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Structural aspects of polyanion and hydrophobically modified polycation multilayers on hydrophilic or hydrophobic surfaces[†]

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Multilayer films of carboxymethylcellulose (CMC), a polyanion, and bromide salts of poly(4vinylpyridine) quaternized with linear aliphatic chains of 2 (ethyl) and 5 (pentyl) carbon atoms, coded as QPVP-C2 and QPVP-C5, respectively, were fabricated by layer-by-layer (LbL) self-assembly onto Si/SiO₂ wafers (hydrophilic substrate) or polystyrene, PS, films (hydrophobic substrate). The films were characterized by means of ex situ and in situ ellipsometry, atomic force microscopy (AFM), contact angle measurements and sum frequency generation vibrational spectroscopy (SFG). Antimicrobial tests were used to assess the exposure of pyridinium moieties to the aqueous medium. In situ ellipsometry indicated that for Si/SiO₂ the chains were more expanded than the PS films and both substrates systems composed of QPVP-C5 were thicker than those with QPVP-C2. For dried layers, the alkyl side group size had a small effect on the thickness evolution, regardless of the substrate. At pH 2 the multilayers showed high resistance, evidencing that the build-up is driven not only by cooperative polymer-polymer ion pairing, but also by hydrophobic interactions between the alkyl side chains. The LbL films became irregular as the number of depositions increased. After the last deposition, the wettability of QPVP-C2 or QPVP-C5 terminated systems on the Si/SiO₂ wafers and PS films were similar, except for QPVP-C2 on Si/SiO₂ wafers. Unlike the morphology observed for LbL films on Si/ SiO₂ wafers, PS induced the formation of porous structures. SFG showed that in air the molecular orientation of pyridinium groups in multilayers with QPVP-C5 was stronger than in those containing OPVP-C2. The exposure of pyridinium moieties to the aqueous medium was more pronounced when the LbL were assembled on Si/SiO2 wafers.

Introduction

The layer-by-layer (LbL) deposition process using polymers was first demonstrated by Decher and co-workers in 1992.1 Since then over 30,000 papers using this technique have been published.² The fundamentals and potential applications are well described in outstanding recent reviews.^{3,4} While in earlier publications the alternated deposition involved polycations and polyanions, over the last years it evolved to the combination of polymers (charged or not) and proteins³⁻⁸ or inorganic particles.⁹⁻¹¹ The driving force for the stable layer association is entropic in nature and results from the release of counter-ions and water.12,13 Issues concerning the dependence of thickness evolution,¹⁴⁻¹⁷ morphology¹⁸ and ionization (in the case of weak

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^dChemistry Department, Faculty of Sciences, University of Chile, Santiago, Chile polyelectrolytes)¹⁹ as a function of alternated deposition are important because they control film stability and, consequently, its final applications.

Protocols used for traditional LbL deposition in aqueous medium often suggest a charged substrate as a solid support because invariably the first layer is oppositely charged. Changing the chemical composition of the substrate might be decisive for the resulting morphology of LbL films,²⁰ particularly when the driving force for the assembly is not electrostatic. For instance, the build-up of multilayers of poly(4-vinylpyridine), PVP, and poly(acrylic acid), PAA, dissolved in methanol, on substrates terminated by OH, COOH or NH2 groups is driven by hydrogen bonds. Upon immersion into basic medium, the microporous structures formed depended on the interaction strength between the substrate and PVP (1st layer), which was COOH > OH > NH₂ groups (due to the acidity and fast ionization). The strongly bound PVP chains were more resistant to aggregation or conformational changes upon immersion in the basic solution and microporous formation was slower.

The primary goal of this work is to investigate how the chemical nature of the substrate affects the structure of LbL assemblies. In order to achieve it, two different substrates were

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chosen, namely, Si/SiO₂ wafers, which are negatively charged and have high surface energy (~80 mJ m⁻²),²¹ and polystyrene (PS) films, which are hydrophobic and have low surface energy $(47 \pm 4 \text{ mJ m}^{-2})$.²² SiO₂ based surfaces are common substrates for LbL deposition because they have an isoelectric point close to 2, so that in aqueous solution the charge density on such surfaces is high. On the other hand, to the best of our knowledge such a hydrophobic surface as PS has seldom been used as support for LbL deposition, probably due to the lack of electrostatic attraction. This drawback can be overcome by adsorbing hydrophobically modified polyelectrolytes, where the hydrophobic interaction is expected to drive deposition onto low surface energy materials such as PS, polyethylene or polypropylene. Thus, our strategy comprised of the alternated deposition of poly(4-vinyl-N-ethylpyridinium bromide), QPVP-C2, or poly(4-vinyl-N-pentylpyridinium bromide), QPVP-C5, and carboxymethylcellulose (CMC) onto Si/SiO₂ wafers or polystyrene (PS) films. In both cases the hydrophobically modified polycation was the first adsorbed layer, so that on Si/SiO₂ wafers the deposition was driven by electrostatic interaction and on PS films it was due to hydrophobic interaction. Ellipsometry, contact angle measurements, atomic force microscopy (AFM) and SFG spectroscopy were used after each layer deposition to assess the thickness evolution profile, surface wettability and homogeneity, morphology and molecular orientation, respectively. Beyond these analytical techniques, which are commonly used for surface and thin film analysis, a standard test for the determination of antimicrobial activity was used as an indirect method to evaluate the exposure of positive charges of hydrophobically modified polycations in multilayers deposited onto Si/SiO₂ wafers and PS films within an aqueous medium.

