Electronic Supplementary Information

Well-defined poly(2-isopropenyl-2-oxazoline) brushes provide fouling resistance and versatility in surface functionalization.

Manisha Singh, Lenka Poláková, Andres de los Santos Pereira, Ognen Pop-Georgievski, Jan Svoboda, Tomáš Riedel, Sachin Gupta, Zdeňka Sedláková, Vladimír Raus and Rafał Poręba*

Institute of Macromolecular Chemistry, Czech Academy of Sciences, Heyrovského nám. 2, 162 00 Prague 6, Czech Republic

Email: poreba@imc.cas.cz

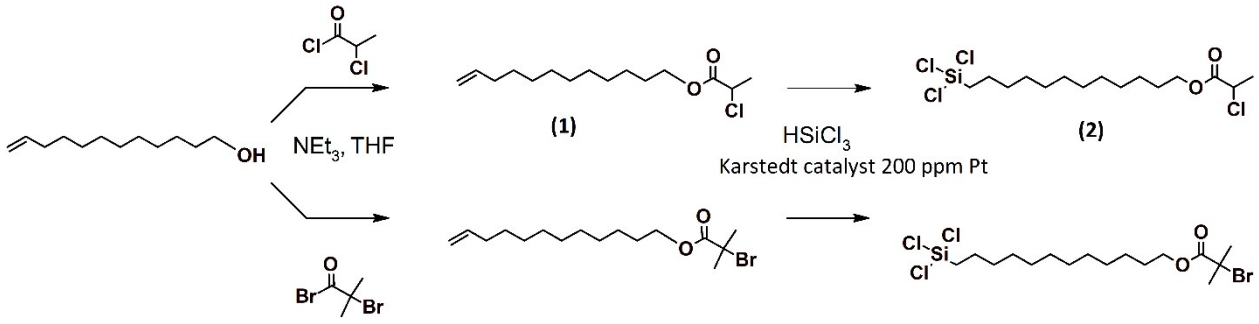
EXPERIMENTAL SECTION

Materials

2-Isopropenyl-2-oxazoline (IPOx; Sigma-Aldrich, 98%) was distilled from calcium hydride under reduced pressure and stored under argon at 4 °C. *N*-(2-hydroxypropyl)methacrylamide (HPMA) was synthesized according to the literature procedure and stored at –20 °C.[1] 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA; Sigma-Aldrich, 99%) was distilled under reduced pressure and stored under argon at 4 °C. Tris(2-pyridylmethyl)amine (TPMA) was synthesized according to a literature protocol,[2] purified by recrystallization,[3] and stored at 4 °C. Tris[2-(dimethylamino)ethyl]amine (Me_6TREN) was synthesized and purified according to a literature protocol and stored under argon at 4 °C.[2] 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA; 97%), copper(I) chloride (99.999%), copper(II) chloride (99.999%), 2-chloropropionic acid (CPA, 99 %), sodium azide (≥99.5%), and propargyl bromide (80 wt. % in toluene; >88.7%) were purchased from Sigma-Aldrich and used as received. 1,4,8,11-Tetramethyl-1,4,8,11-tetraazacyclotetradecane (Me_4Cyclam; ABCR GmbH (Germany), 98%), *N*-methyl-2-pyrrolidone (NMP; Acros Organics, 99.5%, extra dry), anhydrous *N*,*N*-dimethylformamide (DMF; Carl Roth, ≥99.8 %, for peptide synthesis). Hydrochloric acid (Lach-ner (Czech Republic), 35%), 11-azido-3,6,9-trioxaundecanoic acid (AA; TCI Europe, >97%), spectral grade absolute ethanol (VWR Chemicals, ≥99.8%), and anhydrous toluene (VWR Chemicals, ≥99.8%) were used as received. Milli-Q water with resistivity of 18.2 MΩ cm was drawn from a Millipore Direct-Q8 system (Millipore advantage A10 system, Schwalbach, with Milli-Mark Express 40 filter, Merck, Germany). Silicon wafers (orientation<100>) with a native silicon oxide layer were purchased from Siegert Wafer GmbH (Germany). Au-coated SPR chips with a 15-nm-thick SiO_2 top-layer deposited on a 1.5-nm-thick titanium adhesive layer were obtained from the Institute of Photonics and Electronics CAS (Czech Republic). Fibrinogen (Fbg), bovine serum albumin (BSA), and fetal bovine serum (FBS) were purchased from Sigma-Aldrich. Human blood plasma (BP) was kindly provided by the Institute of Hematology and Blood Transfusion (Czech Republic). All samples were obtained in accordance with regulations of the Ethical Committee of the Institute of Hematology and Blood Transfusion, no. EK 3/GA CR/03/2019.

