Electronic Supplementary Material (ESI) for Analyst.

En Route to Traceable Reference Standards for Surface Group Quantifications by XPS, NMR and Fluorescence Spectroscopy

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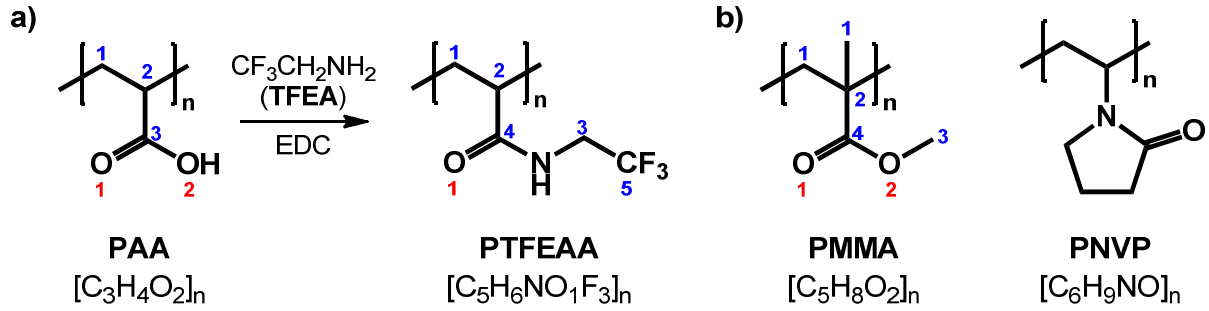
1. Abbreviations

EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride salt; FITC: fluorescein isothiocyanate; FL-A: 1,6-diaminohexyl-FITC; MES: 2-(*N*-morpholino)ethanesulfonic acid; NMR: nuclear magnetic resonance spectroscopy; PAA: poly(acrylic acid); PMMA: poly(methyl methacrylate); PTFEAA: poly(*N*-(2,2,2-trifluoroethyl)acrylamide; PNVP: poly(*N*-vinylpyrrolidone); rcf: relative centrifugal force; TFEA: trifluoroethylamine; XPS: X-ray photoelectron spectroscopy.

2. Supporting Figures



Scheme S1. a) Chemical derivatization reaction of poly(acrylic acid) (PAA, left) grafted microparticles using EDC-mediated amidation with 2,2,2-trifluoroethylamine ($CF_3CH_2NH_2$, TFEA) yielding poly(*N*-(2,2,2-trifluoroethyl)acrylamide (PTFEAA, right). b) Chemical structure of poly(methyl methacrylate) (PMMA) and poly(*N*-vinylpyrrolidone) (PNVP).

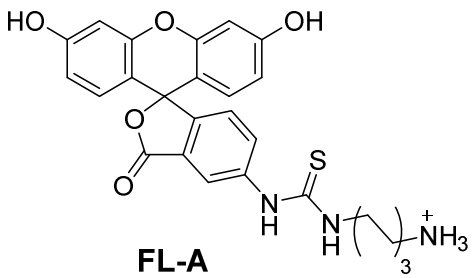


Chart S1. Chemical structure of FL-A.

3. Experimental Details

Materials. If not noted otherwise, all chemicals were purchased from Sigma-Aldrich (Steinheim, Germany), AppliChem (Darmstadt, Germany), J. T. Baker (Deventer, Netherlands), Carl Roth (Karlsruhe, Germany) or Merck (Darmstadt, Germany) and were of highest purity available when used for analytical measurements. PMMA microparticles (diameter 6 µm) with varying amounts of PAA were individually prepared by PolyAn GmbH (Berlin, Germany) for this study. Gold substrates (Georg Albert PVD, Germany) were prepared by thermal evaporation of 30 nm of Au (purity 99.99%) onto polished single-crystal Si (100) wafers that had been pre-coated with a 9 nm titanium adhesion layer.

Sample Preparation. Surface functionalization with 2,2,2-trifluoroethylamine (TFEA)[1-3] was adapted from an established protocol.[4] 10 mg polymer microparticles were washed into 660 µL reaction buffer (0.1 M MES, pH 5.0) by repeated cycles (>5) of centrifugation (5000 rcf), removal of supernatant and refilling of reaction buffer. Subsequently, 50 µL 400 mM TFEA hydrochloride in reaction buffer were added. The reaction was started by adding 80 µL of 100 mg/mL (0.52 M) EDC hydrochloride freshly dissolved in 4 °C cold water. The total reaction volume was 800 µL, final conditions were 12.5 mg/mL polymer microparticles, 25 mM TFEA, and 52 mM EDC. After 3 h reaction time at room temperature, the microparticles were washed into 1 mL neat water.