Experimental methods

Substrates

Silicon wafers (100) (University Wafer, Boston, USA) with a native layer of SiO₂ approximately 2 nm thick were cut in small pieces of 1.0 cm \times 1.0 cm. They were rinsed in a standard manner²³ dried under a stream of N₂ and characterized prior to use. PS ($M_v \sim 100\ 000\ \text{g mol}^{-1}$, BASF, Ludwigshafen, Germany) was dissolved in toluene at the polymer concentration of 20 g L⁻¹ and filtered through a Millipore PTFE membrane (pore size 0.22 µm) to avoid dust in the solution. Then, PS films were spin coated onto Si/SiO₂ wafers, using a Headway Research spinner, Inc. Model PWM32 (Garland, USA), operating at 3000 rpm, for 30 s, at 24.0 \pm 0.5 °C and 55 \pm 5% of relative humidity.

Polyelectrolytes

Carboxymethylcellulose, CMC, (90004-32-4, M_v 243,179 g mol⁻¹, $DS_{cm} = 0.9$) was purchased from Aldrich (USA). Bromide salts of poly(4-vinylpyridine) quaternized with linear aliphatic chains of two and five carbon atoms were prepared from poly(4-vinylpyridine), following method described elsewhere²⁴ and coded as QPVP-C2 and QPVP-C5, respectively. Total quaternization was evidenced by infrared spectra (Fig. S1, see ESI†). QPVP-C2 and QPVP-C5 viscometric average molecular weights (M_v) amounted to 60 970 g mol⁻¹ and 78 260 g mol⁻¹,

respectively. Chemical structures of QPVP-C2, QPVP-C5 and CMC are represented in Scheme 1.

Multilayer build-up

QPVP-C2, QPVP-C5 or CMC was dissolved at concentration 1.0 g L⁻¹ in 0.1 M NaCl, at pH = 6.0 ± 0.2 and 25 ± 1 °C. Multilayers were prepared by alternated deposition. First, the hydrophobically modified polycation (QPVP-C2 or QPVP-C5) were adsorbed onto bare Si wafers or PS films during 10 min, and after that the wafers were rinsed by dipping consecutively into two different 0.1 M NaCl baths for 1 min each. Then the same procedure was done for the deposition of CMC.

Ellipsometry

Ellipsometric measurements of multilayers on Si wafers or PS films were performed *ex situ* (in the air) and *in situ* using a vertical computer-controlled DRE-EL02 ellipsometer (Ratzeburg, Germany). The angle of incidence was set at 70.0° and the wavelength, λ , of the He–Ne laser was 632.8 nm. *In situ* experiments were performed with a fluid cell specially designed for such experiments.²³ For the data interpretation, a multilayer model composed of the substrate, the unknown layer and the surrounding medium should be used. Then the thickness (d_x) and refractive index (n_x) of the unknown layer can be calculated from the ellipsometric angles, Δ and Ψ , using the fundamental ellipsometric equation and iterative calculations with Jones matrices²⁵

$$e^{i\Delta} \tan \Psi = R_{\rm p}/R_{\rm s} = f(n_{\rm k}, d_{\rm k}, \lambda, \phi) \tag{1}$$

where R_p and R_s are the overall reflection coefficients for the parallel and perpendicular waves. They are a function of the angle of incidence ϕ , the wavelength λ of the radiation and of the refractive index and the thickness of each layer of the model, n_k , d_k .

From the ellipsometric angles Δ and Ψ and a multilayer model composed of silicon, silicon dioxide, polymer layer and medium (air or polymer solution) it is possible to determine the thickness of the polymer layer. First of all, the thickness of the SiO₂ layers was determined in air, considering the refractive index for Si as $n = 3.88-0.018^{26}$ and its thickness as an infinite one, for the surrounding medium (air) the refractive index was considered as 1.00. Because the native SiO₂ layer is very thin, its refractive index was set as 1.462^{26} and just the thickness was calculated. The



Scheme 1 Representation of chemical structure of QPVP-C2, QPVP-C5 and CMC (R = H or CH₂COOH).

