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Citation for the original published paper (version of record):
Grenda, K., Idström, A., Evenäs, L. et al (2022). An analytical approach to elucidate the architecture of polyethyleneimines. Journal of Applied Polymer Science, 139(7).
http://dx.doi.org/10.1002/app.51657

Received: 28 April 2021 | Revised: 30 August 2021 | Accepted: 30 August 2021
DOI: 10.1002/app.51657

Journal of Applied Polymer Science WILEY

ARTICLE

An analytical approach to elucidate the architecture of polyethyleneimines

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Funding information
Horizon 2020 Framework Programme, Grant/Award Number: 814496

Abstract
Polyethyleneimine (PEI) is a common polymer used in many industrial applications and in research, especially in surface chemistry. It is available in a wide range of molecular weights and different degrees of branching. It is classified as linear or branched and sometimes the term hyperbranched is also used. This description, however, is quite rough, which limits the possibility to correlate the structure of the PEI to its properties. The aim of this study is to provide analytical tools to characterize the polymer at a level of detail not normally provided by the supplier of PEI. To this end, five commercially available polyethyleneimines were characterized by Fourier transform infrared spectroscopy, thermogravimetric analysis, and nuclear magnetic resonance spectroscopy to gain insight into the structure and the functional groups present in the polymers. Quantitative ^{13}C NMR analysis turned out to be particularly useful, revealing the degree of branching of the polymer based on the ratio of primary, secondary, and tertiary amino groups.

KEYWORDS
dendrimers, hyperbranched polymers and macrocycles, polyelectrolytes, spectroscopy, thermogravimetric analysis

1 | INTRODUCTION

There are several reasons why polyethyleneimines (PEIs) are attractive in many industrial applications. PEI has a high cationic charge density when fully protonated in aqueous solution,[1] it is available in different molecular weights, and it exists in different architectures, linear or branched. PEIs are employed as flocculants (clarification agents) in wastewater treatment, as well as in the paper industry for flocculation of negatively charged fibers (retention and drainage aids).[2] Their chelating properties are taken advantage of in the elimination of metal ions (copper, rhodium, mercury, zinc, and chromium) from sewage.[3,4] PEIs are also employed for air purification, as adsorbents for acidic gases, ozone, and aldehydes[5] and also for CO_2 sequestration.[6] They are used in drilling and completion of oil and gas wells.[7] PEIs have also found use in the biotechnological field, for example, enzyme immobilization[7] and in gene transfection in vitro and in vivo into various cell lines and tissues.[8] In all these varying applications of PEI, it is important to be able to find relationships between performance and structural property. This communication focuses on one important such parameter, the architecture.

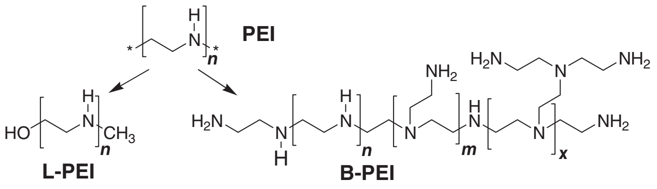

FIGURE 1 Chemical structure of polyethyleneimines (PEIs). Both linear (L-PEI) and branched (B-PEI) polyethyleneimines are commercially available

PEIs are often described as aliphatic polyamines characterized by a —(CH_2CH_2N)— repeating unit.[7] This is true only when the structure is linear (L-PEIs). In the case of branched polyethyleneimines (B-PEIs), which are the most commonly used, besides —(CH_2CH_2N)— several other building blocks are present, as can be seen in Figure 1, (possible structures of PEIs of the general formulas $C_{26}H_{68}N_{14}$ and $C_{58}H_{148}N_{30}$ are presented in Figure S4 and Figure S5). An ideal theoretical dendrimer, i.e. the most branched architecture, will be constituted of primary and tertiary amines while a perfect linear structure will contain only secondary amino groups (see Figures S5, S6 and S7). Also, a decrease of primary and tertiary amino groups leads to an increase in secondary amino groups and increase in linearity of the structure, as is noted for two given theoretical examples with M_w of 577 and 1267 g/mol (structure of $C_{26}H_{68}N_{14}$ and of $C_{58}H_{148}N_{30}$, see Figure S5). Commercial B-PEIs contain all building blocks and the three-term ratio between primary, secondary, and tertiary amines is a very important parameter that influences not only the performance in technical applications but also the toxicity. For instance, it has been found that primary amines in B-PEI are at the origin of an increased cytotoxicity; L-PEIs, which lack primary amino groups, are relatively nontoxic.[9]