Procedures

Synthesis of trichlorosilylated Cu-RDRP initiators



The synthetic pathway for the preparation of Cu-RDRP initiators bearing brominated or chlorinated initiating sites and highly reactive trichlorosilyl groups is shown in the scheme above. The synthesis of the brominated variant, 11-(trichlorosilyl)undecyl-2-bromoisobutyrate (TUBiB), was conducted as previously reported by Rodriguez-Emmenegger et al.[4] The synthesis of the chlorinated variant, 11-(trichlorosilyl)undecyl-2-chloropropanoate (TUCP), was performed in an analogous way, with some improvements in the second step as detailed below.

11-(Trichlorosilyl)undecyl-2-chloropropanoate (TUCP)

In the first step, undec-10-en-1-yl 2-chloropropanoate (1) was synthesized. The solution of 2-chloropropionyl chloride (8.5 mL, 85 mmol) in dry THF (30 mL) was added dropwise to a Schlenk flask containing the solution of 10-undecen-1-ol (15 mL, 75 mmol) and triethylamine (13.5 mL, 90 mmol) in dry THF (75 mL) at 0 °C. The reaction mixture was stirred overnight at r.t. Subsequently, the mixture was diluted with hexane (150 mL) and washed with 2x100 mL of 2 M HCl, 2x100 mL of brine, and 1x100 mL of water, and dried over sodium sulphate. After removal of the solvent under vacuum, the product was purified by vacuum distillation (yield 97%).

In the second step, Karstedt's catalyst (200 ppm Pt equivalent) was slowly added to an Ar-filled, dried, thick-walled flask, containing undec-10-en-1-yl 2-chloropropionate (1) (8.15 g, 31 mmol) and trichlorosilane (31.5 mL, 310 mmol), and the mixture was stirred at r.t. overnight. Afterwards, the reaction mixture was quickly passed through a silica plug wetted with dry dichloromethane to remove the Pt catalyst, and the remaining trichlorosilane and dichloromethane were subsequently removed under reduced pressure. Caution: trichlorosilane boils in contact with silica, therefore the purification should be conducted with the highest care by an experienced chemist. The final product (2) was purified by Kugelrohr distillation at 195 °C, under vacuum ($3 \cdot 10^{-2}$ Torr), resulting in a transparent viscous liquid (yield 56 %).

Immobilization of the Cu-RDRP initiators on Si and SPR chips

First, chips were consecutively washed with spectral grade ethanol and Milli-Q water, dried in a nitrogen stream, and then activated with a UV-ozone cleaner (Model 42 Series, Jelight Company INC., USA) for 20 min. Immediately afterwards, the chips were placed into a Petri dish containing the solution of the TUCP or TUBiB initiator (10 μL) in dry toluene (10 mL), the Petri dish was sealed with parafilm, covered with an aluminum foil, and left for 3 hours at r.t. in a desiccator. Then, the chips were washed by a short immersion in dry toluene and then in non-dried toluene, which was followed by washing with acetone, ethanol, and finally Milli-Q water. Thereafter, the chips were dried in a nitrogen stream and stored in vacuum until use.