mean thickness of the native SiO₂ layer amounted to 2.0 \pm 0.2 nm. PS films presented mean thickness value 105 \pm 5 nm and refractive index as 1.587 \pm 0.004, which were determined by iterative calculations. After the characterization, the thickness of the each polyelectrolyte layer was determined in air or in solution ($n_{\text{solution}} = 1.335$, determined with an Abbé refractometer) considering the nominal refractive index 1.50 for CMC²³ and 1.54 for poly(4-vinyl-*N*-alkyl pyridinium) polycations.²⁴

The stability of multilayers formed by QPVP-C2 or QPVP-C5 and CMC was tested after each deposition by dipping the system into 0.1 M NaCl or 0.01 M HCl solution for one hour at 24 \pm 1 °C. The polymer solutions were filtered through a Millipore PTFE membrane (pore size 0.22 μ m) to remove impurities. After that period of time, samples were rinsed in NaCl 0.1 mol L⁻¹ and gently dried. The layer thickness variation was monitored by ellipsometry.

Atomic Force Microscopy (AFM)

Atomic Force Microscopy measurements were performed in a PICO SPM-LE (Molecular Imaging) microscope in the intermittent contact in air at room temperature, using silicon cantilevers with resonance frequency close to 300 kHz. Scan areas with resolution of 512×512 pixels were obtained. Image processing and the determination of the root mean square (RMS) roughness values were performed with the Pico Scan software.

Contact angle measurements

Contact angle measurements were performed at 25 ± 1 °C in a home-built apparatus.²⁷ Sessile water drops of 8 µL were used for the advancing contact angle (θ_a) measurements. After that the volume of 4 µL was removed from the drop in order to determine the receding contact angle (θ_r). The hysteresis in the contact angle ($\Delta \theta_a$) was calculated as the difference between θ_a and θ_r . Five samples of the same composition were analyzed at two different spots.

Sum frequency generation (SFG)

Sum frequency generation vibrational spectra were obtained with an SFG spectrometer (Ekspla, Lithuania) equipped with a pulsed Nd³⁺:YAG laser at 1064 nm (28 ps pulse duration, 20 Hz repetition rate) and harmonic unit generating second and third harmonics (532 and 355 nm, respectively). The first is the visible beam that excites the sample (pulse energy \sim 950 µJ). The third harmonic and fundamental beams pump an optical parametric amplifier with a difference-frequency stage that generates an infrared (IR) beam tunable from 1000 to 4000 cm^{-1} (pulse energy \sim 30–150 µJ), which overlaps with the visible beam on the sample. The incidence angles and approximate spot sizes on the sample are 51°, 500 μ m and 60°, 1000 μ m for the IR and visible beams, respectively. The sum-frequency signal as a function of IR frequency is collected by a photomultiplier after spatial and spectral filtering. For each scan, data are collected with 100 shots/data point in 3 cm⁻¹ increments. SFG spectra for layer-bylayer films of different layers were obtained with ssp polarization combination, where s and p stand for perpendicular (senkrecht in German) and parallel (to the incidence plane) components of polarized light, respectively, and the first s, second s, and last

p represent the polarizations of SFG, visible, and infrared light, respectively.

Evaluation of antimicrobial effect

The antimicrobial action of multilayers terminated by QPVP-C2 or QPVP-C5 was assessed by a standard protocol used for the determination of lysozyme antimicrobial activity.²⁸ Briefly, first the turbidity (τ_i) of aqueous dispersions (pH 5.7) of *Micrococcus luteus* (ATCC 4698) at 0.15 g L⁻¹ was determined at 25 °C and 650 nm, using a Beckmann Coulter DU-600 spectrophotometer. Then six samples covered with polyelectrolytes, corresponding to a total area of 6 cm², were dipped into the *M. luteus* dispersions and allowed to interact for 15, 30 and 60 min. If a small area is used, the method becomes less sensitive.²⁴ After each interval of time the turbidity of *M. luteus* dispersions was measured (τ_t). Bacteria disruption indicated antimicrobial activity²⁸ and was correlated with the relative decrease of turbidity ($\Delta \tau$); the largest is $\Delta \tau$, the more efficient is the antimicrobial agent:

$$\Delta \tau = (\tau_{\rm i} - \tau_t)/(\tau_{\rm i}) \times 100\% \tag{2}$$

As control experiments, the turbidity of aqueous dispersions of M. *luteus* (τ_i) in the absence of any biocidal agent and in the presence of bare PS films was also measured after 15, 30 and 60 min.