Branched PEI possesses primary, secondary, and tertiary amines in the theoretical ratio 1:2:1.[9] So-called hyperbranched PEI has the approximate ratio 3:4:3; thus, the proportion of secondary amines is decreased in comparison to the situation in normal B-PEI.[7,9,10]

Linear and branched PEIs originate from different synthesis routes. B-PEIs are viscous liquids synthesized by cationic polymerization of aziridine (ethyleneimine)[11] at elevated temperatures in aqueous or alcoholic medium or in bulk at low temperatures,[11,12] typically resulting in a weight average molecular weight in the range 20,000–50,000 g/mol.[1] B-PEI of higher molecular weight can be obtained by use of bifunctional linkers such as dichloroethane or epichlorhydrine.[1] Furthermore, copolymerization with a low molecular weight amine, such as 1,2-ethanediamine, leads to B-PEIs with lower molecular weight.[7] Strictly linear PEIs, on the other hand, are crystalline solids[7] with lower water solubility than B-PEIs (at the natural pH of the polymer).[9] L-PEIs are synthesized either by hydrolysis of poly(2-ethyl-2-oxazoline)[13] or by polymerization followed by hydrolysis of *N*-substituted aziridines.[14] Until recently linear polyethyleneimines were not produced on an industrial scale;[7] however, this has changed and L-PEIs are now commercially available under trademarks such as ExGen 500[15,16] or jetPEI.[13]

Experience has shown that the division into L-PEI and B-PEI is far from sufficient for establishing reliable performance-structure relationships. The aim of the work presented here is to provide a methodology for a more detailed description of the molecular architecture, thus giving the formulator a better tool for selection of the right PEI for a given application.

We have focused on five commercially available PEIs. The degree of branching and the ratio of primary, secondary, and tertiary amines were determined by nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FTIR). The thermal stability of the PEIs was determined using thermogravimetric analysis (TGA).

Among the five tested PEIs, two, with number average molecular weight (M_n) 60,000 and 1200 g/mol, were only described by the supplier as polyethyleneimines without mention of the degree of branching, which one may interpret as the polymers being linear. Two other PEIs, with M_n of 600 and 10,000 g/mol, were described by the supplier as branched and used in this study as examples of B-PEIs. A PEI with M_n 2500 g/mol and described by the supplier as linear was used and confirmed as a true representative of L-PEI.

2 | MATERIALS AND METHODS

2.1 | Materials

L-PEI 2500 (linear; $M_n = 2500$ g/mol [no method disclosed]), B-PEI 600 (branched; $M_w = 800$ g/mol (by light scattering, LS), and $M_n = 600$ g/mol (by gel permeation chromatography, GPC)), B-PEI 10000 (branched; $M_w = 25{,}000$ g/mol (by LS) and $M_n = 10{,}000$ g/mol (by GPC)), PEI 1200 ($M_w = 1300$ g/mol (by LS) and $M_n = 1200$ g/mol (no method disclosed)), and PEI 60000 (average $M_w = 750{,}000$ g/mol (by LS) and $M_n = 60{,}000$ g/mol (by GPC)) were all purchased from Sigma-Aldrich and used without further purification. Fully deuterated methanol, that is, CD_3OD, and D_2O were also purchased from Sigma-Aldrich.

