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**Citation:** Liu, Harris et al. "Aerosol-Assisted Plasma Deposition of Hydrophobic Polycations Makes Surfaces Highly Antimicrobial." Applied Biochemistry and Biotechnology 172.3 (2014): 1254–1264.

**As Published:** http://dx.doi.org/10.1007/s12010-013-0593-4

**Publisher:** Springer US

**Persistent URL:** http://hdl.handle.net/1721.1/107156

**Version:** Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

# Aerosol-assisted plasma deposition of hydrophobic polycations makes surfaces highly antimicrobial

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**Abstract**

The currently used multi-step chemical synthesis for making surfaces antimicrobial by attaching to them hydrophobic polycations is replaced herein by an aerosol-assisted plasma deposition procedure. To this end, *N*,*N*-hexyl,methyl-PEI (HMPEI) is directly plasma-coated onto a glass surface. The resultant immobilized HMPEI coating has been thoroughly characterized and shown to be robust, bactericidal against *E. coli*, and virucidal against human influenza virus.

**Key words:** *N*-alkylated polyethylenimine; immobilization by aerosol-assisted plasma deposition; microbicidal coatings; antibacterial; antiviral

## Introduction

Creating non-leaching surfaces which kill pathogenic bacteria and disinfect viruses on contact is important due to the ever-growing increase in multi-drug resistant microbes (1,2). Previously, we demonstrated that certain hydrophobic polycations based on alkylated polyethylenimines (PEIs) or poly(vinyl pyridines) covalently attached to various solid surfaces efficiently (over 3-log titer reductions) inactivate bacteria and viruses with no resistance developing (3). However, preparing these covalently-modified antimicrobial surfaces is onerous because it requires harsh reaction conditions, involves multi-step syntheses, and is surface specific (3).

In order to explore elimination of these practical drawbacks, in this study we have attached *N*,*N*-hexyl,methyl-PEI (HMPEI) to a solid surface by alternative, new means using an Invexus$^{TM}$ technology — an atmospheric-pressure plasma liquid deposition process (4-6). This method provides a potential route to covalent immobilization of HMPEI via a facile and readily scalable one-step process, which should be surface-independent. The resultant coatings yield surfaces that are nanometer-thin, conformal, durable, wash resistant, thermally stable, and highly antimicrobial.

## Materials and Methods

### Chemicals and Biologicals

Microscope glass slides (75 × 25 × 1 mm) were purchased from Corning Inc. (Corning, NY). Branched PEI (50% (w/w) aqueous solution, average molecular weight of 50,000 g/mol)

and other chemicals and solvents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and used without further purification. HMPEI was synthesized as previously described (7). *Escherichia coli* (ATCC 8739) and Madin-Darby canine kidney (MDCK) cells were obtained from the ATCC (Manassas, VA). The bacterial cells were grown in Luria-Bertani (LB) broth, Lennox (Becton, Dickinson and Co., Franklin Lakes, NJ). Influenza A/PR/8/34 (H1N1) virus was obtained from Charles River Laboratories (Wilmington, MA).

Atmospheric Pressure Plasma Liquid Deposition (APPLD)

HMPEI was deposited using the Invexus™ technology, an atmospheric-pressure plasma liquid deposition process utilizing a non-thermal plasma to polymerize and covalently bond precursor monomers onto surfaces. Specifically, a batch-to-batch platform, SE2100™ Jet PlasmaStream Workstation (4,5), was used. Briefly, the device consists of a plasma deposition head (Figure 1) where the plasma generation and precursor atomization take place, and a computer controlled x-y-z table is used to move the deposition system relative to the surface to be coated. The plasma deposition head utilizes a 19-mm internal-diameter tubular nozzle assembly containing high-voltage RF electrodes that ionize the flowing inert gas and direct the resultant plasma discharge onto the targeted surface. The liquid precursors are introduced via a pneumatic nebulizer as an atomized liquid directly into the plasma discharge within the tubular nozzle. The combination of free-radical and ionized-plasma species activates the liquid precursors sufficiently to initiate surface modification but without significant fragmentation and re-arrangement of the molecules, resulting in nanometer-thick coatings on the targeted surface. The plasma also ensures that the coating is chemically bonded to the surface due to its simultaneous activation during the deposition process.

