

Unlimited growth of host-guest multilayer films based on functionalized neutral polymers

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Experimental section

General

Materials. Poly(allylamine hydrochloride) (PAH), ferrocene carboxyaldehyde, ethynylferrocene, (tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA), 2,2'-azobis(2-methylpropionitrile) (AIBN), methacrylic acid, N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDCI), copper trifluoromethanesulfonate benzene complex were purchased from Aldrich. AIBN was recrystallised in methanol before using. *N*-hydroxypropylmethacrylamide (HPMA; PHPMA – poly(*N*-hydroxypropylmethacrylamide)) monomer was purchased from Polyscience. Sodium ascorbate and CuSO₄·5H₂O were purchased from Alfa Aesar. HS-(CH₂)₁₁-EG₄-OH and HS-(CH₂)₁₁-EG₆-N₃ were purchased from Prochimia. β-Cyclodextrin (β-CD) was kindly supplied by Roquette Frères (Lestrem, France). β-CD monoalcyne and PAH-CD were synthesized respectively from a β-CD monocarboxylic acid derivative and a β-CD monoacetal compound described previously.¹ All other chemical products were purchased from Fluka. When mentioned, Milli-Q water (resistivity = 18.2 MΩ·cm; Millipore system, France) was used as solvent or co-solvent.

NMR spectroscopy. ¹H NMR and ¹³C NMR experiments were performed using a Bruker DRX400 spectrometer operating respectively at 400 MHz and 100 MHz. Chemical shifts (δ in ppm) are given relative to external tetramethylsilane (TMS = 0 ppm) and calibration was performed using the signal of the residual protons of the solvent as a secondary reference. Deuterium oxide, deuterated dimethyl sulfoxide (DMSO) and deuterated chloroform were obtained from SDS (Vitry, France).

Mass spectrometry. MALDI-TOF measurements were performed on a Bruker Daltonics Autoflex apparatus using 2,5-dihydroxybenzoic acid as matrix for the analysis of modified β-CD.

Infrared spectroscopy. Infrared spectra were obtained on a Thermo Electron Corporation Nicolet 380 FT-IR equipped with ATR.

Elemental analysis. Microanalyses were performed by the Service de Microanalyse, Institut Charles Sadron, CNRS.

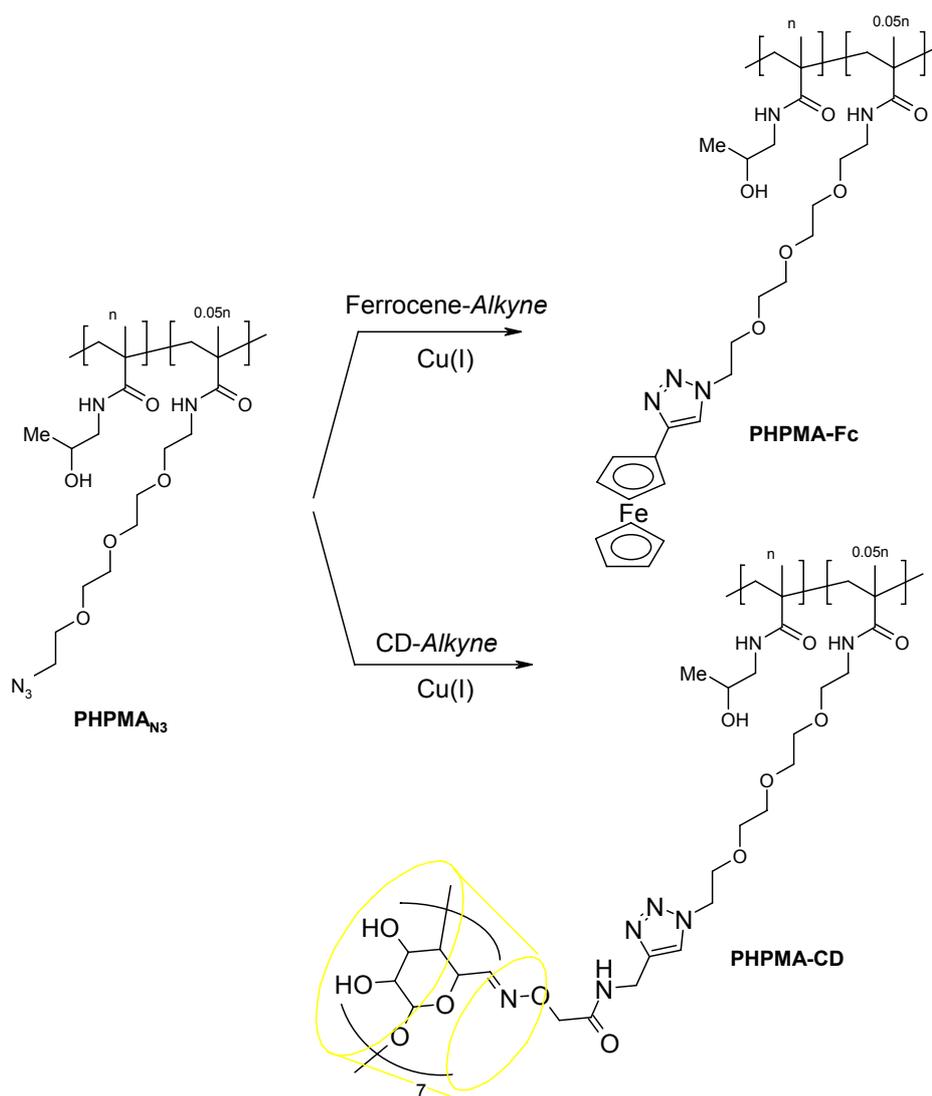
Synthesis of β-CD monoalcyne

To a solution of β-CD monocarboxylic acid (0.2 g, 0.165 mmol) in dry dimethylformamide (DMF; 16 mL), hydroxybenzotriazole (HOBt; 0.046 g, 0.33 mmol), diisopropylcarbodiimide (0.084 g, 0.66 mmol) and propargylamine (0.011 g, 0.215 mmol) were successively added. The resulting mixture was stirred under nitrogen at room temperature overnight. After evaporation of most of the solvent, the residual syrup was poured into acetone (200 mL). The white precipitate was collected by filtration, washed three times with acetone and dried to give pure β-CD monoalcyne (0.172 g).

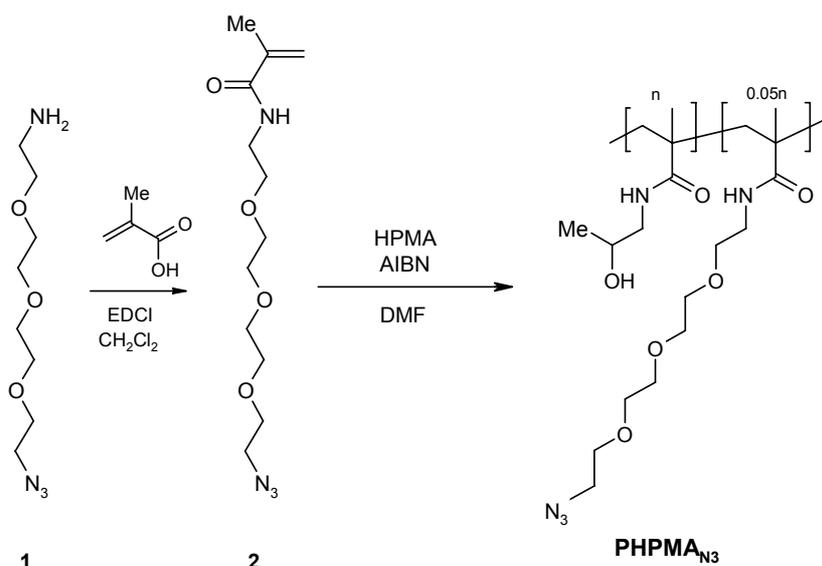
β-CD monoalcyne (yield=79 %).