2.2 | Analytical methods

2.2.1 | Nuclear magnetic resonance spectroscopy

All NMR measurements were conducted on a Varian Inova 500 MHz instrument operating at 11.7 T with a 5 mm HFX-probe, capable of producing magnetic field gradients up to 60 G/cm.

Quantitative inverse-gated ^{13}C-spectra were acquired using a 14 μs ^{13}C-detection pulse, 1 s acquisition time, and 5 s recycle delay.

For self-diffusion measurements the Dbppste (DOSY bipolar pulse pair stimulated echo) solvent suppression sequence was used with parameters including 7.5 μs 90° ^{1}H-pulse, 2 s acquisition time, 10 s recycle delay, 8 scans, gradient pulse length δ of 10 ms, and diffusion time Δ of 200 ms. Gradients ranging from 0.18 to 60 G/cm were used, corresponding to k-values of $1.24{\cdot}10^{6}$ to $1.31{\cdot}10^{11}$ $\mathrm{s{\cdot}m^{-2}}$, in 16 steps.

The NMR measurements of the samples B-PEI 600, PEI 1200, B-PEI 10000, and PEI 60000 were conducted at 0.25 wt% using D_2O as solvent. Since the L-PEI 2500 sample showed poor water solubility, fully deuterated methanol, CD_3OD, was used instead. The inverse-gated ^{13}C-spectra of the B-PEI 600, B-PEI 10000, and PEI 60000 samples are presented in the Figures S1, S2, S3.

2.2.2 | Fourier transform infrared spectroscopy

The IR spectra were measured with a PerkinElmer FTIR spectrophotometer in attenuated total reflectance (ATR) mode, using diamond crystal (GladiATR, Pike Technologies). The optical range was 4000 to 400 cm^{-1} and for all experiments, 64 scans were collected and averaged. The recorded data were subjected to baseline correction. The resolution was set to 1 cm^{-1}.

2.2.3 | Thermogravimetric analysis

Thermal stability of polyethyleneimines was determined by TGA using a TGA/DSC 3+ Stare instrument (Mettler Toledo). During the temperature program, the samples were heated from 25 to 700°C at a heating rate of 10°C/min in a 70 μl alumina sample holder under 50 ml/L air flow. Generally, 5 mg sample was used for each sorbent in the analysis.

3 | RESULTS AND DISCUSSION

3.1 | FTIR analysis

Figure 2 shows the FTIR spectra of PEIs with varying molecular weights and structures. As can be seen from the figure, two regions of the spectra are of particular interest, from ~700 to 1700 cm^{-1} and from 2800 to 3400 cm^{-1}. These regions give valuable information about the structure of the polymer. The much sharper peaks for L-PEI 2500 are most likely due to the highly crystalline nature[17] of this PEI. It can also be seen from Figure 2 that the frequencies have shifted, and new bands have appeared in the spectra of the branched polymers, B-PEI 600 and B-PEI 10000, compared to the spectrum of L-PEI 2500.