Results and discussion

Fig. 1a and 1b show the thickness determined *in situ* and in the air, respectively, by ellipsometry as a function of alternated deposition of QPVP-C2 (circles) or QPVP-C5 (triangles) and CMC layers onto Si/SiO₂ wafers, totalling seven layers. *In situ* measurements (Fig. 1a) evidenced linear thickness growth for



Fig. 1 Thickness evolution as a function of the number of deposited layers onto Si/SiO_2 wafers determined *in situ* (a) and in the air (b) by means of ellipsometry for QPVP-C2/CMC (circle) and QPVP-C5/CMC (triangle).

both systems. However, the mean thickness per layer determined for QPVP-C5/CMC amounted to ~2.7 nm, while QPVP-C2/ CMC was ~1.8 nm. The adsorption of OPVP-C2 or OPVP-C5 onto Si/SiO₂ wafers is favored under high ionic strength^{6,24,29} because under such conditions inter- and intramolecular electrostatic repulsion is screened and polyelectrolyte chains behave more like uncharged chains, forming loops and tails on the substrate. Comparing OPVP-C2 to OPVP-C5 chains, the longer alkyl side groups favored the formation of larger loops due to larger excluded volume effects, yielding thicker layers. Similar effects were reported for LbL films of hydrophobically modified carboxymethylpullulan and polycations deposited onto Si/SiO₂ wafers.³⁰ On the other hand, in the air (Fig. 1b) both dried systems presented exponential growth, which apparently suffered only a small effect of the alkyl group length. The effect is very small because the chains are collapsed in the dried state and, therefore, the differences caused by ethyl or pentyl side groups become less pronounced.

For the sake of comparison to negatively charged Si/SiO₂ wafers, which favor electrostatic interactions with QPVP-C2 or QPVP-C5 chains, hydrophobic PS films were chosen as substrates because they enable hydrophobic interaction with ethyl or pentyl side groups. The first layer to be deposited was QPVP-C2 or QPVP-C5 because CMC chains did not adsorb onto PS films. Fig. 2a and 2b show a linear increase of the mean thickness determined *in situ* and in the air by ellipsometry, respectively, as a function of alternated deposition of QPVP-C2 (squares) or QPVP-C5 (diamonds) and CMC layers onto PS films. *In situ* measurements (Fig. 2a) indicated mean thicknesses of ~1.4 nm and 1.2 nm per layer of QPVP-C5/CMC and QPVP-C2/CMC, respectively. These values are smaller than those found for layers deposited on Si/SiO₂ wafers (Fig. 1a and 1b), evidencing that the hydrophobic interactions induce the



Fig. 2 Thickness evolution as a function of number of deposited layer onto PS films determined *in situ* (a) and in the air (b) by means of ellipsometry for QPVP-C2/CMC (square) and QPVP-C5/CMC (diamond).

QPVP-C2 or QPVP-C5 chains to adsorb in a more compact conformation, since close to the PS surface the chains' hydration is less favored. In the dried state, each adsorbed QPVP-C2 or QPVP-C5 layer was \sim 1.3 nm thick (Fig. 2b), here the effect of the alkyl side group length and the drying process on the average thickness was negligible. On the other hand, the dried layers were slightly thicker on PS than on Si/SiO₂.

In the case of weak polyelectrolytes, the ionization of their functional groups, such as carboxylic acid or amine, depends on the pH and on their location. At constant pH the ionization might be different if chains are free in solution or within the multilayer. For instance, Kharlampieva and Sukhishvili investigated multilayers formed by QPVP with an alkylation degree varying from 12 to 98% and poly(methacrylic acid)¹⁹ on negatively charged surfaces. Their results suggested that QPVP chains with high alkylation degrees (>90%) increased the ionic pair cooperativity, favoring multilayer stability. Such stability was only shaken when carboxylic acid groups were protonated $(pH \sim 2)$ and ionic pairs were disrupted. The stability of multilayers formed by QPVP-C2 or QPVP-C5 and CMC on Si/SiO₂ wafers or PS films was investigated after each layer deposition by dipping the system into 0.1 M NaCl solution for one hour and monitoring the variations in the mean thickness values by means of ellipsometry. For salt solution, the mean thickness variation was under 5%, indicating that the strong electrostatic interactions between CMC and QPVP-C2 or QPVP-C5 impart high stability to these LbL systems, regardless the underlying substrate. The stability tests were also performed at pH 2 after each deposition onto Si/SiO2 wafers or PS films, except for the first one (QPVP-C2 or QPVP-C5). Under acidic conditions ,the CMC carboxylate groups are protonated and electrostatic interactions between CMC and QPVP-C2 or QPVP-C5 were expected to disappear. The mean thickness values of one layer of QPVP-C2, QPVP-C5 or CMC decreased in average $60 \pm 10\%$ or $45 \pm 8\%$ of the original thickness values, respectively, regardless if the substrate was Si/SiO₂ wafers or PS films. This finding evidences that the multilayer build-up is not only due to ionic pair cooperativity but also to hydrophobic interaction between alkyl side chains, which can't be destroyed under acidic conditions. Guyomard and co-workers³⁰ also observed that the stability of multilayers of amphiphilic polyelectrolyes onto Si/SiO₂ wafers is strongly dependent on the hydrophobic interactions between alkyl side chains. Longer chains yielded stronger hydrophobic interactions and thicker films. Therefore, modifying polyelectrolytes with hydrophobic moieties proves to be a simple strategy to improve the stability of polyelectrolytes multilayers deposited onto hydrophilic and hydrophobic substrates, especially when they must be used under extreme acidic or basic conditions.