¹H NMR (400 MHz, D₂O): δ 7.65 (d, 1H, *J* 7 Hz, -CH=), 4.95-4.92 (m, 7H, anomeric protons of β-CD), 4.43-4.25 (m, 2H, CH₂-CO), 4.00-3.39 (m, 50 H, other protons of β-CD, CH₂-C≡H), 2.7 (s, 1H); **MS-MALDI-TOF**: [*M*+Na]⁺ calculated for C₄₇H₇₄N₂O₃₆Na - 1265.39; Found - 1265.56 [*M*+Na]⁺.

Synthesis of PHPMA derivatives, general pathway:



Synthesis of PHPMA-N₃:

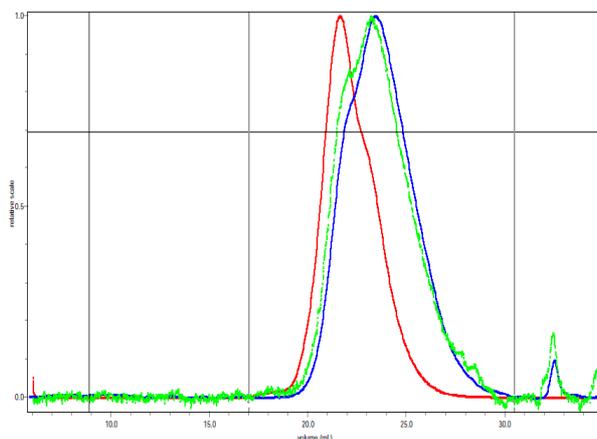


Compound **1**² (1.0 equiv., 150 mg, 5.86 mmol) was dissolved in 5 mL CH₂Cl₂ at 0°C. First 1-hydroxybenzotriazole (HOBt) (0.4 equiv., 40 mg, 0.235 mmol) and EDCI (1.5 equiv., 169 mg, 8.8 mmol) were added as a solid directly to the reaction mixture under stirring. Methacrylic acid (1 equiv., 5.86 mmol, 50 mg, 0.05 mL) was added drop by drop and then a white precipitate appeared in the reaction mixture. Addition of pyridine (0.5 mL) has provided a homogeneous colourless solution. The mixture was stirred overnight and the temperature was increased slowly from 0°C to 25°C. At 0°C, 5 mL of 10% HCl was added drop by drop into the reaction mixture and the organic layer was washed with 2 x 3 mL distilled water, treated with 5 mL of saturated NaHCO₃, washed with 2 x 3 mL distilled water and finally dried with MgSO₄. After removal of organic solvent under the reduced pressure, the residue was purified by chromatography using the following conditions as eluent: ethyl acetate/CH₂Cl₂ (6/4). Monomer **2** was isolated as a colourless oil with 60% yield (101 mg, 3.49 mmol); ¹H NMR (CDCl₃, 400 MHz): 6.30 (broad s, 1H, NH), 5.69 (s, 1H, =CH), 5.32 (s, 1H, =CH), 3.67 (m, 10H, OCH₂-CH₂O), 3.60 (t, ³J=5.5 Hz, 2H, CH₂-N₃), 3.52 (broad q, ³J=5.0 Hz, 2H, CH₂-NH), 3.38 (t, ³J=5.5 Hz, 2H, CH₂-CH₂-N₃), 1.96 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz): 168.67 (CO), 140.25 (CO-C(CH₃)=CH₂), 119.69 (CO-C(CH₃)=CH₂), 70.84, 70.76, 70.73, 70.39, 70.20, 69.91 (6xCH₂O), 50.84 (CH₂-N₃), 39.58 (CH₂-NH), 18.84 (CH₃); m/z (ESI): calculated for C₁₂H₂₂N₄O₄ [M+H]⁺ 287.33, observed 287.39; **Elem. Anal.**: calculated for C₁₂H₂₂N₄O₄ C, 50.34%; H 7.74%; N, 19.57%; found C, 50.42%; H 8.01%; N, 19.91%.

A solution containing a combination of HPMA (300 mg, 2.1 mmol) and monomer **2** (0.21 mmol) and a catalytic amount of AIBN (1.6 mg, 9.74 μmol) in DMF (2.5 mL) was degassed 20 minutes with argon and stirred for 48 hours at 60 °C. The reaction mixture was diluted with 2 mL of methanol and added dropwise to 50 mL of ethyl acetate. This precipitation step was performed twice. Then, the white solid was collected by filtration, washed with ethyl acetate and dried in vacuum. Comparison of ¹H NMR integration signals at 3.60-3.35 ppm (all CH₂ of ethylene oxide spacer) and at 2.88 ppm (NH-CH₂-CH(OH)(CH₃) of PHPMA) gives an effective degree of modification of 5%.

PHPMA-N₃ (yield = 75%)

¹H NMR (DMSO-*d*₆, 400 MHz): 7.20 (br s, NH), 4.71 (br s, OH), 3.70 (br s, CHOH), 3.58 (br s, O-CH₂-CH₂-O), 3.49 (br s, CH₂-NH), 3.42 (br s, CH₂-CH₂-N₃), 2.88 (br s, NH-CH₂-CH(OH)(CH₃)), 1.90-1.59 (br s, C(CH₃)-CH₂-C(CH₃)), 1.00 (br s, CH₃), 0.80 (br s, CH₃); ¹³C NMR (D₂O-*d*₆, 100 MHz): 16.65 (br s), 17.97 (br s), 20.04, 29.22, 44.99, 45.40, 47.09, 50.20, 54.07 (br s), 65.59, 65.79, 69.27, 69.63, 173.32 (br s); **IR** (neat): 3313, 2980, 2925, 2110, 1640, 1590; **SEC**: Mn = 34040, Mw = 92640 Da, PDI = 2.72. The molecular weight was determined using PEG standards.



SEC chromatogram of PHPMA-N₃: refractive index detection (in blue), light scattering detection (in red) and UV absorbance at 254 nm (in green).

Synthesis of PHPMA-Fc

Synthesis of PHPMA-N₃ was described previously.³ PHPMA-N₃ (50mg, 5% of N₃ groups), ethynylferrocene (10 mg, 0.05 mmol) and copper trifluoromethane sulfonate benzene complex (1mg, 0.002 mmol) were dissolved in methanol (2.5 mL) in a Schlenk tube. The mixture was degassed 20 minutes with argon and stirred overnight at 40°C under argon. Then, the reaction mixture was slowly added dropwise to 30 mL of ethyl acetate. The red precipitate was filtered and dissolved again in methanol (2 mL). This precipitation step was repeated twice. The red solid obtained was dissolved in 3 mL of Milli-Q water and pass through a very short amount of celite. Lyophilisation of the aqueous solution provided PHPMA-Fc as red solid. By using IR spectroscopy, no bands corresponding to C≡C bond (2117 cm⁻¹) from ethynylferrocene and no bands from free azide groups (2110 cm⁻¹) were detected. Thus, we considered the PHPMA-Fc substituted by 5% of ferrocene moieties.