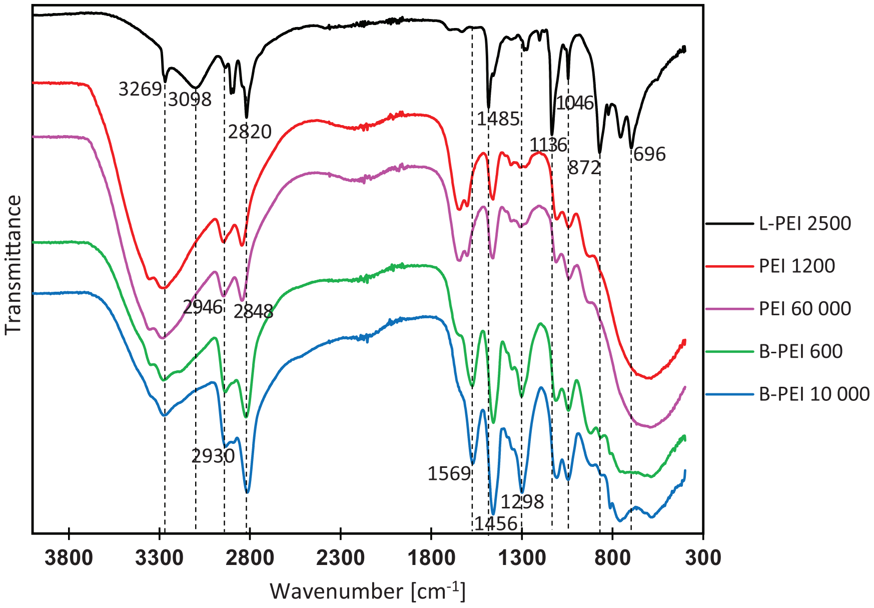

FIGURE 2 FTIR spectra of the different PEIs [Color figure can be viewed at wileyonlinelibrary.com]

The vibrational modes in the region ~700 to 1700 cm^{-1} are primarily a mixture of N—H bending and CH_2 rocking motions. For L-PEI 2500 there are several types of modes: N—H bending at 757 cm^{-1} (literature value 750 cm^{-1} [17]), C—N stretching at 1136 cm^{-1} (literature value 1133 cm^{-1} [17]), and N—H bending mixed with CH_2 scissors at 1485 cm^{-1} (literature value 1485 cm^{-1} [17]). These spectral data clearly show that there is a close correlation between the vibration modes in the linear PEI to those in the branched PEIs (C—N stretching 1110 cm^{-1}, and N—H bending mixed with CH_2 scissors 1456 cm^{-1}). However new absorption bands appear in the branched PEIs in the region between ~1500 and 1650 cm^{-1} and these are attributed to the absorption of N—H bending[18,19] from the primary amines in B-PEI. Bands in this region are not observed in structures with only secondary or tertiary amines.[20] The N—H stretching modes (very sensitive to hydrogen-bonding interactions; an increase in the strength of hydrogen bonding causes the frequencies to decrease[21]) are in the region from 3000 to 3700 cm^{-1}. The much lower frequencies of the N—H stretches in the L-PEI than in the B-PEIs indicate a higher degree of hydrogen bonding for the former. In addition, the C—H stretching and bending vibration peaks of CH_2 groups in PEI can be observed in the region from ~2800 to 2950 cm^{-1}. The peaks in this region, as well as a peak at ~1480 cm^{-1}, were well defined and particularly sharp for the linear PEI due to the abundance of the $—CH_2CH_2NH—$ moiety.[22]

3.2 | Thermogravimetric analysis

The thermal stability and the fraction of volatile material of the PEIs were measured by controlled heating of the polymers in the range 25–700°C, under oxygen. As can be seen from Figure 3, the decomposition profiles are similar for all the polymers except for L-PEI 2500 and the curves are similar to decomposition profiles previously reported.[23] The initial weight loss is due to evaporation of