SI Cu(0)-RDRP of IPOx

A typical procedure was as follows: Into a Schlenk flask equipped with a magnetic stirring bar, CuCl (23.6 mg, 0.238 mmol), and $CuCl_2$ (3.2 mg, 0.0238 mmol) were weighed. After thorough deoxygenation by three vacuum-argon cycles (Schlenk line vacuum of $3 \cdot 10^{-2}$ Torr), water (5 mL), degassed through five freeze–pump–thaw cycles, was added. Subsequently, HMTETA (82 μL, 0.3015 mmol) was added, and the dark blue mixture was stirred for 30 min. (Note: in case a solid ligand is used, it is added together with copper salts). Finally, IPOx (2.5 mL, 23.84 mmol) was added, and the mixture was allowed to stir for 5 min. Afterwards, the polymerization mixture was sampled into individual flask of a deoxygenized custom-built reactor[5] containing the initiator-functionalized Si chips. In kinetic experiments, polymerizations were conducted in parallel at 30 °C for specified time periods. The polymerization was stopped by the addition of fresh, oxygen-rich Milli-Q water. Finally, the samples were consecutively rinsed with spectral grade ethanol and Milli-Q water and dried in a nitrogen stream. The Si chips modified with PIPOx brushes were stored in vacuum until further use.

The procedure where the disproportionation was performed in the monomer presence[6-8] was conducted as follows: A Schlenk flask containing a magnetic stirring bar was loaded with $CuCl_2$ (3.2 mg, 0.0238 mmol), IPOx (2.5 mL, 23.84 mmol), HMTETA (82 μL, 0.3015 mmol), and deoxygenated Milli-Q water (5 mL). Subsequently, CuCl (23.6 mg, 0.238 mmol) was added under a positive argon flow, and the mixture was stirred for 30 min at r.t. The polymerization mixture was then sampled into the individual flasks of a custom-built reactor containing the initiator-functionalized Si chips. The polymerization was then conducted and products were isolated and stored in the same way as in the standard procedure above.

Modification of PIPOx brushes with functional carboxylic acids

The modification procedure with CPA is provided as an example; the reaction with ADA was conducted in the same way (in dry DMF). Into a flask of a custom-build reactor[5] containing a silicon chip coated with a PIPOx brush, dry NMP (4 mL) and CPA (103.2 μL, 300 mM) were added, and the mixture was heated to 60 °C for 24 h. Afterwards, the chip was removed, shortly immersed in dry NMP, then consecutively rinsed with spectral grade ethanol and Milli-Q water, and dried in a nitrogen stream.

Conversion of the chlorine end groups of PIPOx brushes to azide end groups

Into a flask of a custom-built reactor containing a PIPOx-modified Si chip, NaN_3 (5.3 mg, 0.081 mmol) in dry DMF (1 mL) was added, and the flask was heated to 60 °C for 14 h. Subsequently, the chip was shortly immersed in pure DMF, rinsed with spectral grade ethanol, Milli-Q water, and dried in a nitrogen stream.

PIPOx-g-poly(HPMA) brushes

Into a Schlenk flask equipped with a magnetic stirring bar, CuCl (5.92 mg, 0.060 mmol), $CuCl_2$ (1.8 mg, 0.012 mmol), and Me_4cyclam (20.4 mg, 0.078 mmol) were placed. After thorough deoxygenation by three vacuum-argon cycles, degassed water (4 mL) was added, and the dark blue mixture was stirred for 30 min. The obtained mixture was then transferred to another deoxygenated Schlenk flask containing HPMA (476.5 mg, 3.33 mmol) and allowed to stir for 5 min. Subsequently, the polymerization mixture was sampled into individual flasks of a deoxygenated custom-build reactor containing Si chips with the CPA-modified PIPOx brush. Polymerizations were conducted in parallel at 30 °C for specified time periods. The polymerization was stopped by the addition of fresh, oxygen-rich Milli-Q water. Finally, the samples were consecutively rinsed with spectral grade ethanol and Milli-Q water and dried in a nitrogen stream.

PIPOx brush modification via the CuAAC click reaction

CuCl (35 mg, 0.353 mmol) was weighed into a Schlenk flask and deoxygenated by three consecutive vacuum-argon cycles. Then, toluene (3.5 mL), methanol (3.5 mL), propargyl bromide (75 μL, 0.876 mmol), and Me_6TREN (83 μL, 0.310 mmol) were added, and the resulting mixture was stirred overnight. Afterwards, the mixture was transferred to a flask of a custom-built reactor containing an Si chip covered with the ADA-modified PIPOx brush and allowed to react for 1 h at r.t. Subsequently, the Si chip was thoroughly washed with spectral grade ethanol and Milli-Q water and dried in a nitrogen stream.

