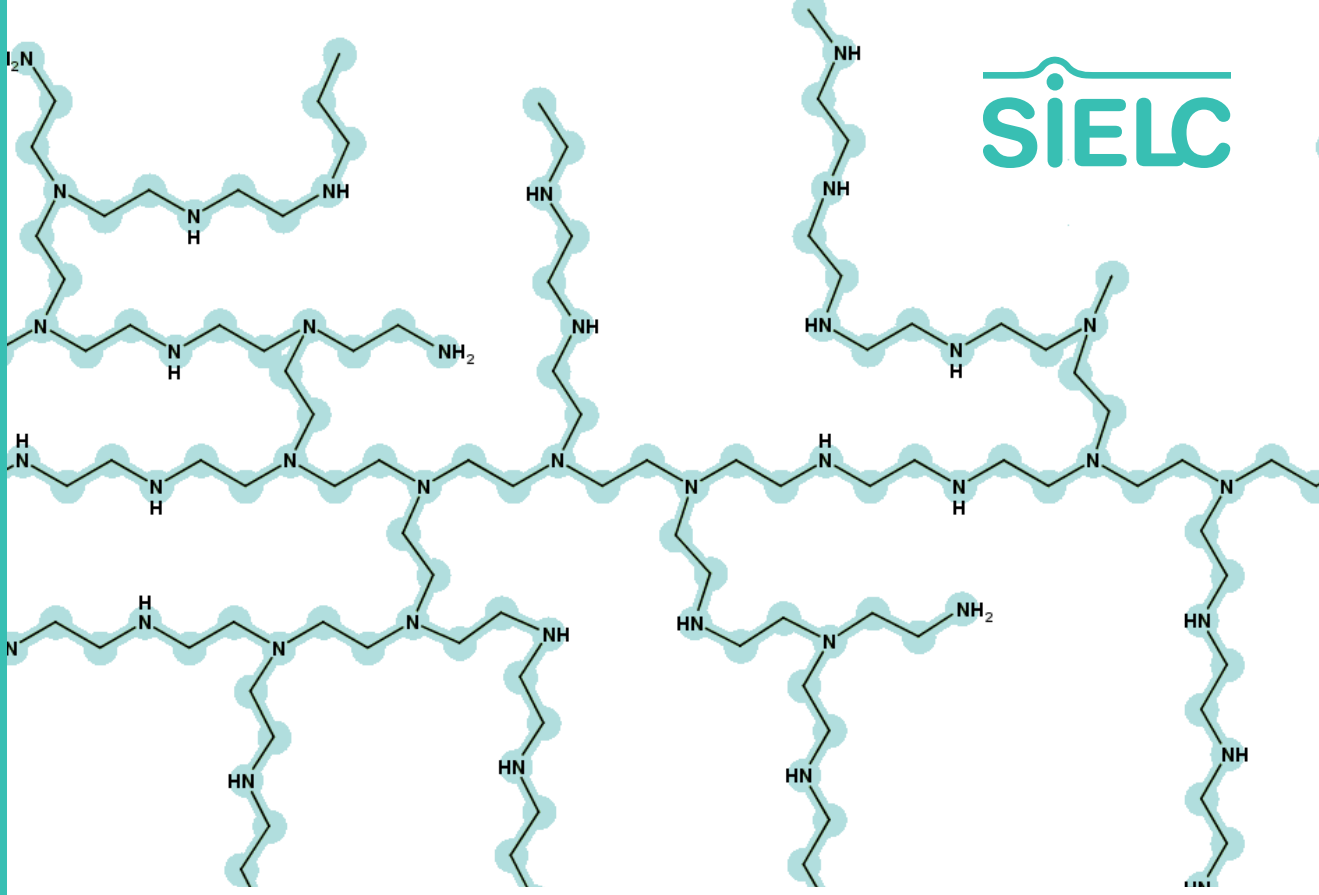
SIELC Technologies, Inc.
Wheeling, IL 60090 USA
P. 847-229-2629 F. 847-655-6079
mail@sielc.com www.sielc.com



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).

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

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).