PHPMA-Fc (yield=30%)

¹H NMR (DMSO-*d*₆, 400 MHz): 7.40 (br s, NH), 4.80 (br s, OH), 4.54 (br s, 2xCH substituted cyclopentadiene), 4.32 (br s, 2xCH substituted cyclopentadiene), 4.05 (br s, 5xCH cyclopentadiene), 3.70 (br s, CHOH), 3.60-3.40 (br m, O-CH₂-CH₂ from the EO linker overlapped with H₂O in DMSO-*d*₆), 3.10 (br s, NH-CH₂-CH(OH)(CH₃)), 2.10-1.60 (br s, C(CH₃)-CH₂-C(CH₃)), 1.20 (br s, CH₃), 0.90 (br s, CH₃).

Synthesis of PHPMA-β-CD

PHPMA-N₃ (50 mg, 5% of N₃ groups), β-CD monoalcyne (25 mg, 0.02 mmol), CuSO₄·5H₂O (2 mg, 0.008 mmol) and sodium ascorbate (3mg, 0.015 mmol) were dissolved in Milli-Q water (3 mL). The mixture was stirred overnight at 35°C. Then, the reaction mixture was slowly added dropwise to 30 mL of acetone/Milli-Q water (19/1). The precipitate was filtered and dissolved in 2 mL of Milli-Q water. This precipitation step was repeated twice. After filtration, a greenish-white solid was isolated and dried under high vacuum 48 hours. Comparison of ¹H NMR integration signals at 5.00 (br s, 7H, anomeric protons of β-CD) and at 3.25 -2.90 (br m, NH-CH₂-CH(OH)(CH₃)) gives an effective degree of modification of 5%.

PHPMA-β-CD (yield=46%)

¹H NMR (D₂O, 400 MHz): 5.00 (br s, 7H, anomeric protons of β-CD), 4.60-4.40 (m, 2H, CH₂-CO overlapped with D₂O signal at 4.75 ppm), 3.80 (br s, CHOH from PHPMA chain overlapped with H from β-CD), 3.70-3.45 (br s, H from β-CD), 3.25-2.90 (br m, NH-CH₂-CH(OH)(CH₃)), 1.85 -1.60 (br m, C(CH₃)-CH₂-C(CH₃)), 1.60 (br s, CH₃), 1.45 (br s, CH₃).

Synthesis of PAH derivatives was performed according to literature methods based on a reductive amination reaction.^{1,4} This reaction is performed under mild conditions that allow avoiding polymer degradation.⁵ Based on these data and the fact that analysis of polymers with amine groups by GPC is very difficult due to their tendency to adsorb on the column, we did not perform GPC analysis for PAH derivatives. In this work, the PAH derivatives were analyzed by ¹H NMR as it is the best technique to check the chemical integrity and purity of modified water-soluble polymers. This is also a powerful method to determine the degree of substitution with good accuracy.

Synthesis of PAH-Fc

PAH (0.1 g, 1.069 mmol repeating units) was dissolved in distilled water (7 mL). After 4 h of stirring, ethanol (EtOH) (4 mL) was slowly added to the polymer solution. Then, ferrocene carboxyaldehyde (0.0183 g, 0.0855 mmol) dissolved in EtOH (1 mL) was added dropwise.

The pH of the mixture was adjusted to 5.1 using a 0.5 M NaOH aqueous solution and NaCNBH₃ (0.1 g, 1.6 mmol) was added. After 24 h of stirring at room temperature, the pH was adjusted to 7.0 using 0.1 N NaOH. Then, the reaction mixture was ultrafiltered through an ultramembrane Amicon YM10 in a 8200 Amicon cell equipped with an Amicon RS4 tank filled with a EtOH/H₂O (2/3, v/v) mixture and then with pure water. The ultrafiltration was stopped when the filtrate conductivity was lower than 10 μS and PAH-Fc was recovered by freeze-drying as a yellow powder (0.095g). The degree of substitution of PAH-Fc was found to be 0.08 ± 0.01 by ¹H NMR.

PAH-Fc (yield=82 %)

¹H NMR (400MHz, D₂O): δ 4.38-4.17 (m, protons of ferrocene), 2.85 (m, CH₂ of PAH), 1.90-0.97 (m, CH and CH₂ of PAH).

Synthesis of PAH-β-CD

The first step consisted in deprotecting the aldehyde function of β-CD monoacetal. Thus, this modified cyclodextrin (0.193 g, 0.149 mmol) was dissolved in water (36 mL) and 0.2 M HCl (6 mL) was added. The resulting mixture was stirred for 3 h at 55°C and then was neutralized to pH 7 to give the desired aldehyde. The latter compound was added to a solution of PAH (0.1 g, 1.069 mmol repeating units) in distilled water (7 mL). The pH was adjusted to 5.1 using 0.1 N HCl and a solution of NaCNBH₃ (0.1 g, 1.6 mmol) in distilled water (1 mL) was added. After stirring overnight, the pH of the reaction mixture was adjusted to 7.0 using 0.1 N NaOH. The modified PAH was purified by ultrafiltration through an ultramembrane Amicon YM 10 in an 8200 Amicon cell equipped with an Amicon RS4 tank filled with pure water. The ultrafiltration was stopped when the filtrate conductivity was lower than 10 μS, and the PAH-CD derivative (0.160 g) was recovered by freeze-drying. The degree of substitution of PAH-β-CD was found to be 0.08 ± 0.01 by ¹H NMR.

PAH-β-CD (yield=78 %)

¹H NMR (400MHz, D₂O): δ 7.62 (m, -CH=), 4.96 (m, anomeric protons of β-CD), 4.54 (m, CH₂-CO), 4.0-3.30 (m, other protons of β-CD), 2.94 (m, CH₂ of PAH), 2.0-1.2 (m, CH and CH₂ of PAH).

SAM-β-CD formation

Gold sensors (gold-coated quartz crystals from Q-Sense, Sweden) were functionalized according to the following procedure. Firstly, self-assembled monolayers (SAMs) were formed at room temperature by dipping overnight gold sensors in the mixture of thiols: 80% HS-(CH₂)₁₁-EG₄-OH and 20% HS-(CH₂)₁₁-EG₆-N₃ (1 mM total thiol concentration in EtOH). After overnight adsorption of SAMs, gold sensors were rinsed with ethanol and dried under nitrogen. Then, “click” reaction was carried out between azide-terminated SAM and β-CD-alkyne in order to produce SAM-β-CD. To perform “click” functionalization, gold electrodes covered with SAM were immersed at room temperature in water/t-BuOH (1:2) solution containing 1 mM β-CD-alkyne, 1 mM CuSO₄, 1mM TBTA and 7 mM sodium ascorbate for 5 hours. After reaction, monolayers were rinsed with ethanol, water, dichloromethane and then ethanol again to ensure that any physisorbed β-CDs were washed off.