For the deposition process, 5% (w/w) HMPEI in ethanol was used as a precursor solution which was delivered to the atomizer at a rate of 15 μL/min with inlet pressure of 50 psi at room temperature. A frequency of 20 kHz with peak voltages of 19.5 to 21.0 kV was used for power supply. Helium was used as the inert gas at a rate of 15 L/min. The target surface explored herein was that of soda lime glass slides which were used as obtained commercially or cut into 10 × 25 mm rectangular pieces. The gap between the nozzle and the surface was approximately 0.5 mm. The plasma deposition head moved at a scan rate of 30 mm/sec, and the gap between raster scans was 1 mm. The glass surfaces were scanned either 10 or 20 times; for a 75 × 25 mm glass slide, one pass takes approximately 66 sec.

Analysis of Quaternary Ammonium Groups

A HMPEI-coated glass slide (55 x 25 mm) was shaken in a 1% solution of fluorescein Na salt in phosphate-buffered distilled water (0.1 M $NaH_2PO_4$, pH 8.0) for 15 min. The slide was then rinsed thrice with the dye-free buffered solution before being placed in 45 mL of 0.1% cetyltrimethylammonium chloride in this aqueous buffer solution and shaken for 15 min to desorb the dye (8). The light absorbance of the resultant aqueous solution was measured at 501 nm, and the concentration of the dye was determined using the previously obtained extinction coefficient in this solution of 77 $mM^{-1}cm^{-1}$ (9).

X-ray Photoelectron Spectroscopy (XPS)

XPS analysis was used to determine the coatings composition and, more specifically, the nitrogen content of the HMPEI coatings. A Kratos Axis Ultra XPS system equipped with a hemispherical analyzer and a 100 W monochromatic Al Kα (1486.7 eV) beam was used. This analysis was performed on 1 × 0.5 mm sampling area at a take-off angle of 90°. The base

pressure in the XPS chamber was held between $10^{-9}$ and $10^{-10}$ Torr. Survey scans and elemental high-resolution scans for C 1s and N 1s were taken in the constant analyzer energy mode of 80 and 20 eV pass energy, respectively. Corrected binding energy for the C 1s and N 1s spectra were set to 285.0 and 399.6 eV, respectively, corresponding to the aliphatic carbon-carbon and the nitrogen-hydrogen, respectively. Deconvolution of high-resolution C 1s and N 1s spectra was carried out through a CasaXPS software using a Gaussian-Lorentzian fit and a full-width half-maximum (FWHM) of 1.6 or less. A total of four spots were scanned for each sample.

Contact Angle and Profilometry

The wettability of the HMPEI-coated glass surfaces was assessed through water contact angles based on the sessile-drop method. Contact angles were measured using a FTA-1000B contact angle and surface tension instrument (First Ten Angstroms, Inc., Portsmouth, VA). The volume of the water droplet was 5 μL. The HMPEI coating thickness was measured using a XP-1High-Resolution Surface Profiler profilometer (AmBios Technology, Inc., Santa Cruz, CA). The coated surfaces were scanned for 2.00 mm at a scan speed of 0.01 mm/sec and stylus force of 0.05 mg.

Durability Test

Plasma-coated glass slides were rinsed thrice with distilled water and then shaken overnight in a 1% Liquinox anionic detergent solution at 55°C. This treatment was followed by rinsing the slides thrice with distilled water again and then titrating them with fluorescein as described above. The quaternary ammonium group densities were subsequently compared with those of the pre-washed samples to determine whether the titer had decreased due to washing.

Bactericidal and Virucidal Activities

An overnight culture of *E. coli* was spun down in a centrifuge tube, and the supernatant was discarded. Phosphate-buffered saline solution (PBS) was then added to the tube, and the cell pellet was re-suspended. Centrifugation and re-suspension steps were repeated, and the final *E. coli* solution was diluted to approximately $6 \times 10^6$ CFU/mL with PBS. This diluted bacterial solution (10 μL) was inoculated onto a coated glass slide (10 × 25 mm), and an uncoated glass slide of the same size was placed on top to spread the inoculum. After a 2-h incubation, the slides were transferred into centrifuge tubes with 10 mL of an extraction solution (0.5% (w/v) lecithin and 4% (w/v) Tween 20 in PBS). The tubes were vortexed to separate the slides. The extraction solution was subsequently plated to determine CFU counts.

Virucidal activity was determined as previously described (10). Briefly, a 10-μL droplet of an influenza virus solution (approximately $10^8$ PFU/mL) was placed in the center of a coated glass slide (25 × 25 mm). A non-coated glass slide was then used to cover and spread the droplet. After a 10-min incubation, the virus-exposed sides of both slides were washed with 0.99 mL of PBS. Two-fold serial dilutions of the washings were then made with PBS, and 200 μL of each dilution was subsequently added to confluent MDCK cells in 6-well tissue culture plates to determine plaque counts.

