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Polarity studies of single polyelectrolyte layers in polyelectrolyte multilayers probed by steady state and life time doxorubicin fluorescence.

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Abstract

Hypothesis

Polarity in polyelectrolyte multilayers (PEMs) may vary from the inner to the top layers of the film as the charge compensation of the layers is more effective inside the PEMs than in outer

layers. Doxorubicin (DX) is used here to sense polarity at the single polyelectrolyte level inside PEMS.

Experimental

DX is complexed electrostatically to a polyanion, either polystyrene sulfonate (PSS) or polyacrylic acid (PAA) and assembled at selected positions in a multilayer of the polyanion and polyallylamine hydrochloride (PAH) as polycation. Local polarity in the layer domain is evaluated through changes in the intensity ratio of the first to second band of spectra of DX (I_1/I_2 ratio) by steady state fluorescence, and by Lifetime fluorescence.

Findings

For PAH/PSS multilayers show a polarity similar to water with DX/PSS as top layer, decreasing to I_1/I_2 ratios similar to organic solvents as the number of polyelectrolyte layers assembled on top increases. For PAH/PAA multilayers, polarity values reflect a more polar environment than water when DX/PAA is the top layer, remaining unaltered by the assembly of polyelectrolyte layers on top. Results show that different polar environments may be present in a PEM when considering polarity at the single layer level.

Keywords: Polarity; Doxorubicin; Life Time Fluorescence; Polyelectrolyte Multilayers; Layer by Layer Technique

Introduction

The Layer by Layer (LbL) technique is a well known approach for the non-covalent functionalization of surfaces and the fabrication of thin films[1,2]. LbL is based on the alternating assembly of oppositely charged polyelectrolytes through electrostatic interactions[3–5] facilitated by the entropic gain through the release of counter ions during assembly[6,7]. Polyelectrolyte multilayers (PEMs) fabricated by the LbL technique have a controlled composition in the nanometric range in the vertical direction[8,9]. Properties of the multilayer can be tuned by varying the number of polyelectrolyte layers assembled and by the choice of polycations and polyanions, which can be complemented by other molecules or nanomaterials, replacing selected polyelectrolyte layers[10–14]. PEMs have found multiple applications in drug delivery[15–20], as protective layers, for sensor fabrication[21–23] and as membranes for nanofiltration[24–27] and reverse osmosis[28], to mention a few. The physicochemical properties of the films and the self assembly mechanism of the PEMs have been subject to multiple studies[29,30]. The kinetics of assembly[31–34], water content of the multilayers[35], mechanical[36] and electrical[37] properties as well as molecular diffusion in the layers[38] have been deeply characterized. However, an aspect of PEMs that has been less studied is their polarity[39]. Polarity plays a significant role in applications of PEMs as separation or filtration membranes, as the inner polarity of the films will determine which type of molecules can diffuse through or be retained in the layers. Polarity is a complex concept to describe. It refers to the character of a molecular environment, if it has characteristics of an organic or an aqueous medium[40]. Indeed, polarity encompasses all interactions that define

the affinity of a molecular entity for the environment[41,42] like electrostatic, dipolar, multipolar, dispersive force interactions, electronic charge transfer, hydrogen bonding, proton transfer, and others. Bulk polarity is usually described by the dielectric constant of the media.

In the LbL technique polyelectrolytes are assembled from a polar environment, an aqueous solution with a defined ionic strength, usually between 100 and 500 mM NaCl[43–45]. When polyelectrolytes assemble on a surface they retain some water associated with the charged groups of the polymer and the chains in the top layer of the PEM remain exposed to the bulk aqueous solution[46]. However, when an additional polyelectrolyte layer is deposited on top, the layer below faces a different environment. The charge from this layer is compensated at least partially by the new layer assembled[47,48]. As the number of layers in the PEM increases, internal layers become more densely packed and most of their charges are compensated by layers on top[49]. It has been shown that the multilayer films become denser as the number of layers increases and defects in the layers are sealed. Polarity must also change during the layer build up and a particular layer will face a change in polarity as subsequent layers are deposited on top due to the charge compensation, a denser arrangement of polymer chains and fewer defects in the multilayer structure trapping water from bulk.

In an excellent paper, Tedeschi et al studied the micropolarity of polyelectrolyte multilayers by pyrene fluorescence[39]. The $\pi^* \rightarrow \pi$ emission spectrum of monomeric pyrene exhibits five well-resolved major vibronic bands between 370 and 400 nm. Due to coupling of the electronic

and vibronic states, the first vibronic band undergoes significant intensity enhancement with increasing solvent polarity compared to the third band[50,51]. This spectroscopic characteristic of pyrene has been extensively used for polarity studies. Tedeschi et al[39] studied the polarity of polyallylamine hydrochloride (PAH)/polystyrene sodium sulfate (PSS) and polydiallyldimethylammonium chloride (PDADMAC)/PSS films. The authors obtained an average value for polarity as they soaked the films in pyrene solution. Pyrene is not charged, it has a very low solubility in water and though it partitions preferentially in the multilayer from water, it does so in very low concentrations. The authors therefore analysed films with many polyelectrolyte layers to assure a concentration of pyrene in the film large enough to obtain detectable spectra. Pyrene diffuses non-specifically in the multilayers. Therefore, it is not possible to use pyrene to study polarity at defined layer positions in the multilayers. Polarity may change for layers placed in the inner region of the PEMs compared with layers in the outer regions. Differences in polarity within a film may play a role in applications related to filtration or separation of molecules. It is also interesting from a fundamental point of view to understand how polarity may change inside the film during the assembly of layers, hinting at how the organization and water content of the inner layers in the film may change with additional layers on top.