QCM-D experiments

All experiments were performed using quartz crystal microbalance with dissipation monitoring (QCM-D) Q-Sense E4 system equipped with four flow chambers (Q-Sense, Sweden). Monitoring the resonance behavior of piezoelectric oscillators allows measuring the mass adsorbed at the surface of the oscillator in real time. Gold-coated quartz crystals (Q-Sense, Sweden) with 5 MHz resonant frequency were used. The overtones $\nu = 3, 5, 7, 9, 11$ and 13 were also recorded to have better stability and higher sensitivity than that obtained

with the fundamental resonance. Before starting the experiment, resonance frequency and dissipation found for each overtone were set equal to zero. QCM-D measurements were done in flow mode (50 $\mu\text{L}/\text{min}$) at 24 $^{\circ}\text{C}$ in 10 mM HEPES with 0.15 M NaCl (pH 7.0). The solutions were also thermostated at 24 $^{\circ}\text{C}$ using Thermomixer (Ependorf). All polymers were dissolved overnight in 10 mM HEPES with 0.15 M NaCl (pH 7.0) at room temperature. Functionalized with SAM- β -CD gold sensors were mounted in the QCM-D chamber. Experimental data were fitted according to the viscoelastic model of Voinova,⁶ using Q-tools modeling software. Table 1 presents the results of fitting QCM-D data obtained for the adsorption of non-modified PAH and PHPMA-N₃ on SAM- β -CD-coated gold sensors.

Table 1 Analysis of the QCM-D response according to the Voinova's model: $\Delta\Gamma$ is the surface mass, μ is the shear elastic modulus and η is the shear viscosity of the non-specifically adsorbed PAH or PHPMA-N₃ film on SAM- β -CD-coated gold surface.

Experiment N ^o	Polymer	μ (Pa)	η (kg/ms)	$\Delta\Gamma$ (ng/cm ²)	ChiSqr
1	PAH	1.5×10^5	1.77×10^{-3}	296	0.3
	HPMA	5.7×10^4	1.61×10^{-3}	224	0.4
2	PAH	1.3×10^5	1.80×10^{-3}	342	0.4
	HPMA	7.2×10^4	1.49×10^{-3}	196	0.2

Fig. 1, 2 illustrate the absence of non-specific adsorption of PHPMA on SAM- β -CD/PHPMA-Fc and SAM- β -CD/PHPMA-Fc/PHPMA-CD films as well as PHPMA-Fc on SAM- β -CD/PHPMA-Fc film and PHMA-CD on SAM- β -CD/PHPMA-Fc/PHPMA-CD film. Fig. 1 shows the QCM-D profile obtained for the subsequent deposition of PHPMA-Fc, PHPMA-N₃, PHPMA- β -CD and PHPMA-N₃ on SAM- β -CD-coated gold surface. The same time was used for adsorption of each polymer layer (15 min) and for rinsing after each adsorption step (10 min). During the injection of the non-modified polymer into the measurement chamber both frequency and dissipation demonstrated the same behaviour as it was obtained in the buffer that proves the absence of non-specific adsorption of PHPMA on both polymer layers.

Fig. 2 illustrates the absence of non-specific adsorption of PHPMA-Fc on SAM- β -CD/PHPMA-Fc film and PHPMA-CD on SAM- β -CD/PHPMA-Fc/PHPMA- β -CD film. As it is shown in the QCM-D profile, during the rinsing steps, a low fraction of polymer is removed from the surface and new injections of the same polymer leads to reaching identical level of the adsorption. Therefore, for both PHPMA-Fc and PHPMA-CD new injections of the same polymers do not produce additional adsorption of the polymers.

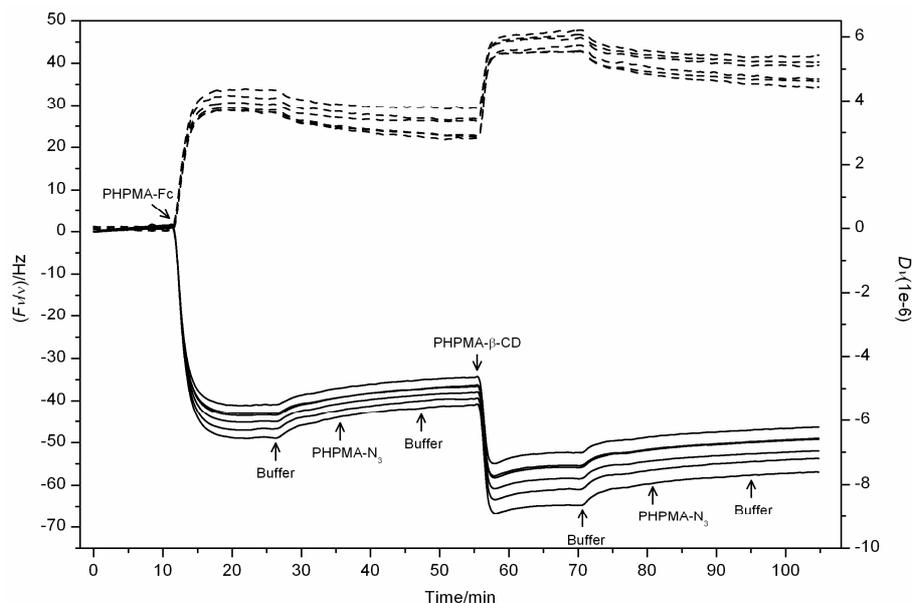


Fig. 1. Frequency F_v/v (solid) and dissipation D_v (dashed) recorded at six overtones $v = 3, 5, 7, 9, 11, 13$ (frequency and dissipation shifts increase with overtone number) during the stepwise injection of PHPMA-Fc, PHPMA-N₃, PHPMA- β -CD and PHPMA-N₃ into the measurement chamber; substrate – SAM- β -CD-coated gold surface, 24 °C, 50 μ L/min, buffer - 10 mM HEPES with 0.15 M NaCl, pH 7.0.