AFM images obtained in the air after alternated deposition of QPVP-C2 and CMC layers onto Si/SiO₂ wafers are presented in Fig. 3. The first deposition, QPVP-C2 layer, is very smooth (RMS = 0.12 nm) and homogeneous (Fig. 3a). However, the deposition of the subsequent three layers revealed the presence of domains \sim 3 nm high, which increased the mean roughness values up to 0.60 nm (see the ESI†). The 4th layer (Fig. 3b) presented domains \sim 8 nm high dispersed on a fine network. Upon further deposition, the domains height and RMS values increased up to 4.1 nm (Fig. 3c) in the last deposition.



Fig. 3 AFM images $(2 \ \mu m \times 2 \ \mu m)$ obtained for the (a) 1st (QPVP-C2), (b) 4th (CMC) and (c) 7th (QPVP-C2) deposition onto Si/SiO₂ wafers, along with the corresponding root mean square (RMS) roughness values and cross sections in the position indicated by the dash lines.

In comparison to QPVP-C2, the first QPVP-C5 adsorbed layer onto Si/SiO₂ wafers presents tiny aggregates 5 ± 1 nm high dispersed on a smooth surface, causing larger RMS values (0.42 nm), as shown in Fig. 4a. These small aggregates probably were already in the polymer solution, although all solutions were previously filtered, and adsorbed on the substrate. However, the underlying film is very smooth, as can be seen in the height profile. As the alternated deposition proceeded, the mean roughness value was kept constant, indicating that the chains adsorbed homogeneously (Fig. 4b). After the fifth deposition (see Fig. S3, ESI[†]) the chains seem to have formed a fine network. The structures observed after the last deposition of QPVP-C5 (Fig. 4c) resemble those observed after the last deposition of QPVP-C2 (Fig. 3c).

PS films spin-coated from toluene are very smooth and homogeneous (Fig. S4, ESI[†]). However, after the 1st QPVP-C2 layer

adsorption, PS presented structures resembling self-assembled chains (Fig. 5a), which might be driven by multipoint hydrophobic interactions between ethyl pendant groups and the PS surfaces. These structures evolved to fibrils after 2nd layer (CMC) deposition (see Fig. S5, ESI[†]), showing that the CMC chains preferred to adsorb onto pre-adsorbed QPVP-C2 chains than to PS free area, since CMC chains have very weak hydrophobic character. As a control experiment, bare PS films were immersed into CMC solution keeping the same conditions used for the deposition of QPVP-C2. No adsorption of CMC onto PS could be detected by ellipsometry. The subsequent deposition of QPVP-C2 and CMC led to thicker fibrils (Fig. 5b), as if the arriving chains kept the preference to adsorb onto the already formed structures, instead of occupying the vacancies (see Fig. S5, ESI[†]). After the last deposition (Fig. 5c) a porous structure could be observed in the topographic image and the corresponding height profile. Such



Fig. 4 AFM images $(2 \ \mu m \times 2 \ \mu m)$ obtained for the (a) 1st (QPVP-C5), (b) 4th (CMC) and (c) 7th (QPVP-C5) deposition onto Si/SiO₂ wafers, along with the corresponding root mean square (RMS) roughness values and height profiles in the position indicated by the dash lines.



Fig. 5 AFM images obtained for the (a) 1st (QPVP-C2), (b) 4th (CMC) and (c) 7th (QPVP-C2) deposition onto PS films, along with the corresponding root mean square (RMS) roughness values and height profiles in the position indicated by the dash lines.

porous structures were more evident in the systems involving QPVP-C5. Already after the 1st layer (QPVP-C5) deposited onto PS films (Fig. 6a), a network is clearly observed. This network seems to be formed by interconnected fibers that became thicker with the next depositions (see Fig. S6, ESI[†]). After the 4th deposition the pores were ~ 8 nm deep (Fig. 6b), but they were not observed over large areas. After the last deposition (Fig. 6c) the mean pore depth did not change, but the porous structure appeared over areas larger than 50 μ m \times 50 μ m. The microporous structure was predominantly caused by hydrophobic interactions between the side groups and the substrate. A similar behavior was observed for poly(maleic anhydride-alt-styrene) modified with phenyl groups deposited onto methyl- and amino-terminated surfaces; the layers adsorbed onto methyl-functionalized substrates were thinner and more porous than those adsorbed onto amino-terminated surfaces.31

Thus, comparing the morphological features observed for multilayers deposited onto the Si/SiO₂ wafers (Fig. 3 and 4) with those observed on the PS films (Fig. 5 and 6), the hydrophobic interaction between the PS and QPVP-C2 or QPVP-C5 chains and subsequent layers led to the formation of films with cavities, while for Si/SiO₂ wafers, where the electrostatic interaction and counter-ions release are expected to control the LbL formation, such porous structures were not observed.