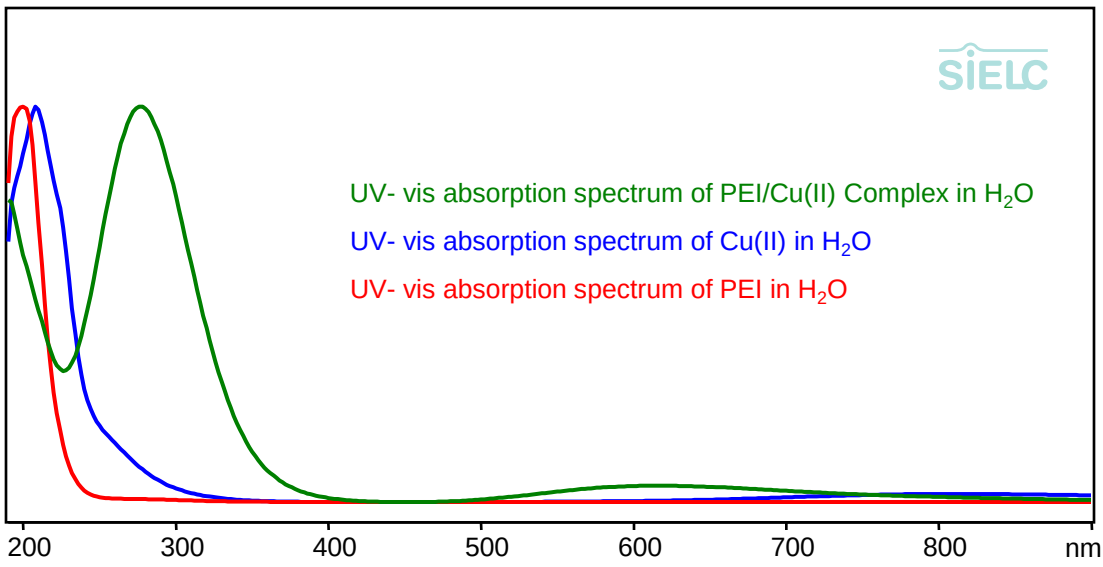


Fig. 1. UV spectra of PEI • Cu^{2+} complex in water (a); Cu^{2+} spectra in water (b); PEI spectra in water (c)

SIELC Technologies, Inc.
www.sielc.com email: mail@sielc.com ph. 847-229-2629 fax 847-655-6079

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).

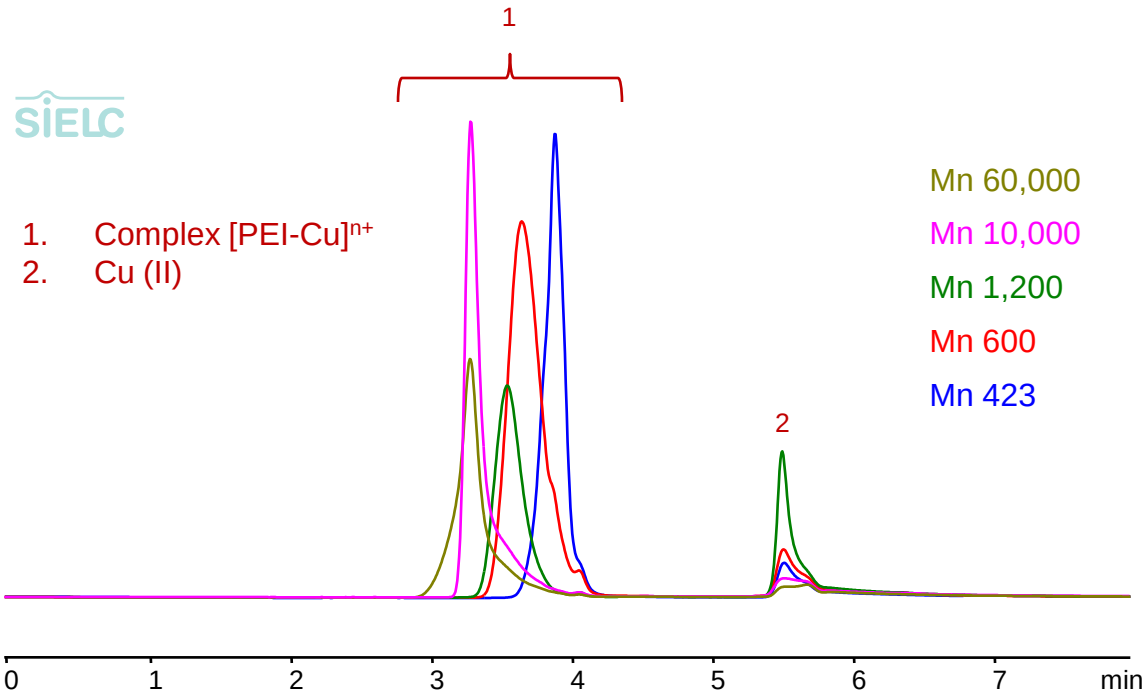


Fig. 2. Chromatograms of PEI complex with Cu(II). Different molecular weight PEI materials were used supplied by Sigma-Aldrich. Analytical column: PEI 4.6 x 250 mm, 5 µm. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: UV 285 nm. Injection: 10 µL of PEI standard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

SIELC Technologies, Inc.