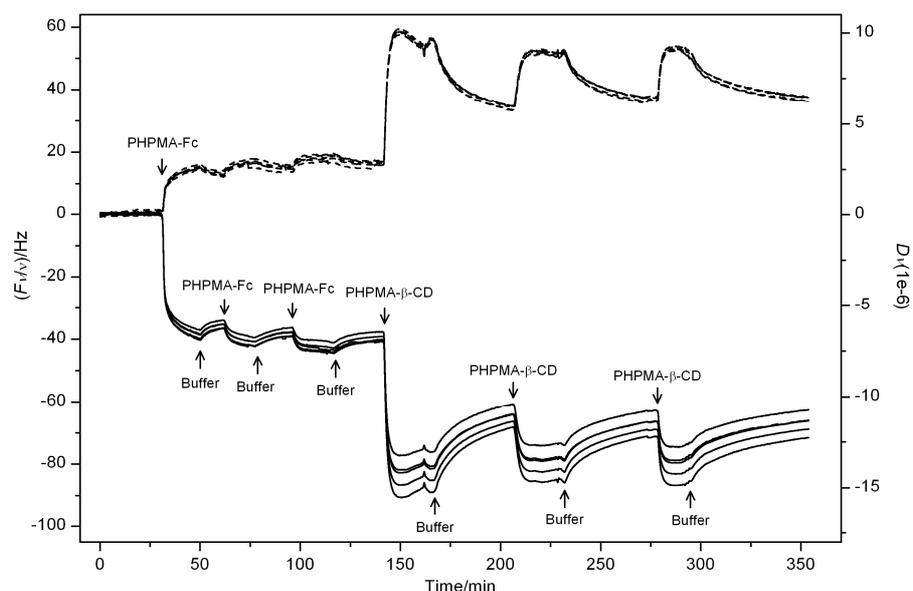


Fig. 2. Frequency F_v/v (solid) and dissipation D_v (dashed) recorded at five overtones $v = 5, 7, 9, 11, 13$ (frequency and dissipation shifts increase with overtone number) during the stepwise injections of PHPMA-Fc, PHPMA-Fc, PHPMA-Fc, PHPMA- β -CD, PHPMA-CD and PHPMA-CD into the measurement chamber; substrate – SAM- β -CD-coated gold surface, 24 °C, 50 μ L/min, buffer - 10 mM HEPES with 0.15 M NaCl, pH 7.0.

Fig. 3-5 represent the QCM-D profiles obtained for (PAH-Fc/PAH- β -CD)_n, (PAH-Fc/PAH- β -CD/PHPMA-Fc/PHPMA- β -CD)_n and (PAH-Fc/PAH- β -CD/(PHPMA-Fc/PHPMA- β -CD))₂_n films built on SAM- β -CD-coated gold surface. In each case total number of studied layers was 30. The same time was used for adsorption of each layer (10 min) and for rinsing after each adsorption step (5 min).

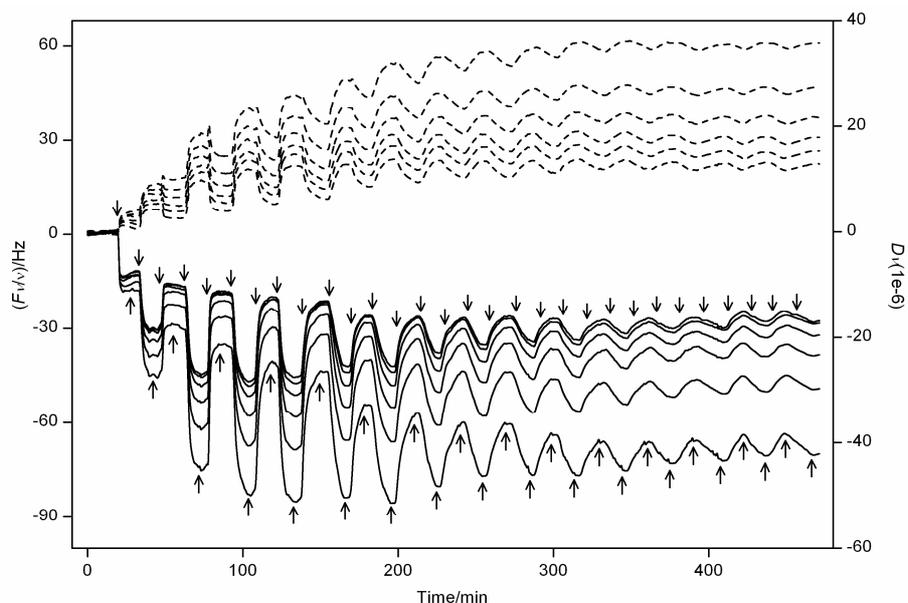


Fig. 3. Frequency F_v/v (solid) and dissipation D_v (dashed) recorded at six overtones $v = 3, 5, 7, 9, 11, 13$ (frequency and dissipation shifts increase with overtone number) during the $(\text{PAH-Fc}/\text{PAH-}\beta\text{-CD})_n$ film deposition on SAM- $\beta\text{-CD}$ -coated gold surface; \downarrow - polymer injection, \uparrow - rinsing; 24 °C, 50 $\mu\text{L}/\text{min}$, 10 mM HEPES with 0.15 M NaCl, pH 7.0.

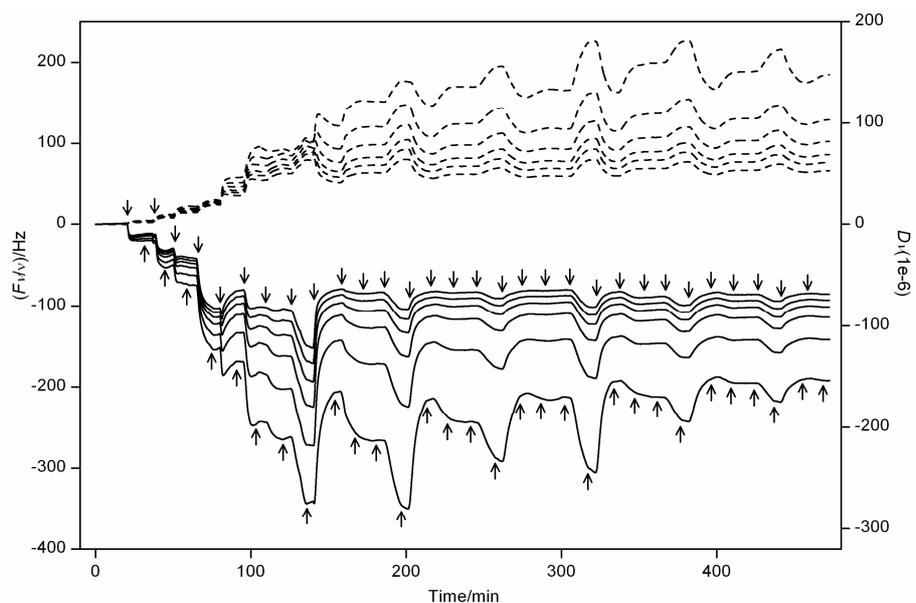


Fig. 4. Frequency F_v/v (solid) and dissipation D_v (dashed) recorded at six overtones $v = 3, 5, 7, 9, 11, 13$ (frequency and dissipation shifts increase with overtone number) during the $(\text{PAH-Fc}/\text{PAH-}\beta\text{-CD}/\text{PHPMA-Fc}/\text{PHPMA-}\beta\text{-CD})_n$ film deposition on SAM- $\beta\text{-CD}$ -coated gold surface; \downarrow - polymer injection, \uparrow - rinsing; 24 °C, 50 $\mu\text{L}/\text{min}$, 10 mM HEPES with 0.15 M NaCl, pH 7.0.