In this work we have used doxorubicin hydrochloride (DX) instead of pyrene to study polarity. DX is an anticancer antibiotic belonging to the anthracycline family, whose spectroscopic

properties, fluorescence in particular, are known to be affected by solvent polarity[52]. The ratio of the intensities of the fluorescence bands of DX located around 550 nm (I_1) and 590 nm (I_2) have been shown to be strongly influenced by the dielectric constant of the solvent[53]. Doxorubicin has been used to sense micro polarity and an empirical scale of polarity has been developed for the dye based on the I_1/I_2 ratio. Doxorubicin in water shows an I_1/I_2 ratio of 0.8, which increases in environments more non-polar than water and decreases in more polar ones. Since DX is positively charged, it can be also electrostatically assembled between polyelectrolyte layers or complexed with negatively charged polyelectrolytes[54]. Therefore, it would be possible to selectively place DX at a defined position in the multilayers, just by assembling DX on top of a chosen polyanion layer or by complexing the polyanion with DX before assembly. By precisely placing DX in a selected layer position in the PEM we show that it is possible to sense polarity locally. We report micro polarity in films of PAH/PSS and PAH/polyacrylic acid (PAA) at the 4th and 8th layers of the films by assembling DX/PSS and DX/PAA respectively at the two positions. Steady state fluorescence and fluorescence lifetime studies have been conducted to study the evolution of polarity in the films with the assembly of polyelectrolyte layers. Our results provide fundamental information on the physicochemical properties of LbL films at single layer level, which are otherwise not accessible.

Experimental

Materials

Poly(allylamine hydrochloride) (PAH, Mw 15 kDa), poly(styrene-sulfonate sodium salt) (PSS, Mw 70 kDa), polyacrylic acid (PAA, Mw 15 kDa), Doxorubicin hydrochloride (DX, Mw 579.98), NaCl were purchased from Sigma Aldrich. Chemical formula of DX and the polyelectrolytes are provided in Figure 1.

PEM Preparation

Glass coverslips were cleaned in a UV chamber for 20 minutes. After that, the glass supports were immersed in ethanol, H₂O for 3 minutes. The last washings were done in a 0.5 M NaCl solution for 3 minutes. Solutions of PAH, PAA, and PSS were prepared at a 1 mg/mL concentration in 0.5 M NaCl solution. All solutions were left to equilibrate at room temperature for 30 min and then filtered through a membrane with 0.22 mm pore size. An aqueous stock solution of 10⁻³ M DX was prepared. Doxorubicin/anionic polyelectrolyte complexes were prepared at a molar ratio of 2, with the molarity of the polyanions corresponding to the concentration of monomers. Glass supports were immersed in polyelectrolyte solutions, starting with PAH, and left to equilibrate for 15 minutes, then rinsed with water three times before the assembly of the following polyelectrolyte layer.

Steady state fluorescence

Steady state fluorescence measurements were performed on a Fluorolog®-3 spectrofluorometer (Horiba-Jobin Yvon) using a solid sample holder with an incident angle of 50 degrees. The excitation wavelength was 500 nm with an excitation slit of 10 nm. The

corresponding emission spectra were acquired from 5 to 800 nm with a scan speed of 1200 nm.min⁻¹ and a slit of 10 nm. Samples were dried before measurements by fluxing nitrogen for 5 minutes and then putting the specimens under reduced pressure for 1 hour.

Lifetime measurements were performed with the time correlated single photon counting (TCSPC) technique using an instrument of local design as previously described[55]. Samples were inserted into the cuvette holder at an angle of approximately 45°, paying attention to prevent the direct reflection of excitation laser from reaching the detector.