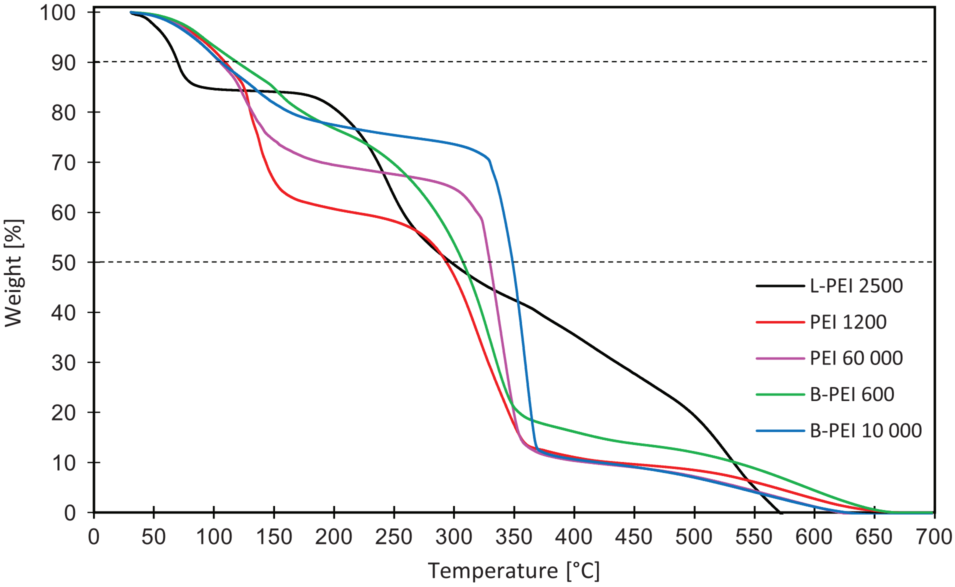

FIGURE 3 Thermogravimetric analysis curve of the different PEIs [Color figure can be viewed at wileyonlinelibrary.com]

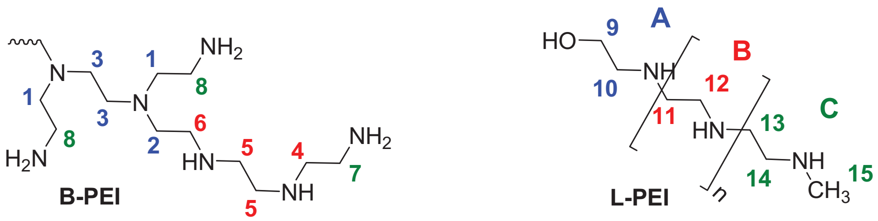

FIGURE 4 Characteristic building elements of branched (B-PEI) and linear (L-PEI) polyethyleneimine. In B-PEI carbons adjacent to a primary amine (carbons 7 and 8) are colored green, carbons adjacent to a secondary amine (carbons 4, 5, and 6) are colored red, and carbons adjacent to a tertiary amine (carbons 1, 2, and 3) are colored blue. In L-PEI carbons close to an OH end (carbons 9 and 10) are colored blue, carbons in the repeating unit (carbons 11 and 12) are colored red, and carbons close to or at the terminal CH_3 group (carbons 13, 14, and 15) are colored green [Color figure can be viewed at wileyonlinelibrary.com]

adsorbed water/moisture and possibly impurities. This weight loss is highest for PEI 1200 and PEI 60000, probably because these polymers were supplied in aqueous solution and therefore contain more adsorbed water. The polymers are then relatively intact to around 200°C for L-PEI 2500 and to higher temperatures for the other PEIs. By comparing the profiles for L-PEI 2500 with those for B-PEI 600 and B-PEI 10000 one may conclude that branching appears to increase the temperature stability.

The decomposition curves also indicate a relationship between molecular weight and thermal resistance. PEI 60000 and B-PEI 10000, which are the polymers with the highest molecular weight, are the most thermally stable, being relatively intact until above 300°C. Thus, branched and high molecular weight PEIs are the most thermally stable.

3.3 | NMR spectroscopy

Figure 4 shows characteristic building elements of L-PEI and B-PEI. A quantitative NMR ^{13}C-spectrum of PEI 1200 is displayed in Figure 5. The NMR signals originating from the different carbons are assigned to the primary, secondary, and tertiary structure. The spectra obtained for the four samples PEI 1200 (Figure 5), B-PEI 600 (Figure S1), B-PEI 10000 (Figure S2), and PEI 60000 (Figure S3) corresponded well with spectra previously obtained for similar