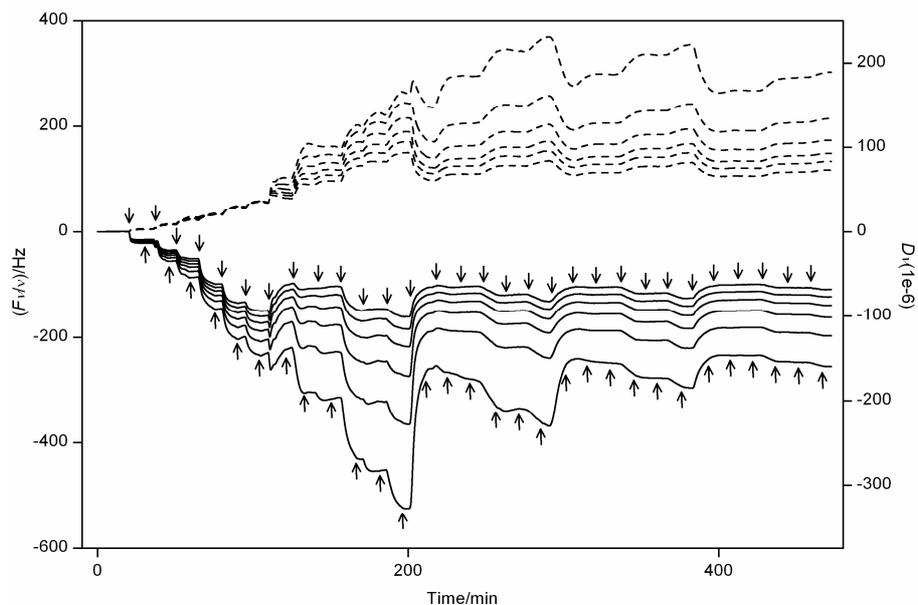


Fig. 5. Frequency F_v/v (solid) and dissipation D_v (dashed) recorded at six overtones $\nu = 3, 5, 7, 9, 11, 13$ (frequency and dissipation shifts increase with overtone number) during the (PAH-Fc/PAH- β -CD/(PHPMA-Fc/PHPMA- β -CD)₂)_n film deposition on SAM- β -CD-coated gold surface; \downarrow - polymer injection, \uparrow - rinsing; 24 °C, 50 μ L/min, 10 mM HEPES with 0.15 M NaCl, pH 7.0.

Tables 2-5 present the results of fitting QCM-D profiles obtained during the adsorption of multilayer polymer films on gold surface covered with SAM- β -CD. Shear elastic modulus was fitted like a scalar parameter for multilayer assemblies: according to the viscoelastic model one value was found by Q-tools software for all rows (layers) assuming that this parameter should not vary a lot at different adsorption steps. Presented in Tables 1-5 ChiSqr is defined by Q-tools software as $\chi^2 = \sum_i [(Y_{\text{theory},i} - Y_{\text{meas},i})/\sigma_i]^2$, where the sum is taken over all measured points (indexed i), σ_i is the measurement error, or standard deviation of the i -th data point, $Y_{\text{theory},i}$ and $Y_{\text{meas},i}$ are the calculated and measured Y -values, respectively. The values of share viscosity and shear elastic modulus obtained in this report are in a good correlation between the same polymer assemblies. The one order of magnitude lower shear elastic modulus obtained for the neutral films as compared to films containing charged layers (cationic and neutral-cationic films) can be explained by the absence of electrostatic interactions and thus lower hydration level in case of the neutral films. The values obtained for cationic polymer film are in a good agreement with hydrogel layer values obtained for multilayer assembly of chitosan derivatives in 0.3 M CH₃COOH with 0.1M CH₃COONa.⁷

Table 2 Analysis of the QCM-D response according to the Voinova's model: $\Delta\Gamma$ is the surface concentration, μ is the shear elastic modulus and η is the shear viscosity of the adsorbed (PHPMA-Fc/PHPMA- β -CD)_n film on SAM- β -CD-coated gold surface.

Layer number	Polymer	μ (Pa)	η (kg/ms)	$\Delta\Gamma$ (ng/cm ²)	ChiSqr
1	HPMA-Fc	2.2x10 ⁴	1.78x10 ⁻³	1736	3.0
2	HPMA- β -CD		1.79x10 ⁻³	2431	8.1
3	HPMA-Fc		1.81x10 ⁻³	2937	9.2
4	HPMA- β -CD		1.91x10 ⁻³	3082	13.8
5	HPMA-Fc		1.88x10 ⁻³	3453	13.7
6	HPMA- β -CD		1.97x10 ⁻³	3535	13.7
7	HPMA-Fc		1.92x10 ⁻³	3916	19.1

8	HPMA- β -CD		2.01×10^{-3}	3970	14.9
9	HPMA-Fc		1.95×10^{-3}	4348	25.0
10	HPMA- β -CD		2.05×10^{-3}	4501	32.3
11	HPMA-Fc		2.02×10^{-3}	4722	37.8
12	HPMA- β -CD		2.11×10^{-3}	4875	33.5
13	HPMA-Fc		2.08×10^{-3}	5041	19.9
14	HPMA- β -CD		2.15×10^{-3}	5345	39.1
15	HPMA-Fc		2.16×10^{-3}	5295	21.5
16	HPMA- β -CD		2.21×10^{-3}	5404	50.8
17	HPMA-Fc		2.14×10^{-3}	5799	30.2
18	HPMA- β -CD		2.24×10^{-3}	5768	75.1
19	HPMA-Fc		2.19×10^{-3}	6007	63.3
20	HPMA- β -CD		2.29×10^{-3}	6129	45.3
21	HPMA-Fc		2.24×10^{-3}	6318	87.9
22	HPMA- β -CD		2.35×10^{-3}	6405	38.7
23	HPMA-Fc		2.31×10^{-3}	6699	58.7
24	HPMA- β -CD		2.36×10^{-3}	6873	89.9
25	HPMA-Fc		2.37×10^{-3}	6746	104.6
26	HPMA- β -CD		2.40×10^{-3}	7105	141.4
27	HPMA-Fc		2.36×10^{-3}	7111	60.8
28	HPMA- β -CD		2.39×10^{-3}	7630	59.5
29	HPMA-Fc		2.48×10^{-3}	7312	63.3
30	HPMA- β -CD		2.48×10^{-3}	7537	107.8

Table 3 Analysis of the QCM-D response according to the Voinova's model: $\Delta\Gamma$ is the surface mass, μ is the shear elastic modulus and η is the shear viscosity of the adsorbed (PAH-Fc/PAH- β -CD)_n film on SAM- β -CD-coated gold surface.

Layer number	Polymer	μ (Pa)	η (kg/ms)	$\Delta\Gamma$ (ng/cm ²)	ChiSqr
1	PAH-Fc	1.4×10^5	1.67×10^{-3}	663	1.9
2	PAH- β -CD		1.69×10^{-3}	1602	0.4
3	PAH-Fc		1.40×10^{-3}	1200	4.9
4	PAH- β -CD		1.61×10^{-3}	2858	2.2
5	PAH-Fc		1.35×10^{-3}	1513	9.3
6	PAH- β -CD		1.59×10^{-3}	3333	3.5
7	PAH-Fc		1.33×10^{-3}	1815	12.7
8	PAH- β -CD		1.56×10^{-3}	3549	4.6
9	PAH-Fc		1.32×10^{-3}	1999	21.9
10	PAH- β -CD		1.52×10^{-3}	3698	3.8
11	PAH-Fc		1.34×10^{-3}	2609	22.6
12	PAH- β -CD		1.50×10^{-3}	3919	7.0
13	PAH-Fc		1.33×10^{-3}	2826	30.1
14	PAH- β -CD		1.45×10^{-3}	3845	15.4
15	PAH-Fc		1.33×10^{-3}	3040	33.2
16	PAH- β -CD		1.42×10^{-3}	3828	16.4
17	PAH-Fc		1.33×10^{-3}	3107	35.1
18	PAH- β -CD		1.41×10^{-3}	3844	25.6
19	PAH-Fc		1.33×10^{-3}	3322	28.2
20	PAH- β -CD		1.40×10^{-3}	3900	26.9
21	PAH-Fc		1.34×10^{-3}	3437	32.9