Thermal Stability of HMPEI Coatings

The HMPEI-coated glass slides were exposed to 90 °C or 150 °C for 2 h in an oven and then tested for antimicrobial activity to determine thermal stability of the coatings. Differential scanning calorimetry (DSC) (Q20, TA Instruments, New Castle, DE) of the raw precursor of HMPEI (not of the resultant coatings) was performed by equilibrating it at -40 °C, heating it up to 150 °C at 10 °C/min, holding it at 150 °C for 0.25 min (the first scan), cooling it to -40 °C at

10 °C/min, holding it at -40 °C for 0.25 min, and heating it up to 150 °C at 10 °C/min (the second scan).

**Results and Discussion**

The goal of this study was to explore a new, single-step procedure for attaching hydrophobic polycations, as exemplified by HMPEI, to solid surfaces using atmospheric-pressure plasma liquid deposition. A key benefit of the facile plasma deposition process used herein is that a degradation of the precursor molecules (HMPEI in this case) in the plasma is minimized by introducing it as a liquid aerosol into a non-thermal plasma (4). Consequently, the resultant coating retains the chemical structure of the precursor molecules, thus achieving characteristics similar to those reported for pulsed-vacuum plasmas (11, 12) or in wide-area aerosol-assisted atmospheric-pressure plasma coatings (6). Also, exposure to the He discharge leads to further cleaning of the target surface, followed by plasma-induced functionalization that promotes better adhesion of the coating. Furthermore, the deposition process takes place at room temperature making it suitable for a variety of surfaces, including those of soft materials.

The extent of plasma coating of glass slides with HMPEI was initially characterized by quantifying the surface density of the grafted quaternary ammonium groups using a fluorescein assay. As seen in Table 1 (first data column), 4.7 nmol/cm$^2$ of quaternary ammonium group were introduced after 10 plasma-coating passes each lasting about a minute or less. This value has not changed appreciably after 10 additional plasma coating cycles (Table 1) indicating that saturation of the surface was already achieved. Importantly, these quaternary ammonium group densities

were comparable to those determined by us previously for glass slides covalently derivatized using the conventional 5-step surface-specific chemical synthesis (9).

To assess the robustness of the surface-immobilized HMPEI prepared herein, we subjected the plasma-coated glass slides to exhaustive washing with water and a Liquinox detergent at an elevated temperature (55°C). As seen in Table 1 (last column), there was no detectable difference in the quaternary ammonium group densities before and after the washing. These results indicate that plasma deposition of HMPEI results in a covalent attachment, as opposed to a physical adsorption, of the hydrophobic polycations to the glass surface; this conclusion was further confirmed using the detailed surface analysis techniques described below.

Next, surface compositions of the HMPEI coating were investigated by means of XPS analysis. An example of a survey scan for each sample is depicted in Figure 2, and the surface compositions are shown in Table 2. Trace elements not associated with the HMPEI deposition process were identified from XPS and attributed to residue due to handling; therefore, they were disregarded in the elemental considerations of the coatings. As expected, the nitrogen content rose with the number of passes of the plasma-coating process. Likewise, the Br content (from the HMPEI's counter-ion) approximately doubled as the number of coating passes was raised from 10 to 20. Since Br is an artifact from HMPEI synthesis, it is expected to increase similarly to the N content. The Si peaks were from the underlying glass surface. For thin coatings (fewer passes by the plasma-coating process), more intense Si signals from the underlying glass surface were detected in agreement with previous studies of plasma deposition (4).

To determine the carbon-based functional groups, deconvolution of the C1s peaks was employed, which is depicted in Figure 3. T-fitted peak binding energy (B.E.) and chemical

group identifications are listed in Table 3. Of particular note is the presence of the peak at 284.3 eV attributed to a C-Si bond in both samples. This peak further confirms that the coatings are chemically bonded to the glass surface via a direct carbon-to-silicon bond, whose presence explains the high level of wash resistance exhibited by the coatings. In addition, the curve fitting revealed a series of peaks attributed to the hydrocarbon and nitrogen species of the starting hydrophobic polycation, indicating that the functional chemistry of the precursor was retained in the coating. Additional weaker peaks at 287.2 and 288.6 eV (Fig. 3) are attributed to the presence of O in the coating. This may be due to minor oxidation of the precursor HMPEI (9), or reflect the formation of chemical bonds between the coating and the $SiO_x$ layer, or be a combination of both. The N1s signal peak shape for HMPEI coating deposited over 10 passes is distinct from that deposited over 20 passes. Deconvolution revealed three peaks at 399.6, 401.4, and 402.5 eV, as depicted in Figure 4, corresponding to C-N, quaternary N, and protonated amine groups, respectively. This analysis shows that cationic nitrogen moieties are indeed present in the HMPEI coatings.