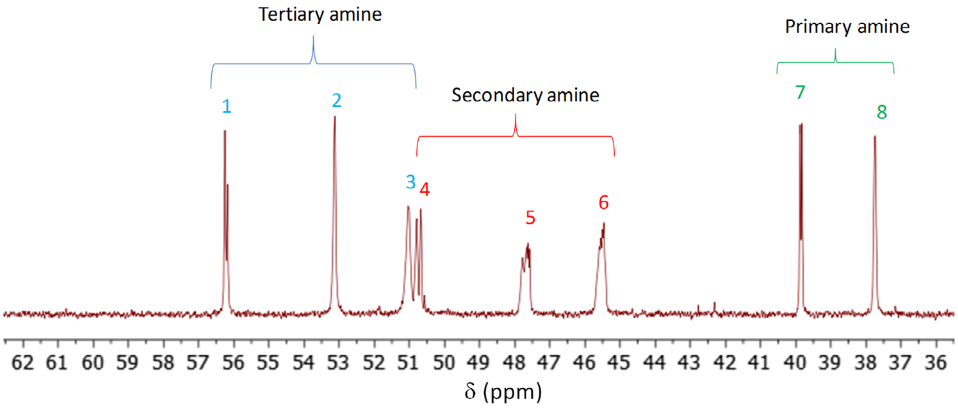

FIGURE 5 Inverse-gated ^{13}C-spectrum of the PEI 1200 sample showing signals originating from the different carbons of the PEI-structure [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Content of primary, secondary, and tertiary amines and self-diffusion coefficients for the PEIs. The numbers within the parentheses refer to the carbon numbering, see Figure 5

	B-PEI 600	PEI 1200	B-PEI 10000	PEI 60000
Primary amine (7,8)	41.9 ± 0.3%	37.8 ± 0.5%	32.2 ± 0.0%	33.0 ± 0.2%
Secondary amine (4,5,6)	35.0 ± 0.3%	37.3 ± 0.4%	41.3 ± 0.5%	39.7 ± 0.8%
Tertiary amine (1,2,3)	23.1 ± 0.1%	24.9 ± 0.9%	26.5 ± 0.5%	27.3 ± 0.6%
Ratio of 1°:2°:3° amines	1.81:1.52:1	1.52:1.50:1	1.22:1.56:1	0.88:1.06:1
Self-diffusion coefficient	2.4E-10 m^2/s	2.0E-10 m^2/s	0.52E-10 m^2/s	0.24E-10 m^2/s

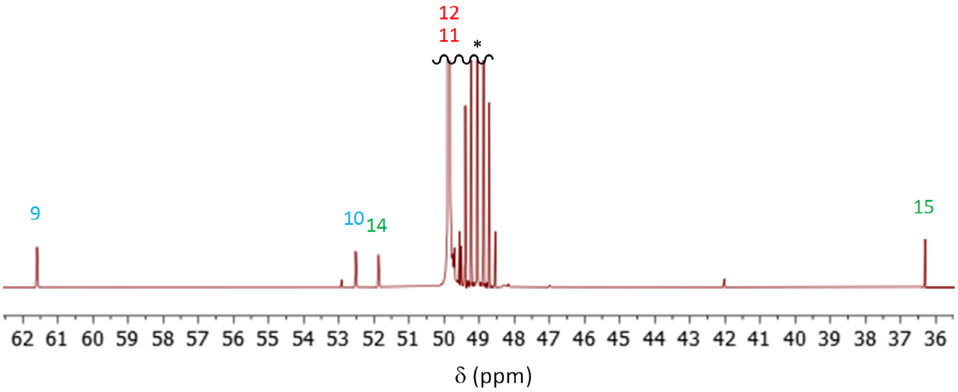

FIGURE 6 Inverse-gated ^{13}C-spectrum of L-PEI 2500 sample showing signals originating from the different carbons of the PEI-structure. The signals from the solvent, CD_3OD, are marked with an asterisk (*) [Color figure can be viewed at wileyonlinelibrary.com]

materials.[24] Using the signal integral intensity of each carbon, the fractions of the different amines could be calculated. To obtain the fraction of primary amines, the integrals of the signals from carbon 7 and carbon 8 were summed. For the secondary amines, the integrals from the signals from carbons 4 to 6 were used and the sum of these integrals was divided by two. Similarly, the sum of the integrals from the signals from carbons 1 to 3 was divided by three to obtain the fraction of tertiary amines. The composition of the PEI polymers is summarized in Table 1.