22	PAH- β -CD		1.38×10^{-3}	3858	27.4
23	PAH-Fc		1.33×10^{-3}	3429	37.1
24	PAH- β -CD		1.36×10^{-3}	3735	34.0
25	PAH-Fc		1.33×10^{-3}	3399	36.5
26	PAH- β -CD		1.35×10^{-3}	3673	29.7
27	PAH-Fc		1.31×10^{-3}	3375	36.5
28	PAH- β -CD		1.34×10^{-3}	3693	26.2
29	PAH-Fc		1.31×10^{-3}	3385	33.1
30	PAH- β -CD		1.34×10^{-3}	3641	31.2

Table 4 Analysis of the QCM-D response according to the Voinova's model: $\Delta\Gamma$ is the surface mass, μ is the shear elastic modulus and η is the shear viscosity of the adsorbed (PAH-Fc/PAH- β -CD/HPMA-Fc/HPMA- β -CD)_n film on SAM- β -CD-coated gold surface.

Layer number	Polymer	μ (Pa)	η (kg/ms)	$\Delta\Gamma$ (ng/cm ²)	ChiSqr
1	PAH-Fc	1.3×10^5	1.68×10^{-3}	635	0.9
2	PAH- β -CD		1.73×10^{-3}	1638	6.1
3	HPMA-Fc		1.74×10^{-3}	2586	7.8
4	HPMA- β -CD		2.34×10^{-3}	4683	13.2
5	PAH-Fc		1.91×10^{-3}	6162	34.8
6	PAH- β -CD		2.20×10^{-3}	9399	50.0
7	HPMA-Fc		2.31×10^{-3}	10399	187.7
8	HPMA- β -CD		2.89×10^{-3}	12297	78.4
9	PAH-Fc		1.95×10^{-3}	9691	26.3
10	PAH- β -CD		2.18×10^{-3}	13164	23.7
11	HPMA-Fc		2.19×10^{-3}	13374	61.3
12	HPMA- β -CD		2.66×10^{-3}	17346	318.1
13	PAH-Fc		2.02×10^{-3}	11442	71.5
14	PAH- β -CD		2.15×10^{-3}	13667	37.2
15	HPMA-Fc		2.15×10^{-3}	13832	20.9
16	HPMA- β -CD		2.39×10^{-3}	16711	45.1
17	PAH-Fc		2.03×10^{-3}	12087	99.3
18	PAH- β -CD		2.07×10^{-3}	12995	79.6
19	HPMA-Fc		2.06×10^{-3}	12844	98.7
20	HPMA- β -CD		2.67×10^{-3}	20474	86.4
21	PAH-Fc		2.05×10^{-3}	12514	212.1
22	PAH- β -CD		2.32×10^{-3}	17260	190.6
23	HPMA-Fc		2.32×10^{-3}	17559	145.4
24	HPMA- β -CD		2.60×10^{-3}	21228	68.7
25	PAH-Fc		2.08×10^{-3}	13148	250.1
26	PAH- β -CD		2.24×10^{-3}	16071	222.9
27	HPMA-Fc		2.24×10^{-3}	16169	196.8
28	HPMA- β -CD		2.51×10^{-3}	20054	111.2
29	PAH-Fc		2.14×10^{-3}	14309	235.1
30	PAH- β -CD		2.22×10^{-3}	15751	224.7

Table 5 Analysis of the QCM-D response according to the Voinova's model: $\Delta\Gamma$ is the surface mass, μ is the shear elastic modulus and η is the shear viscosity of the adsorbed (PAH-Fc/PAH- β -CD/(HPMA-Fc/HPMA- β -CD)₂)_n film on SAM- β -CD-coated gold surface.

Layer number	Polymer	μ (Pa)	η (kg/ms)	$\Delta\Gamma$ (ng/cm ²)	ChiSqr
1	PAH-Fc	1.5x10 ⁵	1.71x10 ⁻³	677	2.6
2	PAH- β -CD		1.74x10 ⁻³	1728	16.3
3	HPMA-Fc		1.79x10 ⁻³	2755	30.6
4	HPMA- β -CD		2.36x10 ⁻³	4310	33.7
5	HPMA-Fc		2.68x10 ⁻³	5789	33.5
6	HPMA- β -CD		2.81x10 ⁻³	6741	44.6
7	PAH-Fc		2.12x10 ⁻³	7964	64.8
8	PAH- β -CD		2.42x10 ⁻³	12020	151.9
9	HPMA-Fc		2.48x10 ⁻³	12675	384.1
10	HPMA- β -CD		3.24x10 ⁻³	16702	402.4
11	HPMA-Fc		3.35x10 ⁻³	18417	629.0
12	HPMA- β -CD		3.83x10 ⁻³	21794	1013.0
13	PAH-Fc		2.39x10 ⁻³	13981	217.2
14	PAH- β -CD		2.68x10 ⁻³	19112	186.0
15	HPMA-Fc		2.68x10 ⁻³	19248	125.8
16	HPMA- β -CD		3.09x10 ⁻³	23395	237.9
17	HPMA-Fc		3.07x10 ⁻³	22991	259.9
18	HPMA- β -CD		3.36x10 ⁻³	25627	296.9
19	PAH-Fc		2.59x10 ⁻³	17541	301.7
20	PAH- β -CD		2.73x10 ⁻³	20010	261.9
21	HPMA-Fc		2.72x10 ⁻³	19866	238.2
22	HPMA- β -CD		3.03x10 ⁻³	23455	288.3
23	HPMA-Fc		3.01x10 ⁻³	23078	320.0
24	HPMA- β -CD		3.20x10 ⁻³	24849	340.3
25	PAH-Fc		2.54x10 ⁻³	17003	265.3
26	PAH- β -CD		2.54x10 ⁻³	17361	245.3
27	HPMA-Fc		2.67x10 ⁻³	18907	230.2
28	HPMA- β -CD		2.74x10 ⁻³	19886	220.5
29	HPMA-Fc		2.78x10 ⁻³	20354	247.7
30	HPMA- β -CD		2.84x10 ⁻³	21004	249.6