As seen from the data in Table 4, the HMPEI coatings were moderately hydrophobic and their thickness was approximately 30 nm. Note that both contact angle and coating thickness were comparable regardless of the number of plasma coating passes.

Having achieved a permanent attachment of HMPEI to the glass surface by plasma deposition and characterized the resultant coatings, we then examined their antimicrobial properties. Bactericidal activity was tested against waterborne *E. coli*. As seen in Table 5 (first data column), the plasma-coated slides afforded an over 2.5-log reduction in the bacterial titer when compared to the uncoated (control) slides under the same conditions. Thus, greater than 99% reduction in bacterial numbers was achieved by both the 10- and 20- pass coating samples

(Table 5). This antibacterial potency is comparable to that of the HMPEI immobilized via a conventional 5-step procedure (9).

We also assessed the antiviral properties of the HMPEI-coated glass slides against human influenza H1N1 virus. As seen in the last column of Table 5, the plasma-coated slides resulted in a greater than 4-log reduction in the viral titer when compared to the control. These data demonstrate that both antibacterial and antiviral properties of plasma-coated HMPEI slides are at least on par with those previously prepared using multi-step and surface-dependant covalent attachment of this hydrophobic polycation to the same surface.

A 2-h incubation of the immobilized HMPEI, deposited by either 10 or 20 coating passes, at 150 °C resulted in no reduction and, in fact, a slight increase of its antibacterial activity. This observation further attests to good heat resistance of the plasma-deposited hydrophobic polycation.

Finally, differential scanning calorimetry was used to probe the thermal properties of the HMPEI prior to its plasma immobilization onto a glass slide. As seen in Figure 5, the hydrophobic polycation features a glass transition temperature ($T_g$) at approximately 70 °C in the first DSC scan. There was also a broad exotherm between 110 °C and 150 °C, which might indicate some polymer cross-linking in this temperature range. Indeed, a second scan of the same sample showed that the $T_g$ value rose to about 100 °C, indicating a change in properties. It is noteworthy, however, that this putative cross-linking or another molecular re-arrangement had likely occurred upon exposure of the plasma-coated slide to 150 °C for 2 h and, as noted above, no adverse effect on the antibacterial efficacy was observed.

In closing, in the present work we have examined and validated a facile and scalable atmospheric-pressure plasma liquid deposition method for readily attaching hydrophobic polycationic coatings to a solid surface in a single step to endow it with strong and permanent antimicrobial properties.

**Acknowledgment**

This research was financially supported by the U.S. Army Research Office under contract W911NF-07-D-0004.

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**Table 1.** Quaternary ammonium group densities of glass slides plasma-coated with HMPEI before and after detergent washing as a function of the number of coating passes.

| No. of passes | Quaternary ammonium group density (nmol/cm$^2$) | |
|---|---|---|
| | Before wash | After wash |
| Uncoated | 0 | 0 |
| 10 | 4.7 ± 0.3 | 4.6 ± 0.4 |
| 20 | 4.5 ± 0.4 | 4.5 ± 0.4 |

**Table 2.** The surface composition of HMPEI coating on glass slides after 10 and 20 plasma coating passes.

| Number of passes | Spot no. | Atomic content (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | C 1s | O 1s | N 1s | Ca 2p | Br 3p | Si 2p |
| 10 | 1 | 23.37 | 47.98 | 2.72 | 0.99 | 0.89 | 24.05 |
| | 2 | 23.39 | 51.27 | 2.40 | 1.11 | 0.66 | 21.17 |
| | 3 | 24.03 | 46.34 | 2.53 | 0.92 | 0.75 | 25.44 |
| | 4 | 21.95 | 50.49 | 3.30 | 0.76 | 1.09 | 22.39 |
| | Avg. | 23.19 | 49.02 | 2.74 | 0.95 | 0.85 | 23.26 |
| 20 | 1 | 37.05 | 37.01 | 4.77 | 0.53 | 2.08 | 18.56 |
| | 2 | 34.70 | 42.15 | 4.57 | 0.37 | 2.13 | 16.09 |
| | 3 | 38.43 | 36.40 | 4.48 | - | 2.65 | 18.04 |
| | 4 | 39.35 | 35.17 | 4.88 | 0.38 | 1.85 | 18.37 |
| | Avg. | 37.38 | 37.68 | 4.68 | 0.43 | 2.18 | 17.77 |