Fig. 7a and 7b show the advancing contact angle (θ_a) values and the contact angle hysteresis ($\Delta \theta$) determined after each layer deposition onto Si/SiO₂ or PS films, respectively. The last deposited layer corresponds to modified polycation. The $\Delta \theta$ values are useful to gain insight about the chemical and/or topological heterogeneities at a macroscopic level. For Si/SiO₂ wafers (Fig. 7a) the θ_a values increased from 5° (bare substrate) to a plateau value of $41 \pm 4^\circ$ or $62 \pm 5^\circ$ after the 4th deposition of



Fig. 6 AFM images obtained for the (a) 1st (QPVP-C5), (b) 4th (CMC) and (c) 7th (QPVP-C5) deposition onto PS films, along with the corresponding root mean square (RMS) roughness values and height profiles in the position indicated by the dash lines.



Fig. 7 Advancing contact angle (θ_a) and contact angle hysteresis ($\Delta \theta$) determined after each alternated deposition of QPVP-C2 or QPVP-C5 (1st layer) and CMC onto (a) Si/SiO₂ wafer or (b) PS films as a function of layer number. Squares and circles stand for assemblies with QPVP-C2 and QPVP-C5, respectively. The data represent the average over ten measurements (two different spots on five samples of same composition) with the corresponding standard deviation.

QPVP-C2/CMC or QPVP-C5/CMC systems, respectively. The $\Delta\theta$ values increased with the number of layers for both systems; after the last deposition of QPVP-C2 or QPVP-C5 $\Delta\theta$ amounted to 15° or 18°, respectively. Thus, LbL systems made of polycations modified with pentyl groups were more hydrophobic, due to the more favored exposure of alkyl groups to the air, and were slightly more heterogeneous than those composed of polycations modified with ethyl side groups.

The θ_a values determined for bare PS as $90 \pm 2^{\circ}$ (Fig. 7b) decreased to $70 \pm 3^{\circ}$ or $71 \pm 4^{\circ}$ after the 4th deposition of QPVP-C2/CMC or QPVP-C5/CMC systems, respectively, due to partial exposure of polymer charged segments to the medium. Similar to the LbL systems deposited onto Si/SiO₂ wafers, for PS the $\Delta\theta$ values also increased with the number of layers, so that after the last deposition of QPVP-C2 or QPVP-C5 $\Delta\theta$ amounted to 13° or 17°, respectively.

A comparison between the last deposition onto Si/SiO₂ and PS shows that in the case of QPVP-C5, the θ_a values tended to converge to a mean value of $(65 \pm 5)^\circ$, in spite of their different topographies. On the other hand, in the case of QPVP-C2, the θ_a value for the last deposition onto PS films came along with a mean value of $(65 \pm 5)^\circ$, but it was ~20° smaller when Si/SiO₂ was the substrate. The trends in $\Delta\theta$ observed in Fig. 7a and 7b corroborated with the increase in the RMS values as the number of layers increased, although RMS values derived from topological analyses at microscopic level. Tables T1 and T2 in the ESI† comprise θ_a , $\Delta\theta$ and RMS values determined for all layers on both substrates.

SFG spectroscopy is a powerful tool to reveal molecular orientation at interfaces. The pyridinium group is responsible for

the antimicrobial properties of OPVP-C2 or OPVP-C5 films. Thus, in the present study we are especially interested in the orientation of the pyridinium group after each deposition onto the quartz (SiO₂) slides up to five layers. For this reason, SFG spectra of dried films were obtained in the pyridinium characteristic absorption range (~1645 cm⁻¹, see FTIR spectra obtained for QPVP-C2 or QPVP-C5 presented in the ESI[†]). SFG in situ measurements were avoided due to the water bending frequency in the range of 1630 cm⁻¹ to 1640 cm⁻¹, which would overlap with the pyridinium absorption band. Fig. 8a and 8b show the SFG spectra obtained for OPVP-C2/CMC and OPVP-C5/CMC systems, respectively, The ssp spectra collected for the first adsorbed layer on the quartz slides, QPVP-C2 or -C5, have a peak at ~ 1645 cm⁻¹, associated with C=C stretches of the pyridinium ring. As observed previously for PAH/PSS films,32 the SFG intensities for the QPVP-C5/CMC multilayers alternate from high (when terminated by OPVP-C5) to low values (when terminated by CMC). This finding suggests that the first QPVP-C5 layer is well ordered, with the pyridinium groups probably pointing on average towards the substrate due to electrostatic interaction. The adsorption of CMC (2nd layer) partially reorientates the pyridinium groups and their net preferential orientation is reduced (lower SFG signal). The 3rd layer (QPVP-C5) also adsorbs with the pyridinium groups towards the 2nd layer, which is negatively charged, increasing again the SFG signal. The 4th layer (of CMC) once more re-orientates the 3rd layer of QPVP-C5, leading to a reduction is SFG signal. This alternation continues, but with gradually less contrast between each adsorption. This implies that the average orientation of the pyridinium groups towards the substrate, in the outermost layer, decreases with the film thickness, probably due to increased random orientation of the polymer chains.