Results

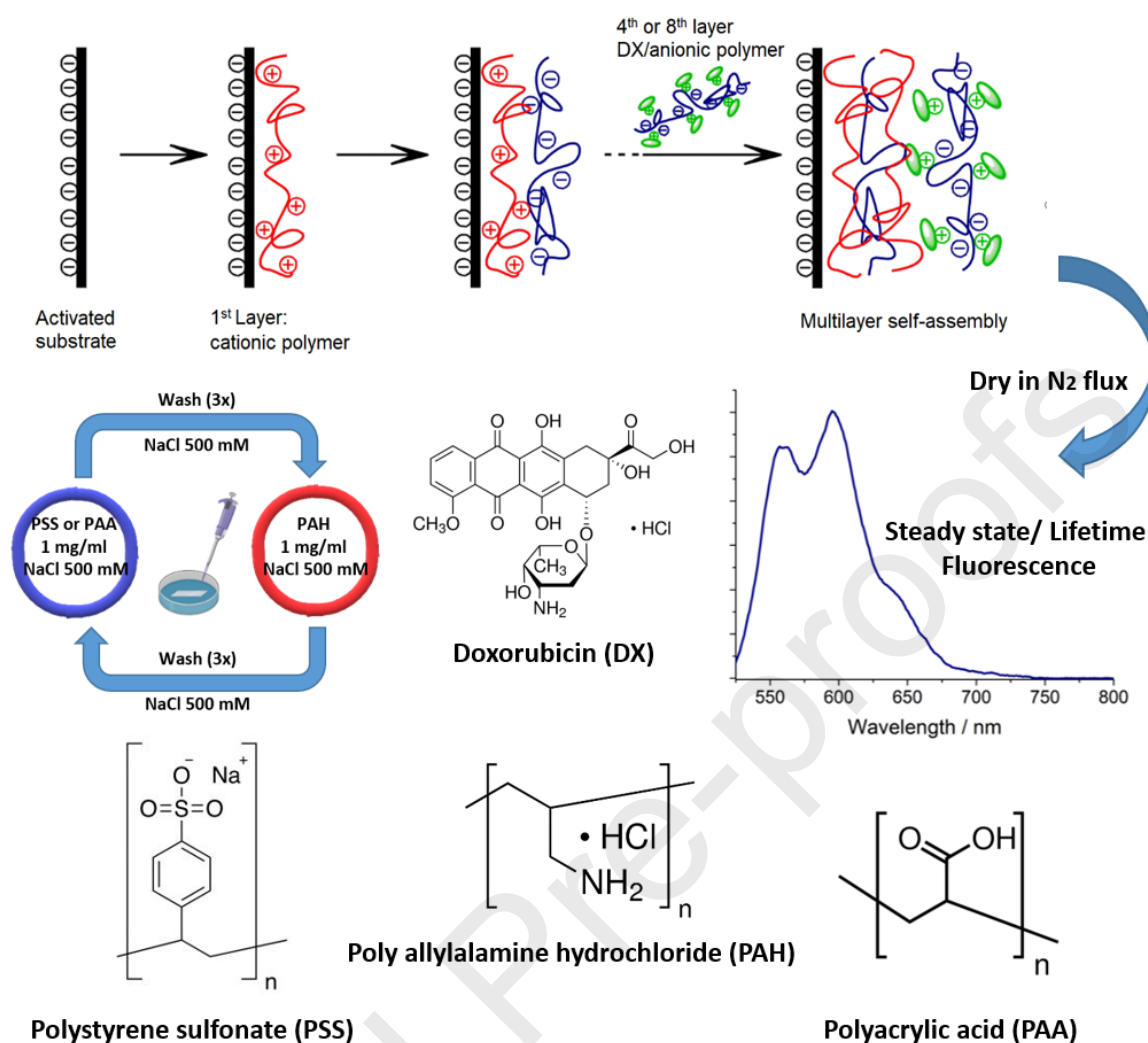


Figure 1. Scheme of the LbL assembly of DX/anionic layers (at the 4th or 8th layer) and the experimental work carried out in this paper. After the assembly of the multilayers with doxorubicin, PEMs are dried in nitrogen flux and then steady state and life time fluorescence measurements are performed. The scheme also summarizes the assembly procedure of the polyelectrolytes as described in the experimental part. Chemical formula of doxorubicin

hydrochloride (DX) and of the polyelectrolytes (PSS; PAH; PAA) used in this work are also shown.

Complexes of polyanions, PSS or PAA, with DX were formed at a molar ratio of DX to polyanion (monomers) of 2 and assembled in the PEMs in different positions in the multilayers, then dried and Steady State and Lifetime fluorescence measured as sketched in Figure 1. DX/polyanion complexes were assembled either at the 4th or the 8th layer in the film to probe polarity in two distinct positions in the PEMs. The 4th layer is close to the supporting glass surface, where it is in the initial build up of the multilayer characterized by less defined polyelectrolyte layers. Conversely, at the 8th layer the film is expected to have reached a regular growth and a densification of the layers[49].

Steady state fluorescence was measured immediately after the assembly of the DX/polyanion layer (4th layer) and after each layer deposited on top. Films were always measured in dry state. A sketch of the PEMs is shown together with the corresponding fluorescence spectra of PAH/PSS with a layer of DX/PSS at the 4th layer without additional layers on top, with three layers of polyelectrolyte on top and with 6 layers on top (Figure 2). The fluorescence spectra change as an increasing number of polyelectrolyte layers are added on top. While the intensity of the DX emission band located around 590 nm does not change, the intensity of the first band (at around 560 nm) increases progressively. An I_1/I_2 ratio of 0.8 is obtained when the DX/PSS

layer is the top layer, coincident with the value measured for DX in water. This result implies that the DX/PSS complex is facing an environment with a polarity analogous to water, which is understandable as the DX/PSS complexes have no layers on top to compensate the negative charges that retain water molecules in the layer even after drying the film (besides some condensation water from the air that can be expected). The addition of three layers of polyelectrolytes on top results in a significant increase in the I_1/I_2 ratio, to a value of 1.12. This value reveals the exposure of DX to quite a non-polar environment, now trapped between oppositely charged polyelectrolyte layers. The additional assembly of another three layers on top results in an even higher I_1/I_2 value of 1.2 meaning that the environment probed by the DX located at the 4th layer is now even more apolar. A value of $I_1/I_2 = 1.2$ is obtained for DX dissolved in *n*-heptane, thus indicating that the inner regions of the multilayer become quite apolar as the PEM grows.