**Table 3.** Fitted peak binding energies (B.E.) and chemical group identification (13,14,15).

| Peak no. | B.E. (eV) | Assigned chemical group |
|---|---|---|
| C1 | 284.3 | C-Si |
| C2 | 285.0 | C-C |
| C3 | 285.6 | C-C-C / C-N |
| C4 | 286.5 | C-O, C-C-C |
| C5 | 287.2 | N-C=O |
| C6 | 288.6 | C=O |
| N1 | 399.6 | C-N, cyano |
| N2 | 401.4 | quaternary nitrogen |
| N3 | 402.5 | $-N^{+}-$, protonated amine |

**Table 4.** Physical properties of HMPEI coatings.

| Number of passes | Contact angle with water[a] (°) | Coating thickness[b] (nm) |
|---|---|---|
| 10 | 55.9 ± 1.8 | 32 ± 19 |
| 20 | 50.3 ± 0.8 | 34 ± 13 |

[a] Contact angle was measured once from each sample and averaged from three samples.

[b] Coating thickness was measured in three different locations for each sample and averaged from three samples (a total of 9 measurements).

**Table 5.** Antimicrobial efficiencies of glass slides plasma-coated with HMPEI against *E. coli* and influenza viruses as a function of the number of coating passes.

| Number of passes | Titer reduction (logs) | |
|---|---|---|
| | *E. coli* | Influenza virus |
| 0 (control) | 0 | 0 |
| 10 | > 2.8[a] | 4.5 ± 0.3 |
| 20 | > 2.6[a] | 4.3 ± 0.4 |

[a] No bacterial colonies were observed on agar plates, and the numbers given indicate detection limits of the test based on dilutions and plated volumes.

**Table 6.** Antimicrobial efficiencies of glass slides plasma-coated with HMPEI after heat treatment against *E. coli* as a function of the number of coating passes.

| Number of passes | Titer reduction (logs) | |
|---|---|---|
| | After 2 h at 90 °C | After 2 h at 150 °C |
| 0 (control) | 0 | 0 |
| 10 | 1.2 ± 0.4 | > 3.2[a] |
| 20 | 1.7 ± 0.4 | 3.3 ± 0.4 |

[a] No colonies were observed on agar plates, and the number given indicates the detection limit of the test.