Fig. 8 SFG spectra obtained in the air for (a) QPVP-C2/CMC films and (b) QPVP-C5/CMC films as a function of number of layers.

For QPVP-C2/CMC films, after the deposition of the 2nd layer (CMC), the SFG signal decreased considerably, because the CMC carboxylate groups probably screened the pyridinium groups. The subsequent deposition of 3rd layer (QPVP-C2) had no effect on the SFG signal intensity, indicating that pyridinium groups of this 3rd layer are randomly oriented. The deposition of CMC on the 3rd layer (QPVP-C2) suppressed completely the pyridinium SFG signal. The next deposition of OPVP-C2 (5th layer) yielded SFG spectra with signal intensities, which varied as a function of sample position. Such irregular signal intensity is probably due to the increase of surface heterogeneity evidenced by AFM images and $\Delta \theta$ values. Thus, SFG spectra were not recorded for the 5th, 6th and 7th layers. Regarding the multilayers built on PS films, they were not adequate for SFG measurements because their absorption bands are close to those of pyridinium.

The techniques presented so far provided information about the thickness, wettability, topography and molecular orientation of deposited layers onto Si/SiO₂ or PS films. Considering the outstanding biocidal properties of QPVP-C2 and QPVP-C5,^{24,33-36} a standard test for the determination of antimicrobial activity after each deposition of QPVP-C2 and QPVP-C5 was used as an indirect method to assess the exposure of pyridinium groups in aqueous medium, since it is reliable and specific to the hydrophobically modified polycations.

The biocidal action of pyridinium salts for Gram-positive and Gram-negative bacteria is well reported in the literature.^{24,33-36} The mechanism is briefly described. The adsorption of bacteria onto cationic surfaces is driven by electrostatic interaction between cationic charges present on the substrate and the bacterial membrane's negative charges. Upon adsorbing, the compensation of the negative charges of the bacterial envelope is provided by the cationic charges of the substrate, and the bacteria lose their natural counter ions, which are released, increasing the entropic gain. Thus, the solid substrate becomes biocidal when the number of cationic sites is large enough to remove counter ions from bacteria, inducing disruption of the bacteria envelope.^{33–35} In the present study, the biocidal activity of OPVP-C2 or OPVP-C5 layers deposited onto Si/SiO2 wafers or PS films was tested against M. luteus, Gram-negative bacteria, which was correlated with the relative decrease of turbidity ($\Delta \tau$). Table 1 shows the $\Delta \tau$ values measured after interacting for 15, 30 and 60 min with each polycation deposition. Regarding the results found for LbL onto Si/SiO2 wafers, some important information can be extracted from Table 1. After 60 min, systems composed of QPVP-C2 chains behaved similarly to those containing QPVP-C5 chains, with maximal $\Delta \tau$ values of ~95%, which is in agreement with previous results,24 and indicate a similar amount of pyridinium groups exposed to the aqueous dispersion. The biocidal activities of the 1st, 3rd and 5th layers of QPVP-C2 or QPVP-C5 could be detected after 15 min. Nevertheless, they increased at least twofold after 60 min. This behavior shows that pyridinium groups need some time either to orientate towards the aqueous dispersion, as if the chain segments had to diffuse out of the LbL structure, or to undergo conformational changes. The antimicrobial effects observed for the OPVP-C2 or OPVP-C5 7th layer were already the highest after 15 min, suggesting that the surface charge density for a film with 7 layers is higher compared to the others.

Table 1 Relative decrease of turbidity ($\Delta \tau$, %) measured for dispersions of *M. luteus* as a function of the time and deposition layer of polycation onto Si/SiO₂ or PS films. The data are mean values obtained for three different sets of the same system with the corresponding standard deviations

		15 min	Δτ (%) 30 min	60 min
	QPVP-C2 1st	45 ± 5	60 ± 7	91 ± 7
	QPVP-C2 3rd	30 ± 4	55 ± 7	90 ± 6
	QPVP-C2 5th	52 ± 6	63 ± 6	93 ± 7
	QPVP-C2 7th	80 ± 9	88 ± 9	96 ± 4
Si/SiO ₂				
	QPVP-C5 1st	37 ± 4	63 ± 6	89 ± 9
	QPVP-C5 3rd	45 ± 5	56 ± 5	97 ± 3
	QPVP-C5 5th	58 ± 6	70 ± 7	90 ± 8
	QPVP-C5 7th	75 ± 7	86 ± 8	94 ± 6
	QPVP-C2 1st	11 ± 1	15 ± 2	15 ± 2
	QPVP-C2 3rd	24 ± 2	23 ± 2	25 ± 2
	QPVP-C2 5th	23 ± 1	25 ± 2	27 ± 2
	QPVP-C2 7th	16 ± 2	16 ± 3	23 ± 3
PS				
	QPVP-C5 1st	8 ± 1	14 ± 2	15 ± 2
	QPVP-C5 3rd	23 ± 2	24 ± 1	28 ± 3
	QPVP-C5 5th	15 ± 2	23 ± 1	24 ± 3
	QPVP-C5 7th	9.5 ± 0.8	10 ± 1	23 ± 2