www.sielc.com email: mail@sielc.com ph. 847-229-2629 fax 847-655-6079

Summary

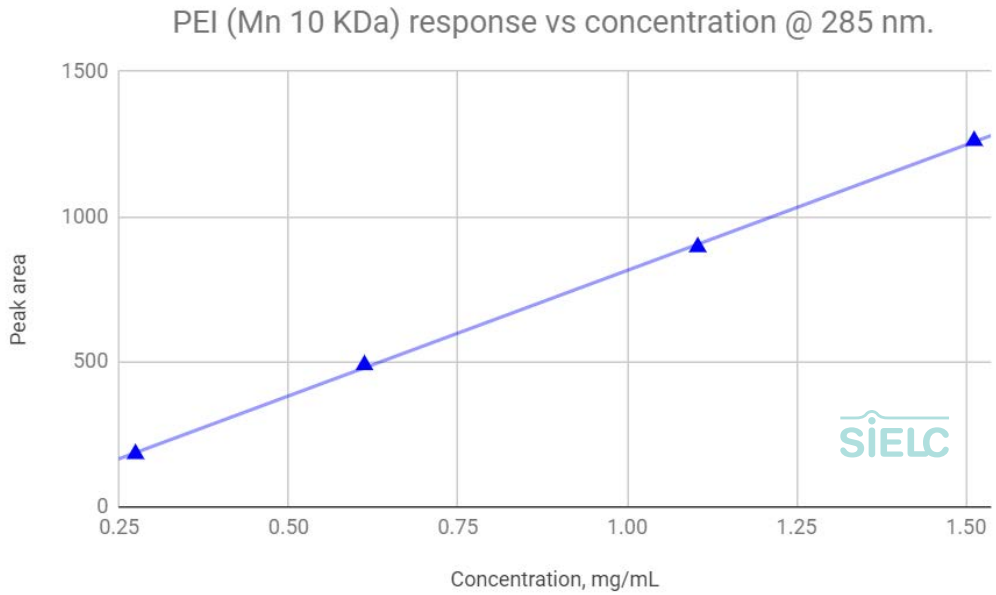


Fig. 3. Linearity study of the PEI analysis quantitation method. Analytical column: PEI 4.6 x 250 mm, 5 µm. Flow rate: 0.5 mL/min. Mobile phase: MeCN – 40% with AmFm buffer pH 3.0, 20 mM. Detection: UV 285 nm. Injection: 10 µL of PEI standards with $CuSO_4$

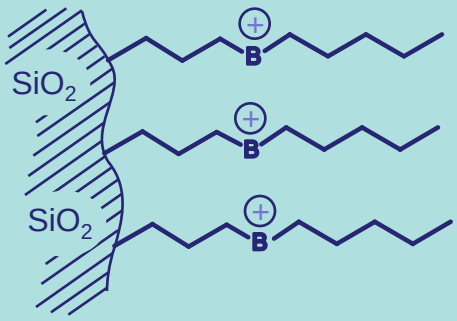


Fig. 4. Schematic structure of PEI columns surface chemistry

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-46.250.0510

To order a column or ask a question send your message to **sales@bgb-analytik.com** or call us at **+41 61 991 00 46**

SIELC Technologies, Inc.
www.sielc.com email: mail@sielc.com ph. 847-229-2629 fax 847-655-6079

SIELC

formerly Allsep Technologies

For decades liquid chromatography stationary phase design has been dominated by the goal to eliminate multiple, or “unwanted”, interactions and to obtain a simple and predictable retention mechanism. Unfortunately, the simplification of the retention process limits the ability to control elution order of the analytes and the scope of available applications this system can be used for. As a response to this limitation, hundreds of different reverse-phase columns were introduced in the last years to cover a variety of analytical situations.

In contrast, PrimesepTM stationary phases were intentionally designed with two major interactions offered on the same column. Both interactions are independently adjustable with mobile-phase composition producing unlimited states of the stationary phase. The hydrophobic interaction is controlled by the amount of organic modifier in the mobile phase (as in any reverse-phase column), while the ion-exchange interaction is controlled by the ion-strength and pH of the mobile phase (as in other ion-exchange columns). This unique property allows using one stationary phase for numerous applications, including analyses of polar and non-polar, ionizable and neutral, organic and inorganic compounds. The behavior of PrimesepTM columns is predictable and reproducible. The method development process is simple and versatile.

BGB GC|LC MS|CE

www.bgb-shop.com

Switzerland BGB Analytik AG • Rohrmattstrasse 4 • 4461 Böckten • Phone +41 61 991 00 46 • Fax +41 61 991 00 25 • sales@bgb-analytik.com

Benelux BGB Analytik Benelux B.V. • Drielandendreef 42 • 3845 CA Harderwijk • Phone +31 341 700270 • Fax +31 341 700271 • sales.benelux@bgb-analytik.com

France BGB Analytik France S.A.S. • 81 Vie de l‘Etraz • 01630 St. Jean de Gonville • Phone +33 450 488567 • Fax +33 450 562378 • sales.fr@bgb-analytik.com

Germany BGB Analytik Vertrieb GmbH • Mühlestraße 1 • 79539 Lörrach • Phone +49 7621 58842 70 • Fax +49 7621 58842 89 • sales.de@bgb-analytik.com

Turkey BGB Analytik Laboratuvar Malzemeleri Ticaret LTD.ŞTİ • Starport Residence • Yenişehir Mah. Osmanlı Bulvarı. • Sümbül Sok. No:10, Daire: 193 • 34912 Kurtköy, Pendik, İstanbul • Phone +90 2169092048 • sales.tr@bgb-analytik.com