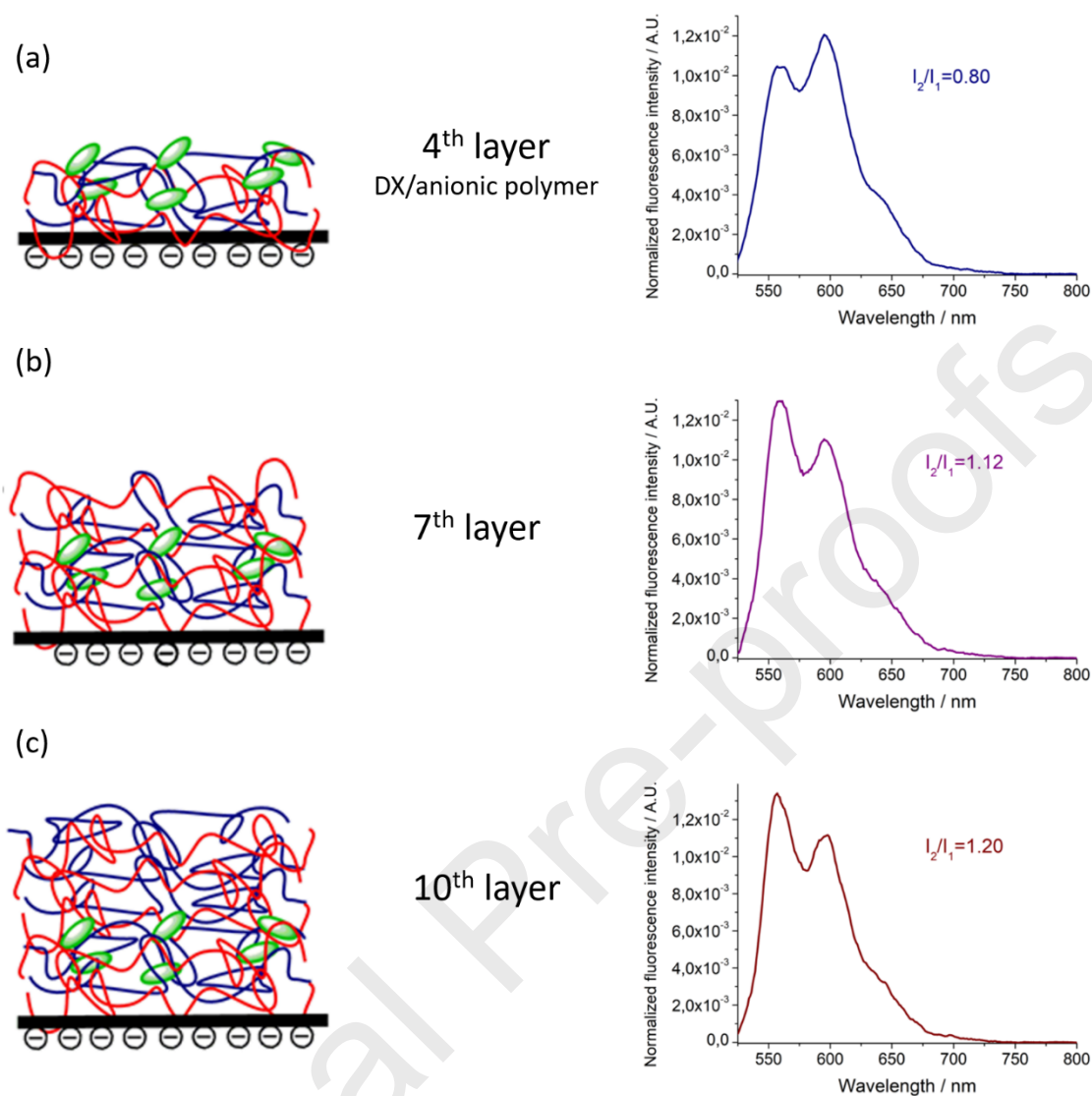


Figure 2. Left: Scheme of LbL assembly with DX/PSS complexes located at the 4th layer position with (a) no additional polyelectrolyte layers, (b) 3 additional polyelectrolyte layers, and (c) 6 additional layers of (PAH/PSS/PAH)₂. Blue lines correspond to polycations, while

red lines indicate polyanions. The green ovals represent DX. Right: DX fluorescence spectra corresponding to the PEMs sketched on the left.

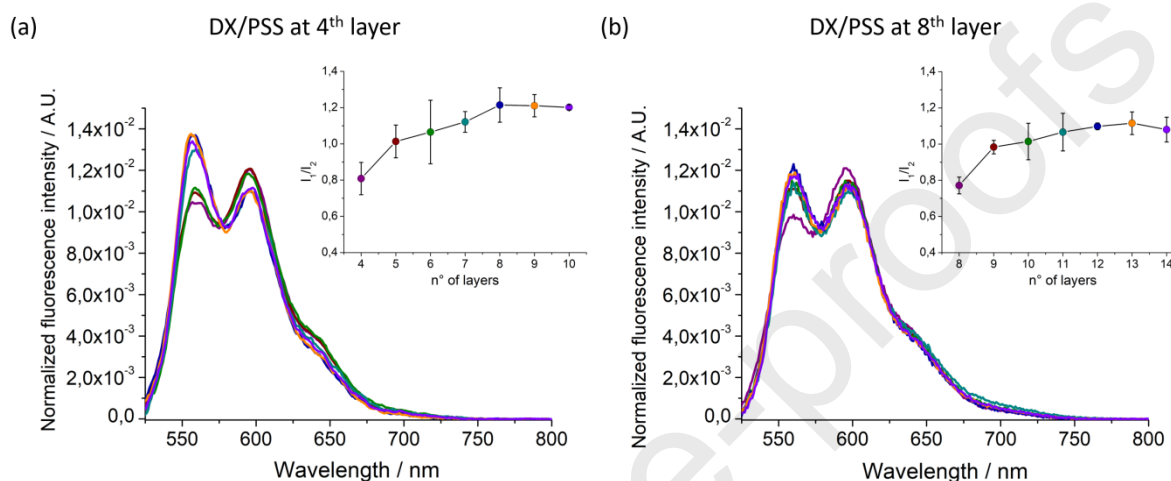


Figure 3. Normalized fluorescence spectra from DX/PSS complexes assembled in PAH/PSS LbL films at (a) the 4th layer position and (b) the 8th layer position. The spectra correspond to the PEMs with the DX/PSS layer on top, and with additional layers of PAH and PSS on top from 1 to 6 layers. In the inset, the ratio of the first and second DX bands (I_1/I_2) from the complex 4th layer and 8th layer, has been plotted as a function of the number of layers in the films, from 0 to 6 layers on top of the probing layer (error bars represent the standard deviation obtained by recording the spectra of different specimens; $n=3$). The spectra correspond to the

I_1/I_2 ratio (number of layers deposited on top of the DX/polyanion layer) shown in inset with the same colour as the spectra.

Fluorescence spectra (normalized area) from PEMs with DX/PSS located at the 4th layer or the 8th layer with an increasing number of layers of PAH and PSS on top are shown in Figure 3 together with a plot of the evolution of the I_1/I_2 ratio for the two cases. For both systems, the same I_1/I_2 ratio of 0.8 was observed when no additional layers were deposited on top, meaning that in both cases the DX is facing an environment with a polarity similar to water. Assembling PAH on top of the DX/PSS layer has the same effect for both systems, increasing the I_1/I_2 ratio to 1. This is the largest variation in the ratio observed after the deposition of a single layer on top. By further increasing the number of deposited layers, a less steep and more continuous increase of the I_1/I_2 ratio is observed. After 4 layers deposited, the ratio does not seem to change, which is in agreement with the reported compensation of charges by an assembled layer for up to 4 layers below[47,56]. After assembling 4 polyelectrolyte layers, the deposition of additional layers does not seem to play any role in the polarity, as both the water content and the polymer arrangement surrounding the layer carrying the DX are not further affected. The final I_1/I_2 value, after adding 6 polyelectrolyte layers on top of the DX/PSS layer, is higher for DX placed at the 4th layer than at the 8th, 1.2 vs 1.1. This difference in the two values may reflect a more densely packed or more interdigitated polymer environment at the 4th layer than