As control experiments, the biocidal activities of 6th layers of CMC, which were deposited either after QPVP-C2 or QPVP-C5, were detected after 15 min and 60 min contact with *M. luteus* dispersions. Regardless of the polycation type and period of contact, the $\Delta \tau$ values were smaller than 15%, indicating that positively charged pyridinium groups were screened by CMC layer. The biocidal activity of bare Si/SiO₂ wafers was tested in the same way. The $\Delta \tau$ values ranging from 5% to 10%, which is within the method accuracy, indicated null activity.

The biocidal activities determined for the QPVP-C2 or QPVP-C5 layer onto PS films were generally weaker than those observed for polycations of Si/SiO₂ wafers, indicating less exposure of pyridinium groups to the aqueous dispersion. The largest $\Delta \tau$ values, ~28%, were observed for the QPVP-C2 3rd layer or OPVP-C5 5th layer after 60 min contact with the M. luteus dispersions. The low biocidal activity of these systems corroborates with their hydrophobicity shown in Fig. 7b and indicates that in water the exposure of positive charges stemming from layers deposited on PS is less favored than from those on Si/SiO₂ wafers. This effect might be explained by the selfassembly driven by the first QPVP-C2 or QPVP-C5 layer and network building, as could be observed in the AFM images (Fig. 5, 6, S5 and S6, ESI[†]). Antimicrobial activity of bare PS films was practically null because the $\Delta \tau$ values ranged from 5% to 10%, which is within the method accuracy.

Conclusions

In situ ellipsometry results revealed that the substrate character (hydrophilic or hydrophobic) has a significant influence on the chain conformation upon adsorption; for Si/SiO_2 wafers the chains are thicker (more expanded) due to better hydration, while for PS films the chains seem to be less hydrated and tend to assume a more compact conformation. The effect of the alkyl side group length was more pronounced when the substrate was

hydrophilic; pentyl groups led to thicker films than ethyl groups, suggesting that QPVP-C5 chains formed larger loops upon adsorbing onto Si/SiO₂ wafers. On the other hand, upon drying, the adsorbed chains collapsed and the effect of the pendant group was negligible, regardless the substrate nature. The acid resistance of multilayers formed by oppositely charged polyelectrolytes, which is generally due to cooperative polymer–polymer ion pairs, was enhanced by additional hydrophobic interactions between the alkyl side chains present in QPVP-C2 or QPVP-C5, for both substrates.

AFM images revealed porous structures only when the multilayers were deposited onto PS films. Such structure formation might be attributed to the first layer deposition, which was caused by multipoint attachment of alkyl pendant groups on the hydrophobic surface. This initial layer guided the next layer's deposition, which led to network building. Although the substrate character affected the morphological features of resulting multilayers, all films became more irregular at the macro- and microscale, as the number of layers increased. The inhomogeneity ($\Delta\theta$) and wettability (θ_a) of QPVP-C2 or QPVP-C5 terminated systems onto Si/SiO₂ wafers and PS films were similar, except for the wettability of QPVP-C2 onto Si/SiO₂ wafers, which was the highest.

SFG spectra obtained in air after each layer deposition onto Si/SiO_2 wafers evidenced that the molecular orientation of pyridinium group was (i) strong after polycation deposition, (ii) more pronounced for QPVP-C5 than for QPVP-C2 and (iii) disappeared after polyanion deposition. The antimicrobial tests were used as a tool to assess the exposure of pyridinium moieties to aqueous medium. A substantial exposure of the pyridinium groups was detected when the deposition was on the Si/SiO₂ wafers, but on PS films it was less evident. This finding corroborates with the *in situ* ellipsometric measurements, which showed more expanded chains when the substrate was hydrophilic. Moreover, for theSi/SiO₂ wafers, the exposure of the pyridinium groups to the medium was time dependent; the chains needed time to diffuse out of the layer and/or to undergo conformational changes.

This study demonstrated that the substrate character (hydrophilic or hydrophobic) plays a decisive role on the adsorption of the first layer, which in its turn affects the structure and function of the resulting multilayer.

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Addition and correction

This article was originally published with incorrect page numbers. This is the corrected, final version.

The Royal Society of Chemistry apologises for these errors and any consequent inconvenience to authors and readers.