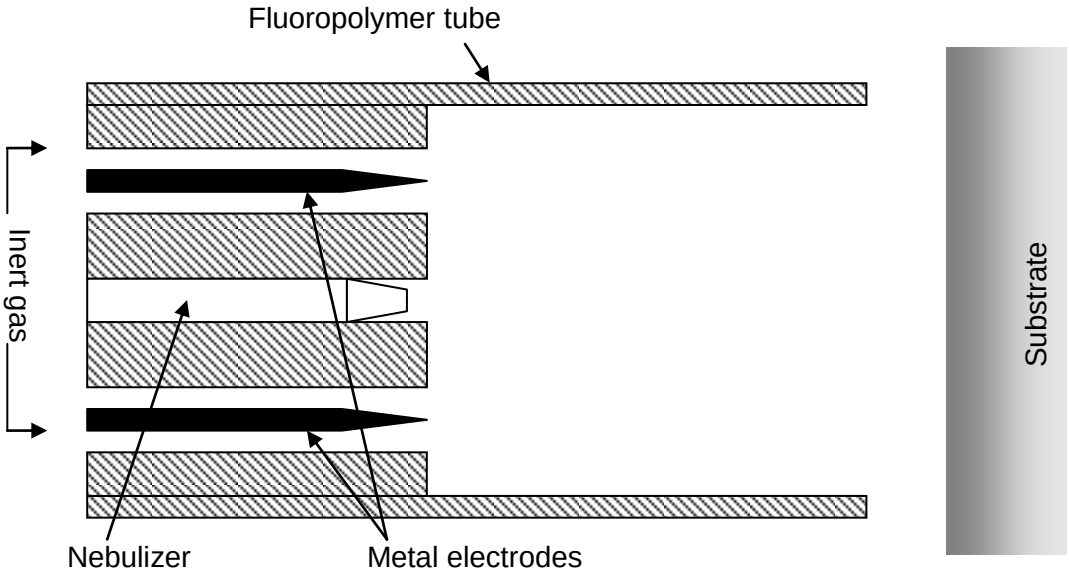


**Figure 1.** Schematic diagram of the plasma head.

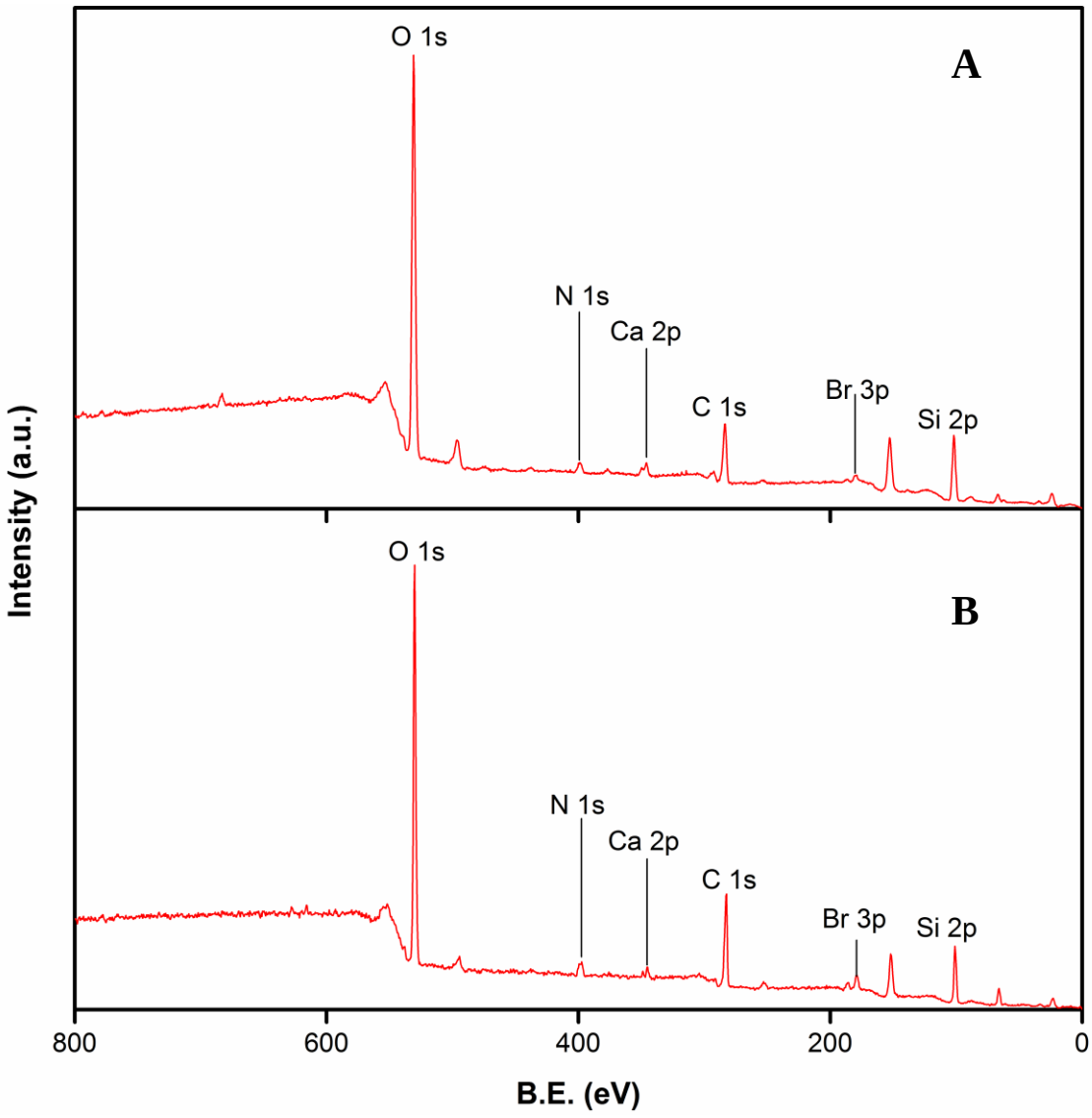


**Figure 2.** XPS survey spectra of HMPEI coatings deposited on glass slides after 10 passes (**A**) and 20 passes (**B**).