Characterization

Spectroscopic ellipsometry

The dry thickness of PIPOx brushes grafted from silicon chips was determined using a J.A. Woollam M-2000X Spectroscopic Ellipsometer at three angles of incidence (60°, 65° and 70°) and in the wavelength range between 245 nm and 1000 nm. A multilayer Cauchy model was used for the fitting of the raw data in the CompleteEASE software. The swelling ratio of PIPOx brushes in water was measured via *in situ* ellipsometry using an immersion cell/cuvette, which allows for variation of the angle of incidence. The device, measurement, and data analysis were carried out as previously described in detail.[9]

Grazing-angle attenuated total internal reflection Fourier transform infrared spectroscopy (GAATR-FTIR)

The chemical structures of (modified) PIPOx polymer brushes were assessed by GAATR-FTIR spectroscopy using a Nicolet Nexus 870 FTIR spectrometer equipped with a VariGATR attachment (Harrick Scientific). The spectra were acquired under continuous purging with dry air at the resolution of 4 cm^{-1}; 256 scans were acquired. A bare Si chip, freshly cleaned by rinsing with ethanol and Milli-Q water, dried under nitrogen flow, and activated in a UV/Ozone cleaner for 20 min, was used as a background.

X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed using a K-Alpha$^+$ XPS spectrometer (ThermoFisher Scientific, UK) operating at a base pressure of 1.0×10^{-7} Pa. The data acquisition and processing were performed using the Thermo Avantage software. To limit the X-ray induced destruction of the thin polymer films and to maximize the signal-to-noise ratio, individual points were measured within areas covering 8 × 8 mm^2. At each point, a high-energy resolution core-level spectrum was measured using microfocused monochromated Al Kα X-ray radiation (an elliptic spot of size 400 µm × 200 µm, pass energy of 100 eV). All reported XPS spectra are averages of 64 individual measurements. The spectra were referenced to the C 1s peak of hydrocarbons at binding energy of 285.0 eV, controlled by means of photoelectron peaks of poly (ethylene terephthalate) PET and metallic Cu, Ag, and Au standards. The atomic concentrations of the different chemical moieties were determined from the respective photoelectron peak areas of the Br 3d, Si 2p, C 1s, N 1s, O 1s, and Cu 2p high resolution spectra after applying Shirley's inelastic background subtraction. Assuming a simple model of a semi-infinite solid of homogeneous composition, the peak areas were corrected for the photoelectric cross-sections, the inelastic mean free paths of the electrons in question, and the transmission function of the spectrometer used.[10] All high-resolution spectra were fitted using Voigt profiles.

Surface plasmon resonance (SPR)

The extent of non-specific protein adsorption from single-protein solutions and undiluted blood plasma was determined via SPR on PIPOx brushes grafted from SiO_2-coated sensor Au chips using a custom-built SPR instrument (Institute of Photonics and Electronics CAS, Czech Republic).[11,12] A 1.5-nm-thick Ti adhesion layer was deposited on the Au sensor surface, followed by the SiO_2 layer of thickness 15 nm. The SPR instrument itself is based on the Kretschmann geometry of attenuated total reflection and consists of a BK-7 glass prism (refractive index 1.51) coupled to an Au-coated glass sensor substrate with index-matching oil. The tested solutions were driven by a peristaltic pump for 15 min through a flow cell containing six independent channels, in which the SPR responses were simultaneously recorded as shifts in the resonant wavelength, λ_{res}. The flow rate was 25 µL/min. The sensor response ($\Delta\lambda_{res}$) was evaluated as the difference between the baselines in PBS before and after injection of the tested solutions: citrated blood plasma (pooled from 10 donors, undiluted), fibrinogen (Fbg, 1 mg/mL), bovine serum albumin (BSA, 5 mg/mL), and fetal bovine serum (FBS, diluted to 10%). All solutions were prepared in PBS. The sensor

response was calibrated to the mass of molecules deposited at the sensor surface; according to former calibration with a shift $\Delta\lambda_{res}$ = 1 nm, corresponding to the deposition of 23 ng/cm^2 of protein mass.[9] The limit of detection (LOD) was estimated to be the sensor response corresponding to three standard deviations of the baseline noise. The average LOD was 0.03 nm, corresponding to ca 0.7 ng/cm^2. All the measurements were performed in triplicate.