Powdered samples for solid-state NMR were prepared by slow evaporation of the microparticle suspensions in a vacuum drying oven at 40 °C and < 50 mbar.

Microparticle samples for XPS were prepared by drop casting several drops (1-10 µl) of aqueous microparticles suspensions (10 mg/ml) on glass or gold-coated silicon substrates and were allowed to dry overnight in a closed petri dish. That was repeated until the substrate was covered homogeneously. PAA reference films for XPS were prepared by spin coating (4000 rpm,

30 sec) of a 3% (w/w) aqueous PAA solution on a 1×1 cm^2 piece of silicon or gold-coated Si wafer.

4. Methods

Fluorescence Spectroscopy. Fluorescence measurements were performed as described previously.[4]

X-ray Photoelectron Spectroscopy (XPS). XPS measurements were carried out with an AXIS Ultra DLD electron spectrometer manufactured by Kratos Analytical, UK. XPS spectra were recorded using monochromated Al Kα excitation at a pass energy of 80 eV for survey spectra and 20 eV for the core-level and valence band spectra. The electron emission angle was 0° and the source-to-analyzer angle was 60°. The binding energy scale of the instrument was calibrated following a Kratos Analytical procedure, which uses ISO 15472 binding energy data.[6] Spectra were taken by setting the instrument to the hybrid lens mode and the slot mode providing approximately a 300 x 700 μm^2 analysis area.

In case of insulating samples, the charge neutralizer was used. The binding energy scale was furthermore corrected for charging[7], using an electron binding energy of 285.0 eV[8] for the C 1s level of aliphatic hydrocarbon. Spectra are decomposed with the CasaXPS peak fit program, version 2.3.15 from Casa Software Ltd. (United Kingdom). A Gaussian/Lorentzian product function peak shape model GL(30) (70% Gaussian, 30% Lorentzian) was used in combination with a Shirley background. Curve fittings of C 1s and O 1s core-level spectra were done according to previous reports.[8,9] Elemental compositions from survey spectra are calculated using peak areas normalized on the basis of acquisition parameters after a Shirley background subtraction, experimental sensitivity factors and transmission factors provided by the manufacturer. Damage of polymer microparticles by X-ray radiation was not observed.

NMR. Solid-state NMR experiments were performed on a Bruker Avance 600 spectrometer (14.1 T). All experiments were carried out at room temperature using a 2.5 mm magic angle sample spinning (MAS) probe for solid-state NMR experiments. All samples were placed with spacers in the middle section (3mm filling height) of a 2.5mm rotor where the B_1 field inhomogeneity is less than 5%. The MAS rotation frequency was 25 kHz. The 90° pulse length was 3.1 μs. The number of scans was: 1024 for P946, 24576 for P35 and 40960 for P99 particles, respectively. The repetition time was 2 sec and ensures full T_1 relaxation of the CF_3 ^{19}F signal. 4-(trifluoromethyl)benzoic acid was used as intensity reference standard. It was filled in another rotor (4.78 mg standard) in the same manner as for the analyte samples. The repetition time here was 20 sec to avoid saturation. 32 scans were accumulated. All spectra were run under the same experimental conditions, specifically with the same receiver gain. To check spectrometer stability first the analyte sample was run followed by the reference rotor experiment. This procedure was repeated three times. The maximum deviation between the signal intensities of the analyte sample (similarly for the standard) was less than 1%. Data analysis was performed with the software TopSpin 2.1 (and 3.0) and DMFIT.[10]

Quantification of surface-bound TFEA. The applied amidation reaction of surface carboxyl (CO_2H) groups on PMMA/PAA core-shell microparticles for quantification of surface-bound TFEA by XPS and ^{19}F-NMR is illustrated in Scheme S1.

XPS. The measurand is here the amount fraction of carboxylic acid carbon atoms before TFEA labeling expressed as percentage, x_{CO_2H}, of the total carbon amount described by equation 1.

$$x_{CO_2H} = \frac{n_{CO_2H}}{n_{C_{total}}} \times 100 \quad (1)$$

with

$$n_{C_{total}} = n_{CO_2H} + n_{C_R} \quad (2)$$

where n_{CO_2H} is the amount of carboxylic acid carbon atoms, $n_{C_{total}}$ the total amount of carbon, and n_{C_R} the amount of all remaining carbon atoms that are not located in CO_2H groups, respectively.