As can be seen from Figure 5, PEI 1200 contains primary, secondary, and tertiary amines, which means that it is branched but also contains linear segments. This is most likely true for all so-called branched polyethyleneimines.

From Table 1 it can be seen that all four PEIs, regardless of their labelling, showed rather similar ratio between primary, secondary, and tertiary amines. This shows that also PEI 1200 and PEI 60000 can be regarded as branched polyethyleneimines, contrary to specifications.

Self-diffusion measurements were carried out to assess the influence of molecular weight on the diffusion of the four PEIs and the values obtained can also be seen in Table 1. The differences in the self-diffusion coefficients showed the expected decreasing trend with increasing molecular weight of the polymer. One would expect to see a difference in the self-diffusion coefficient with PEIs being linear or branched; however, for the chosen samples the self-diffusion coefficient is more dependent on the M_w difference than on the linearity.

In order to verify the branched structures of all four PEIs discussed above, L-PEI 2500 was used as a reference. The quantitative ^{13}C-spectrum of this linear PEI is shown in Figure 6, using the peak assignment shown in Figure 5 for L-PEI. Deuterated methanol was used as solvent. The strongest signal is originating from the carbons in the repeating unit, labeled 11 and 12 in Figure 5, as expected for a completely linear PEI. In addition, signals with significantly lower intensity are present, corresponding to the carbons close to the hydroxyl end (carbons 9 and 10) and close to or at the methyl end (carbons 14 and 15).

The spectrum of L-PEI 2500 only showed secondary amines and no primary or tertiary amines, as expected from a true linear PEI. In addition, by using the integrals of the different carbons, including those at the end-groups, the molecular weight of the L-PEI could be determined. For the calculations, the polymer was divided into three parts; part A constituting the hydroxyl end-group with the carbons 9 and 10 (blue), part B constituting the main repeating unit with carbons 11 and 12 (red), and part C constituting the methyl end-group with carbons 13, 14, and 15 (green). Integrating the different carbon signals gave the ratio 1:54.4:1 for fragments A:B:C. Using the molecular weights 60 for A, 43 for B, and 58 g/mol for C, provided a number average molecular weight of 2458 g/mol for L-PEI 2500, corresponding well to that provided by the manufacturer. The calculation of M_w is only possible for a linear polymer when both end-groups can be quantified in the spectrum and it cannot be performed for a branched PEI without a known number of terminal groups.

4 | CONCLUSIONS

The chemical information most generally provided for commercially available PEIs is a rough and often insufficient way to characterize the polymer, complicating prediction of the performance of the PEI in various applications. A more detailed description would be helpful and could avoid several misinterpretations. In this work we have demonstrated that a combination of readily available spectroscopic methods, in particular quantitative ^{13}C NMR, is a valuable tool to decipher the architecture of PEI. We have shown that the important issue of the degree of branching can be assessed by the NMR technique.

To illustrate the validity of our approach, we analyzed five different PEIs, for which we had limited amount of information – for some only the molecular weight was given, for others it was also stated whether the polymer was linear or branched. For none of the PEIs there was any quantitative information about the degree of branching. The most commonly used characterization techniques, FTIR or TGA, were not able to show qualitative differences between the PEI samples. Only ^{13}C NMR showed to be useful to assess the architecture, that is, the degree of branching. This technique is very well suited to quantitatively determine the ratio between primary, secondary, and tertiary amines in a PEI. Such information can be important in the selection of a polyethyleneimine for a given application, in particular in cases where a specific amino group plays a key role in performance of the material.

ACKNOWLEDGMENTS

The authors would like to thank the European Commission – APACHE project (Active & Intelligent Packaging Materials and Display Cases as a Tool for Preventive Conservation of Cultural Heritage), through Grant agreement 814496 for financial support.

AUTHOR CONTRIBUTION

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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How to cite this article: K. Grenda, A. Idström, L. Evenäs, M. Persson, K. Holmberg, R. Bordes, *J. Appl. Polym. Sci.* **2021**, e51657. https://doi.org/10.1002/app.51657