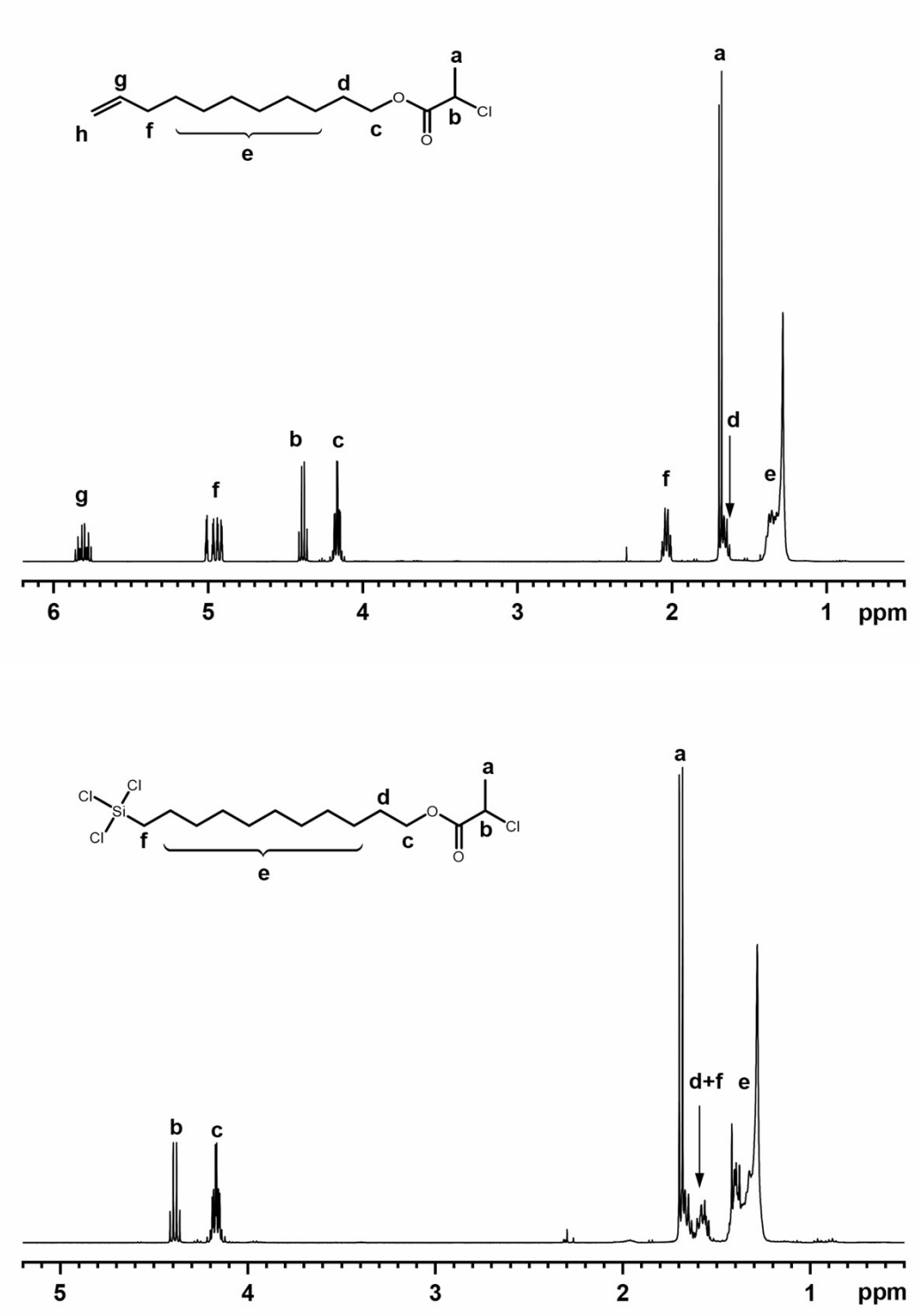


Fig. S1 ^{1}H-NMR spectra of undec-10-en-1-yl 2-chloropropionate (top) and 11-(trichlorosilyl)undecyl-2-chloro-propionate (TUCP, bottom) measured in $CDCl_3$.