at the 8th layer, which can be expected since the first layers are less homogeneous, less stratified, and not covering the whole surface. It has been shown that PAH/PSS systems display a linear growth, where the amount of polymer deposited per layer is practically constant. However, quartz crystal microbalance with dissipation (QCMD) studies demonstrated that the linear growth starts at the 4th layer[35], Figure S1. The first three layers, in fact, show a progressive increase in mass. The 4th layer may therefore be less stratified, and the DX/PSS may occupy free spaces in the assembled layers below the 4th which results in a slightly different environment than at the 8th layer where the multilayer growth is more regular, and a more stratified assembly can be expected. The most striking feature arising from these results is however the increase in the I_1/I_2 ratio from 0.8 to 1 by just adding one layer of PAH, complexing with the top DX/PSS layer. A value of 1 in the ratio means that the environment is becoming largely more apolar for DX. In previous work[35] we have studied the evolution of the water content of PAH/PSS layers by combining QCMD with ellipsometry. While the wet mass of the PEM was measured by QCMD, ellipsometry measurements provided the dry mass. By combining these two values, the hydration of the PEM per layer assembled could be determined. After the 4th layer assembled, hydration of PAH/PSS PEMs remains constant, independent of the number of polyelectrolyte layers further deposited. This means that when a layer is deposited on the PEM bringing its own hydration water, there is a removal of water from the PEM, most likely from the last layer (that is facing the bulk) below the new one. The polarity change for the DX/PSS complex fits quite well with the QCMD/ellipsometry data thus

supporting the assumption based on the DX I_1/I_2 value, that water associated with PSS is removed when PAH is assembled on top.

The fact that the addition of one PAH layer is enough to create an apolar environment for the DX in the top PSS layer indicates that the water associated with PSS is significantly decreased around the sulfonate groups. Tedeschi et al[39] reported a polarity for PAH/PSS films with pyrene that can be placed between methanol and acetonitrile (a moderately polar environment), but it has to be taken into account that the authors prepared a PEM and put it in contact with a methanolic solution of pyrene. This means that pyrene molecules were at the PEM interface as well as inside the PEM and the emission spectra averaged all environments faced by the pyrene. Here, DX is strictly localized to the layer where the DX/PSS complex has been deposited, probing just the polarity of the environment of that particular layer when no further layers were assembled on top of it, or of the inner regions of the PEMs as additional polyelectrolyte layers were further deposited. Tedeschi and coworkers [39] also assembled a layer of poly(acrylic acid) covalently labelled with 1-pyrenylmethylamine (PAA-MePY) onto the PAH/PSS film to sense the local polarity, obtaining values close to those shown for this probe in water. This result is probably due to the fact that PAA is a highly hydrated polymer and the pyrene senses the polarity of PAA and not of the PAH/PSS film.

For further insight into this particular issue, we also fabricated PEMs with PAA as the polyanion instead of PSS, but keeping PAH as the polycation and including DX to sense the

local polarity using the same approach described here for the PAH/PSS system. PAA was complexed with DX and assembled at either the 4th or the 8th layer position and additional PAH/PAA layers were assembled on top. After each layer deposition the steady state fluorescence of DX was recorded.

Differently from our experiments with PAH/PSS, a value for I_1/I_2 of 0.6 for the DX/PAA complex at the 4th layer without additional layers on top hints that DX is placed in an environment more polar than water. PAA is a highly hydrated polymer and at the pH of 6.5 employed for the assembly, it is reasonable to assume that the carboxylate groups are mostly deprotonated[57,58]. There is an increase in the I_1/I_2 ratio as the number of

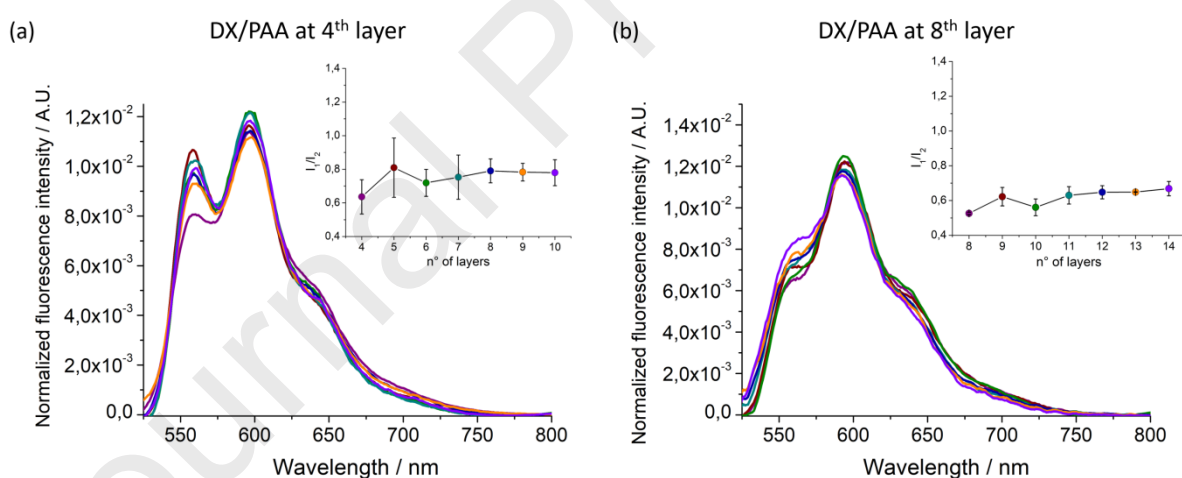


Figure 4. Normalized fluorescence spectra from DX/PAA complexes assembled at the (a) 4th layer position and (b) 8th layer position in PAH/PAA PEMs. The spectra correspond to PEMs with DX/PAA complexes as the top layer, and with additional layers on top of the DX/PAA complex, from 1 to 6 additional layers. The inset graphs show the I_1/I_2 ratio of DX localized at the 4th or 8th layer as a function of the number of layers deposited on top of the probing layer (error bars represent the standard deviation obtained by recording the spectra of different specimens; n= 3).