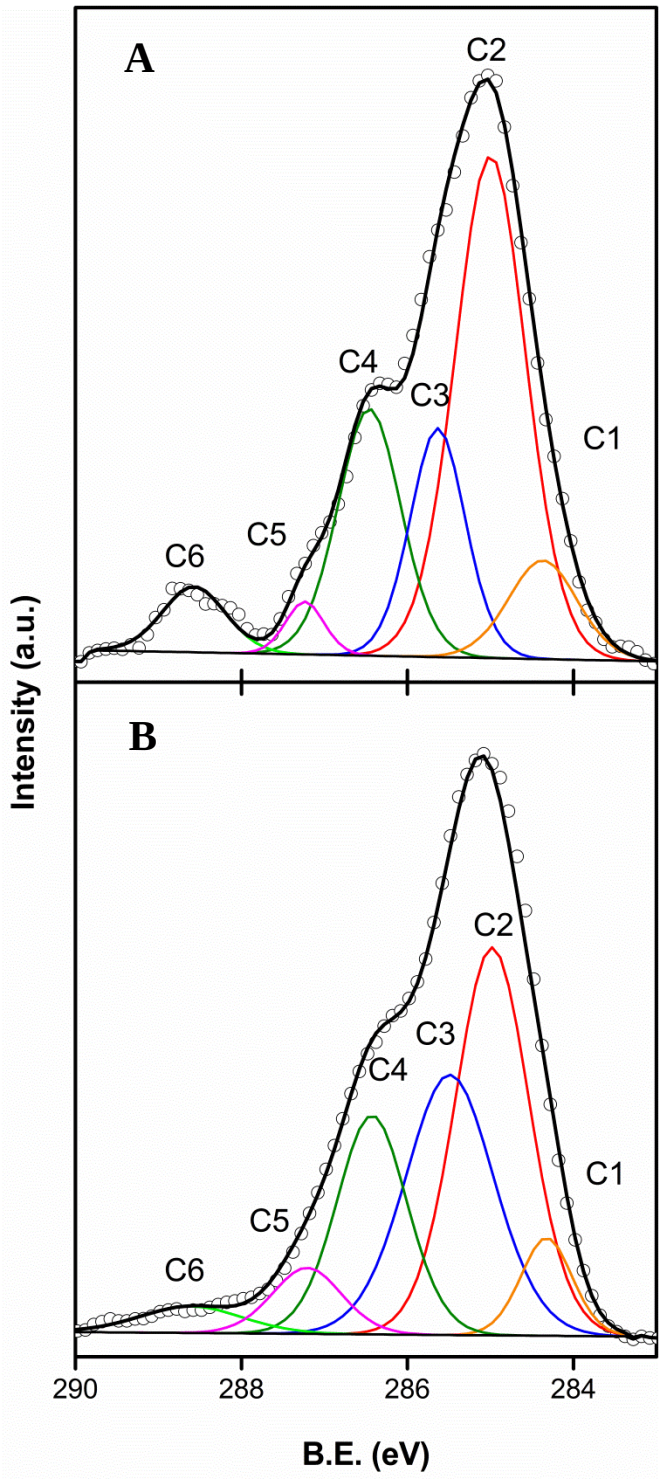


**Figure 3.** Deconvoluted C1s peak of the HMPEI coatings after 10 passes (**A**) and 20 passes (**B**).

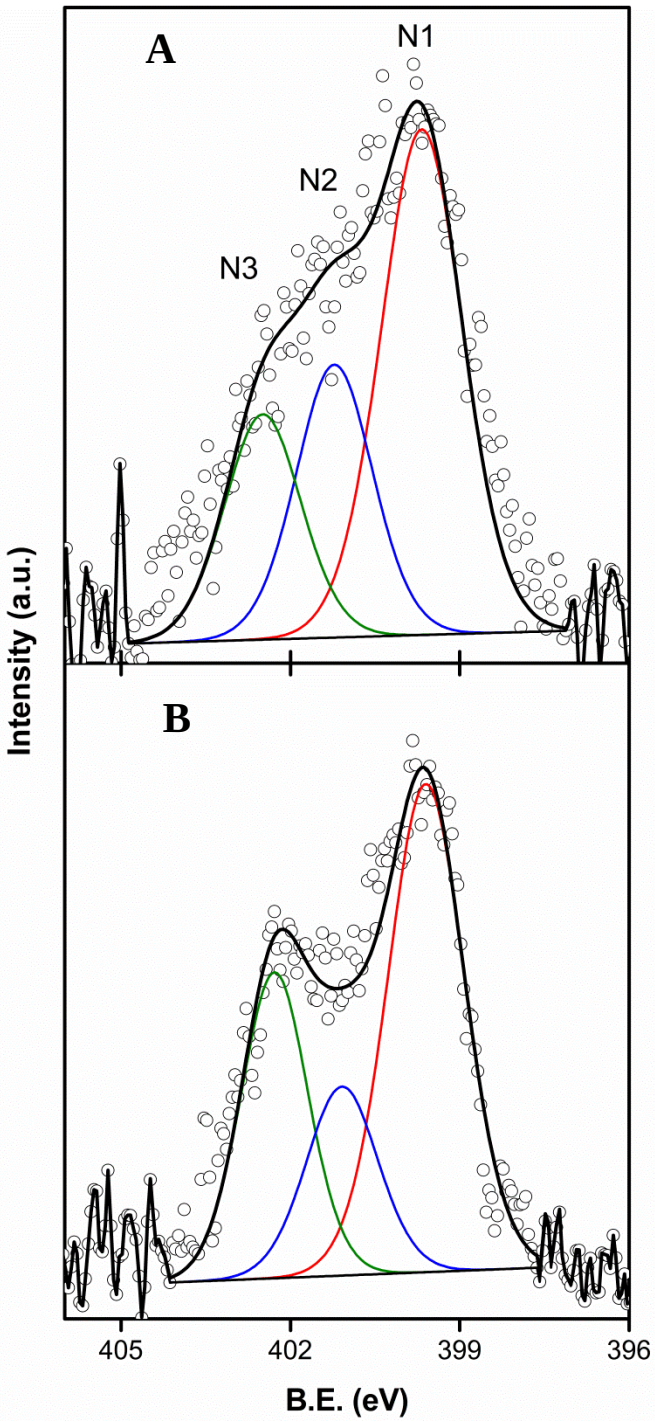