The Quantitative Elemental Analysis (QEA) approach for quantification of x_{COOH} was applied by quantification of XPS survey scans using the intensities of C 1s and F 1s photoelectron peaks (measured in atomic percent, at.%).[11] The QEA data set has to be corrected for additional TFEA related elements (C, N, and F) that are introduced during the derivatization reaction. According to the mass balance law, a more generalized equation follows from Scheme 1.

$$\mathrm{C_x} - (CO_2H)_\mathrm{y} + \mathrm{y\,CF_3CH_2NH_2} \longrightarrow \mathrm{C_x} - (\mathrm{CONHCH_2CF_3})_\mathrm{y} + \mathrm{H_2O} \quad (3)$$

with

$$\mathrm{C_x} - (\mathrm{CONHCH_2CF_3})_\mathrm{y} = \mathrm{C_{x+2y}H_{2y}O_y(NH)_yF_{3y}} \quad (4)$$

where

$$\mathrm{y} = \frac{1}{3} \times \frac{\mathrm{I_{F_{1s}}}}{\mathrm{RSF_{F_{1s}}}} = \frac{[\mathrm{F}]}{3} \quad (5) \quad \text{and} \quad \mathrm{x} = \frac{\mathrm{I_{C_{1s}}}}{\mathrm{RSF_{C_{1s}}}} - 2\mathrm{y} = [\mathrm{C}] - \frac{2[\mathrm{F}]}{3} \quad (6)$$

$I_{F_{1s}}$ and $I_{C_{1s}}$ are the XPS intensities of F 1s and C 1s determined from the survey scan after TFEA derivatization, and $RSF_{F_{1s}}$ $RSF_{C_{1s}}$ are the relative sensitivity factor of F 1s and C 1s taken from the Kratos element library. Finally x_{COOH} can be calculated from eq. 1–6 according to eq. 7.

$$\mathrm{x}_{CO_2H} = \frac{\mathrm{y}}{\mathrm{x}} \times 100 = \frac{[\mathrm{F}]}{3[\mathrm{C}] - 2[\mathrm{F}]} \times 100 \quad (7)$$

The derivatization reaction yield (*X*) can then be calculated by taking into account that 1/3 of all carbon atoms in PAA are located in carboxylic acid (CO_2H) moieties.

$$\mathrm{X} = 3 \times \mathrm{x}_{CO_2H} \quad (8)$$

Alternatively the derivatization reaction yield (X) can be calculated using relative CF_3 component peak areas from the C 1s core level spectra according to eq. 9.

$$X = \frac{CF_{3,exp.}}{CF_{3,stoich.}} \times 100 \quad (9)$$

Here $CF_{3,exp.}$ refers to the CF_3 relative peak area as obtained from the fit of measured C 1s core-level spectra, while $CF_{3,stoich.}$ is the fraction derived from the theoretical stoichiometry of a completely derivatized PAA shell (*cf.* Scheme 1). And from the chemical structure of PTFEAA (the quantitatively derivatized PAA) shown in Scheme 1 it follows $CF_{3,stoich.} = 20\%$.

NMR. The CF_3 signal position of the analyte samples appears at -70.5 ppm (c.f. Fig. 1). The CF_3 group concentration of the analyte samples N_{Sample} is calculated in the usual fashion[12,13] from the weight of the sample m_{Sample}, the signal intensity I_{Sample}, the actual number of scans A_{Sample}, as well as the weight of the standard m_{Std}, the molar weight M_{Std} (190,42 g/mol), the signal intensity I_{Std} and the number of scans A_{Std} (here 32 scans).

$$\frac{N_{Sample}}{m_{Sample}} = \frac{I_{Sample}}{I_{Std}} * \frac{A_{Std}}{A_{Sample}} * \frac{m_{Std}}{M_{Std}} / m_{Sample} \quad (10)$$

The results are listed in Table S1. The uncertainty is about 5%.

Table S1. Sample weights and detected concentrations of CF_3 groups at -70.7 ppm.

Sample	Sample weight [mg]	CF_3 concentration [µmol/g]
P35	3.39	10.4±0.5
P99	1.94	26±1
P946	3.15	367±18

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