The spectra correspond to the I_1/I_2 ratio (number of layers on top of the DX/polyanion layer) shown in inset with the same colour as the spectra.

layers of PAH/PAA are assembled on top of the DX/PAA layer (Figure 4). The I_1/I_2 ratio then remains close to a value of 0.8 even after 6 layers assembled on top of the probing layer. For the PAH/PAA system, QCMD experiments showed a more slow growth of the PAH/PAA multilayer compared with the PAH/PSS, Figure S2. The assembly of PAA in multilayer is very dependent on the pH and at pH 6.5 we can expect the PAA molecules to be deprotonated highly hydrated and less prone to assembly as in the bulk they will be hydrated than in the PEM[59]. On total we observe an increase of frequency of 700 Hz for PAH/PAA while for the PAA PSS for the same number of layer there is an increase in 2000 Hz. The carboxylate groups of PAA are highly hydrated and it can be expected that they retain more water and also absorb

water molecules from the air than PSS. It seems reasonable that PAA would provide a polar aqueous environment for the DX in addition to the contribution to the polarity coming from the carboxylate groups of PAA to which DX is bound. Carboxylate groups in comparison with sulfonates, have a more localized charge that may also affect the polarity sensed by DX. For DX/PAA placed at the 8th layer the I_1/I_2 remains practically unchanged and close to 0.6, confirming an environment surrounding the DX that is more polar than water. The substantial difference in polarity in the PAH/PAA and PAH/PSS films must come from the high capacity of the carboxylate groups to tightly bind water[60,61], which is not so altered by the interaction with PAH for the carboxylate groups complexing DX.

From steady state fluorescence measurements however, it is not possible to discriminate if the DX faces more than one environment obtaining just an average spectral response. To get more insight into the environment probed by the DX in the different PEMs, fluorescence lifetime measurements were conducted. DX lifetime is extremely sensitive to the environment polarity. In water, DX fluorescence decays with a typical lifetime of 1.0 ns[62]; in apolar environments lifetimes can be as long as 4.3 ns[63] or as short as 170 ps in the densely packed cylindrical aggregates formed by DX at high salt and drug concentrations[64]. A multiexponential fluorescence decay of a dye implies its exposure to more than one environment with different polarity. The fitting of the decays makes it possible to obtain both the lifetimes and the relevant weight contribution of each component describing the decay. The fluorescence decay curves

measured for all the PEMs with DX are in any instance described by two exponential components. Due to the low amount of DX incorporated into the PEMs, the signal-to-noise ratio of the decays was not very high, preventing us from obtaining an accurate description of the fluorescence relaxation process of DX in the PEMs. Fitting of the fluorescence decay data was performed in two steps. In the first step, the data were fitted to the sum of two exponentials allowing all parameters to freely adjust to obtain the best χ^2 and randomly distributed residuals. Since the lifetimes relevant to the fast component obtained with this procedure did not differ too much one from the others, their values were averaged and assigned as a fixed parameter in the second fitting step, leaving free to adjust the lifetime of the slow component. To increase the statistic relevance of this analysis, PEMs were placed at different heights in the cell holder in order to probe different portion of each specimen (this is because the impinging laser covers a very tiny spot - 1 mm², approximately - of the PEM surface) and three measurements were taken for each sample. The presence of a fast component, with a lifetime slower than 1.0 ns suggests that a fraction of DX is in close contact with the charged groups (carboxylates or sulfonates for PAA and PSS, respectively) and the overall increase in the average lifetime, $\langle\tau\rangle$, hints at a reshaping of the environment of DX upon increasing the layers deposited on top of the DX/PAA or DX/PSS probing layer. The change in average life time for DX upon addition of polyelectrolyte layers for both DX at the 4th and 8 layer are shown in Figure 5 together with a representative fluorescence life time decay curve. The changes observed suggest that DX is experiencing a less polar environment upon increasing the layers deposited on top. In the case

of the PAH/PSS system, comparable results were obtained for samples where the probing DX/PSS layer was at the 4th or at the 8th layer position.

From the data of Figure 5 some conclusions can be drawn. We can indeed observe that the average DX lifetime increases with the number of layers on top of the probing layer. When DX/PSS is on top at the 4th layer, the average lifetime is shorter than when it is on top at the 8th layer. After deposition of 6 polyelectrolyte layers on top of DX/PSS, the situation reverses with the lifetime becoming longer for the DX/PSS complex at the 4th layer with 6 layers on top. The reason for a shorter DX average lifetime for the top 4th layer is probably a consequence of a less homogeneous polymer film than at the 8th layer, as described in the steady state fluorescence measurements.