Optical Waveguide Light Mode Spectroscopy experiments

We used Optical Waveguide Lightmode Spectroscopy (OWLS) to follow the film thickness and the mass of the films during their buildup. This optical technique allows for the determination of the optical thickness and the refractive index of films deposited on a Si_{0.8}Ti_{0.2}O₂ waveguide. The technique has been extensively described elsewhere⁸ and experimentally applied to polyelectrolyte multilayers⁹. It will only be briefly described here. A laser beam is shined on a grating imprinted in the waveguide and one determines the incoupling angles for both TE and TM waves into the guide. To each incoupling angle corresponds an effective refractive index, respectively $N(\text{TE})$ and $N(\text{TM})$. The homogeneous and isotropic monolayer model is then applied to characterize the refractive index, the (optical) thickness and the (optical) mass of the film. The film deposited on the waveguide is sensed by an evanescent wave with a penetration depth typically of the order of 200 nm. Mass data are calculated according to the De Feijter's formula with $dn/dc = 0.170 \text{ cm}^3/\text{g}$. The studied film was built on a different precursor film than for QCM-D experiments. A precursor film of PEI_{N3}-PAA_{C=C} (PAA_{C=C} = poly (acid acrylic) bearing 5% of alkyne groups³) was

performed in order to anchor the first layer of a special PHPMA- β -CD- N_3 bearing 5% of both - β -CD and free azide groups. This precursor film was obtained by first adsorbing a PEI $_{N_3}$ monolayer on the surface followed by an electrostatic deposition of PAA $_{C\equiv C}$. The anchorage of PAA $_{C\equiv C}$ is reinforced by a covalent coupling using click chemistry process. Polymer (PEI $_{N_3}$ and PAA $_{C\equiv C}$) dipping solutions were made up in a constant volume ratio of 3(Polymer at 0.83 mg mL $^{-1}$): 1(CuSO $_4$ at 0.36 mg mL $^{-1}$): 1(Sodium ascorbate at 8.8 mg mL $^{-1}$). The rinsing solutions were made up in a similar ratio, however, replacing polymer solution by water. Another rinsing step is realized with 10 mM HEPES with 0.15 M NaCl (pH 7.0). The first PHPMA- β -CD- N_3 monolayer was anchored by click-chemistry between free azide functions of PHPMA- β -CD- N_3 and alkyne functions present on the surface through PAA $_{C\equiv C}$. This step allows anchoring the neutral polymer on the surface of the Si $_{1.8}$ Ti $_{0.2}$ O $_2$ waveguide and then monitoring the following alternated adsorption of PHPMA-Fc and PHPMA- β -CD by OWLS. The deposition step of HPMA-CD and HPMA-Fc were obtained by alternated injection of 500 μ L of 0.1 mg mL $^{-1}$ polymer solution prepared in 10 mM HEPES with 0.15 M NaCl (pH 7.0) separated by a rinsing step (2 mL) with 10 mM HEPES with 0.15 M NaCl (pH 7.0). Fig. 6 shows the evolution of the refractive index, optical thickness and optical mass of the (PHPMA-Fc/PHPMA- β -CD) $_n$ film. The film increases steadily with the number of layers deposited. The optical mass adsorbed is two times lower than the one obtained from QCM-D data: this could be due to the hydration of the film. Indeed, the OWLS senses the optical mass and the QCM-D senses the hydrated mass¹⁰. After 10 pairs of layers, the film has a refractive index of 1.387. The hydration of the film, evaluated by a simple calculation from reference¹¹, is of 95%.

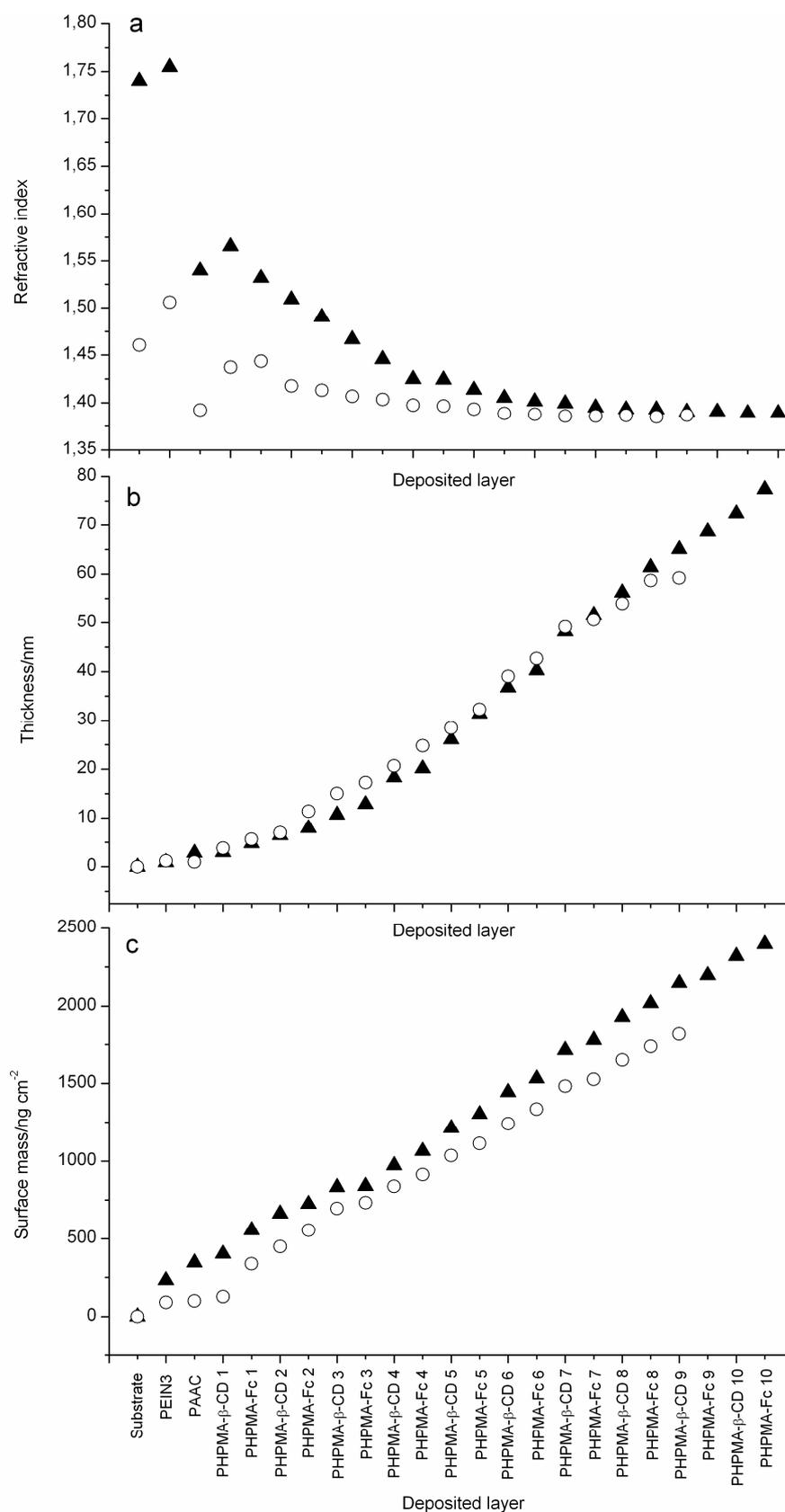


Fig. 6. Evolution of the refractive index (a), optical thickness (b) and optical mass (c) obtained from OWLS monitoring of the (PHPMA-Fc/PHPMA-β-CD)_n film deposition on PEI₃-PAA_{C=C}-coated Si_{0.8}Ti_{0.2}O₂ waveguide; ▲ - experiment N°1, ○ – experiment N°2; In 10 mM HEPES with 0.15 M NaCl, pH 7.0.

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