**Figure 4.** Deconvoluted high-resolution N1s peak of the HMPEI coatings after 10 passes (**A**) and 20 passes (**B**).

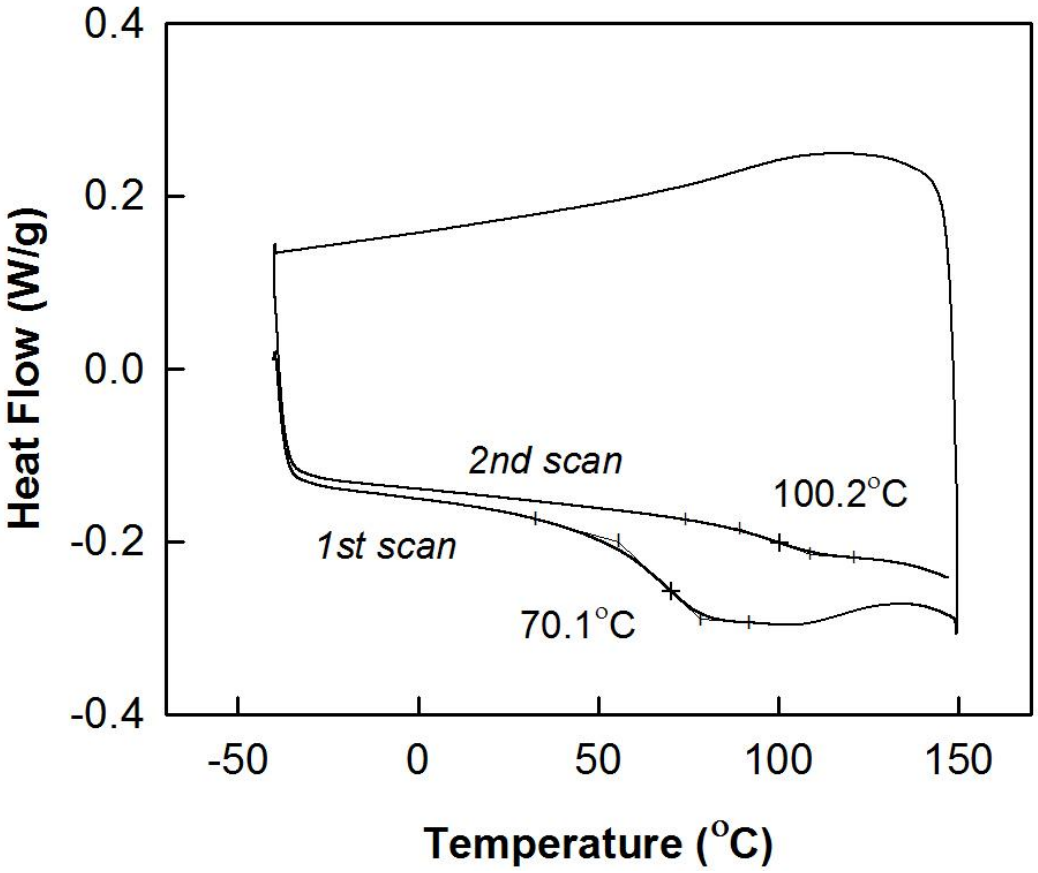


**Figure 5.** Differential scanning calorimetry of HMPEI.