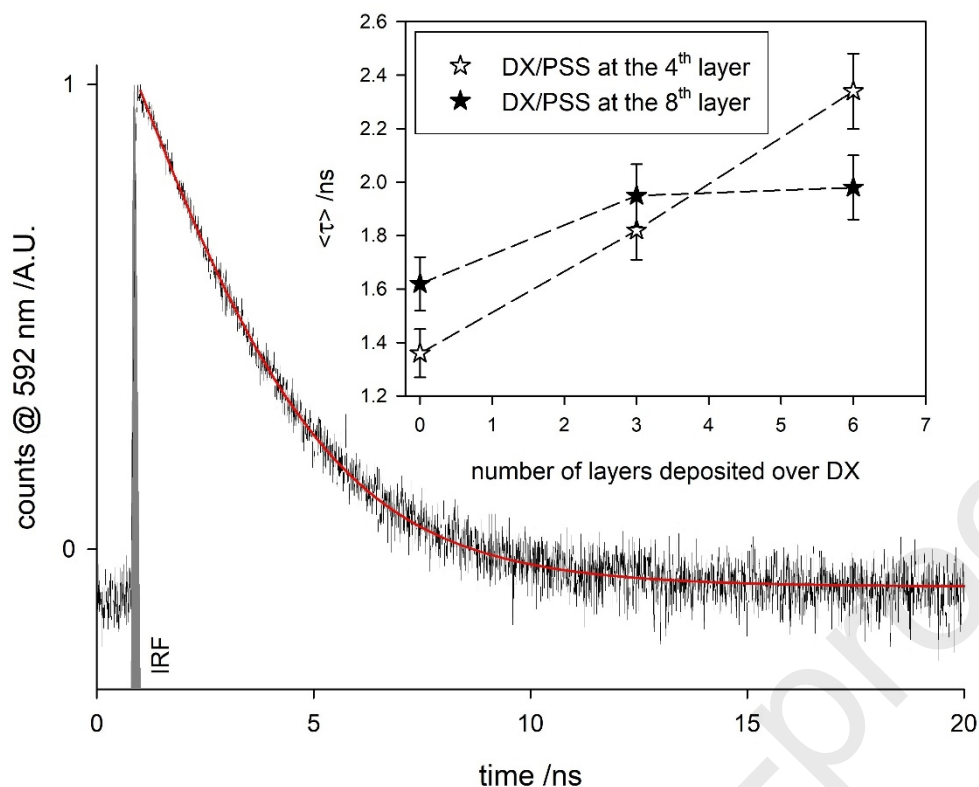


Figure 5. Characteristic lifetime decay curve corresponding to DX/PSS at the 4th layer coated with 6 polyelectrolyte layers on top. The inset shows the intensity weighted average lifetime as a function of the polymer layers deposited on top of the DX/PSS- layer for PAH/PSS PEMs (error bars represent the standard deviation - $n=3$ - obtained by measuring the same specimen but placed at different heights respect to the impinging laser beam).

The longer lifetime after depositing 6 layers on top of the DX/PSS probing layer, implies that the dye at the 4th layer faces a more apolar environment with respect to when it is located at the

8th layer, even after the assembly of the same number of additional layers, again in agreement with the results from steady state fluorescence.

Overall, the results from DX lifetime measurements are in good agreement with the DX steady state fluorescence, both revealing a polar environment for the top layer of the PAH/PSS PEMs and a largely more apolar environment for the inner regions of the PEMs.

DX lifetime was also measured for the PAH/PAA films, although due to a lower amount of DX deposited, a high quality fit could only be obtained for the PEMs with 6 layers on top of the DX/PAA layer. In both cases, for the DX/PAA complex at the 4th and 8th layer, the average DX lifetime value was below 1 ns, indicating that the DX/PAA complex is facing a more polar environment, again in agreement with the steady state fluorescence data.

Our results clearly show that polarity in the inner layers of the PEMs varies with the nature of the chosen polyanion. Top layers in the PEMs face polar environments resulting from uncompensated charges and the water associated to these charges. By choosing PAA as polyanion, a polymer capable of holding large amounts of water, a polar environment is retained in the layer formed by the DX/PAA complex even after assembly of 6 layers of PAH/PAA on top. For PSS/PAH PEMs we observe instead that the deposition of PAH/PSS layers on top of the DX/PSS layer results in a decrease in the polarity associated to the DX/PSS layer. Indeed, the top DX/PSS layer shows I_1/I_2 ratio of DX in water and after deposition of 6 polyelectrolyte layers on top, the ratio corresponds to an alkane solution, n-heptane. The

assembly of polyelectrolyte layers on top of the DX/polyanion will decrease the water associated with the anionic groups of PSS, creating a more organic environment through charge compensation and densification of the layers. Charge compensation is also taking place for PAA and PAH assembly, however, the carboxylate groups of PAA bear a large capacity to retain associated water as additional polyelectrolyte layers are assembled on top. The sketch in Figure 6 summarizes the findings of this work and our hypothesis. The choice of the polycation should also impact on the water retained in the layers and in the hydrophobicity of the DX/polyanion layer as different pairs of polyanion and polycations can result in different degrees of charge compensation, which in turn will affect the amount of water retained and hydrophobicity of the layer pairs. It can be expected that quaternary amines as in PDADMAC create a more hydrophobic environment than the primary amines of PAH, which can also interact with water through hydrogen bonding. Future studies varying the composition of the PEMs, both anionic and cationic polyelectrolytes, are needed to fully understand how the chemistry of polyelectrolyte and interaction between specific polycation and polyanion pairs affects polarity.