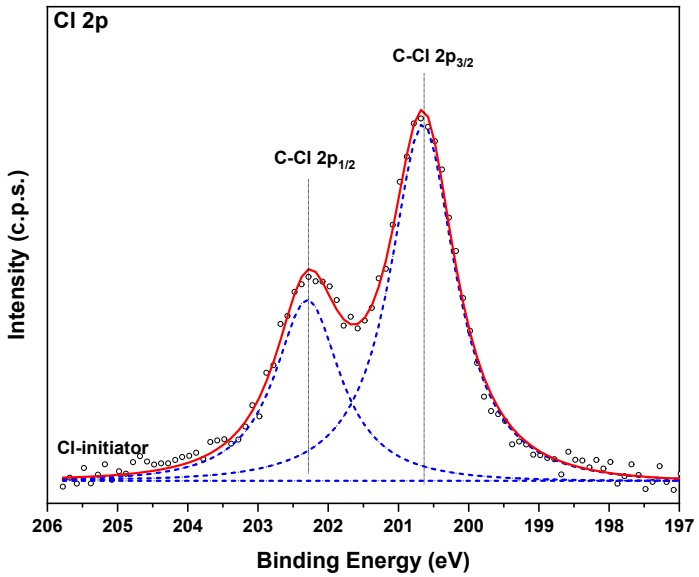


Fig. S2 Representative high-resolution core-level spectrum of the TUCP initiator SAM acquired in the Cl 2p region. Open circles – the measured spectrum, red line – fitting of the spectral points, blue line - the individual contributions of different functional groups.

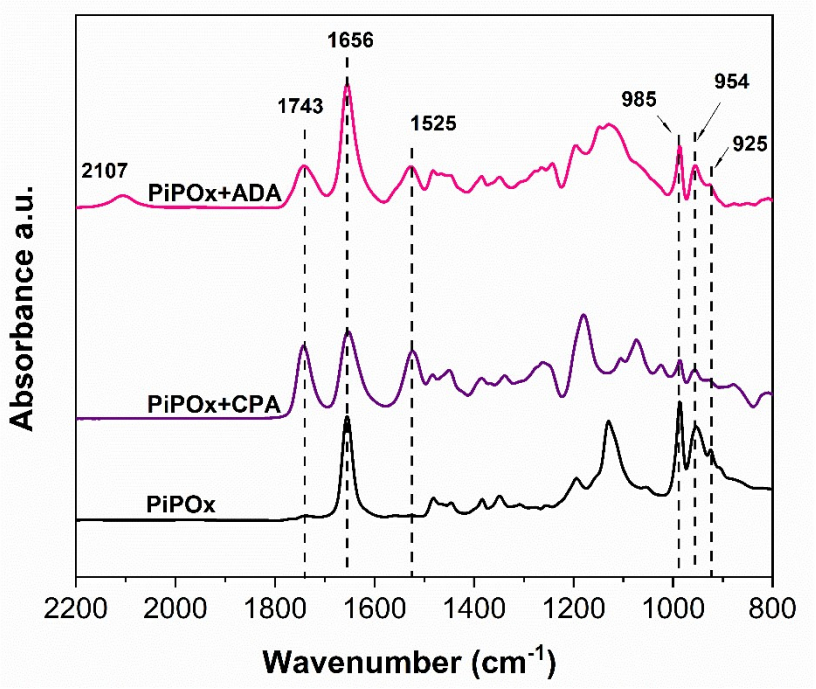


Fig. S3 GAATR FTIR spectra of the PIPOx brush before and after modification with CPA and ADA (0.3 M acid, 60 °C, 24 h).

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