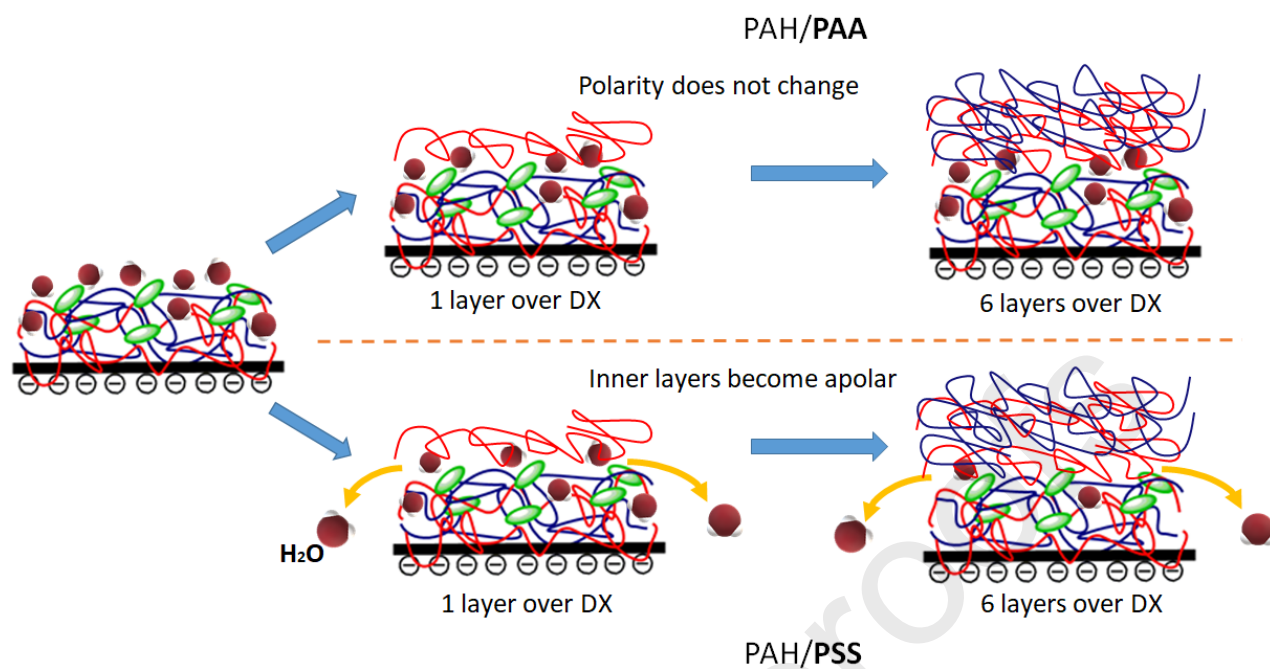


Figure 6 Sketch summarizing experimental findings and mechanistic explanations. PEMs with DX on the top layer (green ovals) retain water (red spheres). For PAH/PSS PEMs the addition of additional layers of PAH and PSS on top of DX triggers the removal of water from the PEM, resulting in more apolar environments for the inner layers of the PEMs compared with the top layer.- For PAH/PAA PEMs water is retained in the PEM after the addition of additional PAH and PAA layers and the inner layers keep a practically constant polarity.

Conclusions

By measuring the polarity of single polyelectrolyte layers at different positions in PEMs we have gained insight into the way the architecture of the multilayers changes during

polyelectrolyte assembly. For PAH/PSS PEMs, we have learned that the densification of the layers and charge compensation make the inner regions of the multilayers more apolar, while for PAH/PAA PEMs the highly hydrated PAA layers create a very polar environment that remains practically unchanged with the number of layers. In the case of PAH/PSS PEMs there are appreciable differences in polarity from the interface of the PEM with water when DX/PSS is the top layer facing an apolar environment, compared to when DX/PSS is located in the inner regions, where the environment is largely more apolar. In both PEM systems that we tested, PAH acts as the polycation; however by changing the polyanion, PSS or PAA, a dramatic difference in polarity inside the PEM is obtained. This polarity difference is due to differences in a number of properties of the PAH/PSS and PAH/PAA PEMs including the organization of the pair of polyelectrolytes, the packing of the layers, the water associated with the chains, and the electrostatic interactions. The combination of all these factors makes the inner layers of PAH/PSS apolar while those of PAH/PAA very polar.

There are few examples of polarity studies in PEMs in literature. Our work differentiates from previous studies of polarity of PEMs [39] in that here we were able of detecting polarity at the level of single polymer layers and tracing the evolution of polarity with the multilayer build up. Our work bring insight in the inner structure and properties of the PEMs, and how properties of the layers change during the assembly.

Future work will focus on the study of the polarity of other polyelectrolyte combinations in PEMs, i.e. using other polycations besides PAH, and polyanions and polycations from

biological origin, and to design PEMs with gradients of polarity in the vertical direction. We believe that these studies could be of relevance for nanofiltration and reverse osmosis applications of PEMs.

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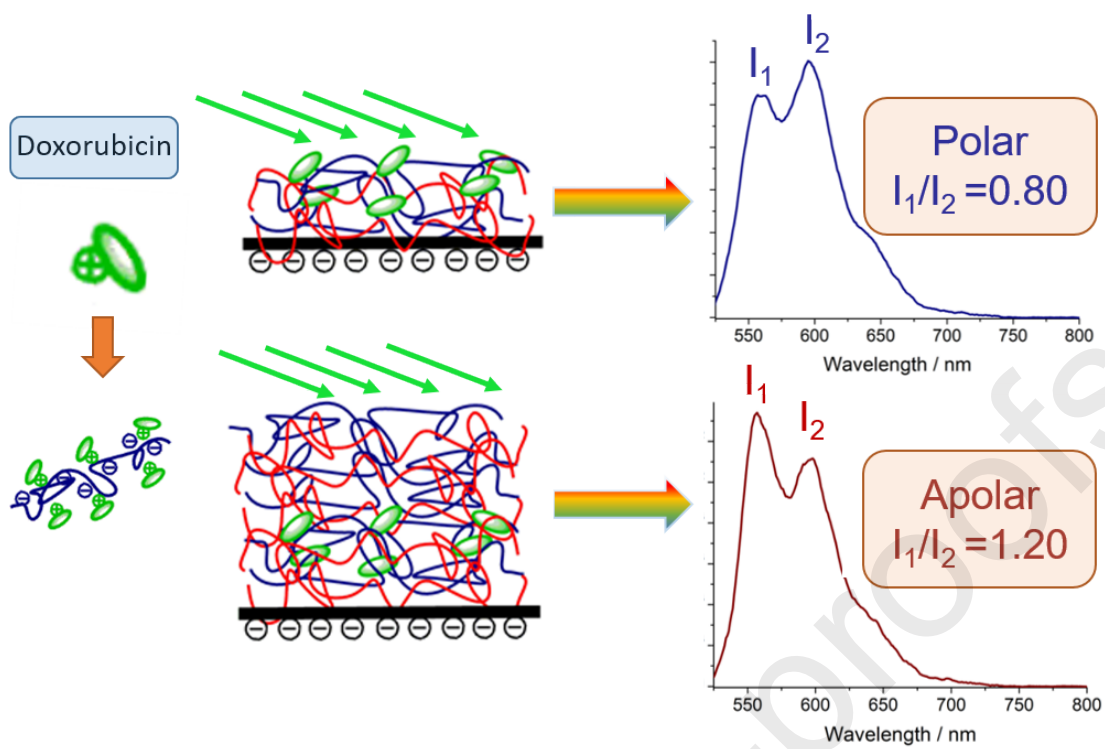
Hernan Martinelli: Investigation , Writing- Reviewing and Editing. **Elisamaria Tasca:** Investigation , Writing- Reviewing and Editing. **Patrizia Andreozzi:** Investigation. **Sara Libertone:** Investigation; **Hernan Ritacco:** Supervision. **Mauro Giustini:** Conceptualization, Writing- Reviewing and Editing. **Sergio E. Moya:** Conceptualization, Original draft preparation, Writing- Reviewing and Editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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