

## Multilayers Built from Two Component Polyanions and Single Component Polycation Solutions: A Way To Engineer Films with Desired Secondary Structure

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Polyelectrolyte multilayers were built up layer by layer from a polycation (poly(L-lysine) (PLL)) solution and polyanion solutions containing simultaneously poly(L-glutamic acid) (PGA) and poly(L-aspartic acid) (PAA). Quartz crystal microbalance-dissipation (QCM-D) measurements revealed that, for a given number of bilayers, the mass of the films is a nonlinear function of the composition of the polyanion build-up solution: it remains independent of the composition up to a PGA content of about 50% and increases strongly at higher PGA contents. The secondary structures of these polyelectrolyte multilayers were monitored by Fourier transform infrared (FTIR) spectroscopy in the attenuated total reflection mode. The “pure” PLL/PAA films exhibited a large extent of  $\beta$ -structure, characterized by a band at around 1609  $\text{cm}^{-1}$ . In contrast, no such  $\beta$ -structure elements were found in the “pure” PLL/PAA multilayers. The FTIR spectra of the polyelectrolyte multilayers constructed from PGA/PAA solutions of various compositions were analyzed by linear combinations of “pure” PLL/PAA and PLL/PAA film infrared spectra. It was found that the proportions of the two polyanions in the multilayers differed from those in the build-up solutions, with a preference toward the incorporation of PAA. At all film compositions, the  $\beta$ -sheet content was higher than expected on the basis of the assumption that PGA and PAA interact independently with PLL. This indicates a cooperative effect between PGA and PAA for the formation of  $\beta$ -sheets. This study may open up a route toward the utilization of the self-assembly of polyelectrolyte multilayers constructed from mixed polyanion and mixed polycation solutions, leading to films whose properties are directly related to the local interactions between the polyanions or the polycations constituting each layer of the film; such structures could not be attained by multilayers constructed from pure polyanion and polycation solutions.

### Introduction

By deposition of a polycation and a polyanion layer by layer on a charged surface, polyelectrolyte films called polyelectrolyte multilayers can be constructed.<sup>1</sup> This deposition process appears very versatile and allows the construction of films ranging in thickness from nanometers to microns. Since their discovery 10 years ago, many different polyelectrolyte multilayers have been reported, with potential applications in fields as different as electrooptical devices,<sup>2,3</sup> biomaterial coatings,<sup>4,5</sup> or drug delivery.<sup>6,7</sup> Through a change of the nature of the applied polyelectrolytes and/or the build-up conditions, the physico-chemical properties such as film thickness<sup>8,9</sup> or other properties (e.g. those affecting cellular adhesion) can be finely tuned.<sup>5,10</sup> The effects of parameters such as the pH or the ionic strength of the build-up polyelectrolyte solutions on the film structure have also been reported.<sup>9,11,12</sup> However, films have usually been constructed only from homo-polyanion and homo-polycation

solutions. One way to change the properties of the multilayers could be the use of mixtures of polyanions and/or polycations of different chemical natures during the build-up. The different constituents of the polyelectrolyte solutions could then interact upon being incorporated simultaneously into each polyanion/polycation bilayer. To the authors' knowledge only two studies report the use of mixed polyanion or polycation solutions. Leporatti et al. have constructed films using poly(styrene sulfonate)-chitosan sulfate (both being negatively charged) and poly(allylamine hydrochloride)-chitosan (both being positively charged) mixtures.<sup>13</sup> Very recently, Sui and Schlenoff reported the use of poly(diallyldimethylammonium chloride) mixed with poly(diallyldimethylammonium chloride)-*co*-sodium polyacrylate alternately deposited with poly(styrene sulfonate).<sup>14</sup> However, in both these studies, the authors neither investigated the effect of changing the mixture composition nor characterized the composition of the resulting multilayer film. The aim of this article is to provide a first systematic study of such multilayers constructed from a homo-polycation, poly(L-lysine) (PLL), solution and a hetero-polyanion solution containing simultaneously poly(L-glutamic acid) (PGA) and poly(L-aspartic acid) (PAA) where the properties of the film are varied continuously by changing the composition of the polyanion-

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containing solution. Moreover, attention is also focused on the influence of the composition of the polyanion build-up solution on the composition of the resulting polyelectrolyte film.

When polypeptides are applied as polyelectrolytes, films are obtained that have secondary structures similar to those observed in proteins.<sup>15,16</sup> Polyelectrolyte films with biologically relevant ( $\alpha$ -helical,  $\beta$ -sheet) structures on their surfaces could be very important in biomedical applications. However, the secondary structure of polypeptide films can be a source in itself of unique information concerning the local interactions between polyelectrolytes inside a multilayer.

Fourier transform infrared (FTIR) spectroscopy has been successfully used for the study of such film secondary structures<sup>16</sup> and also to determine the structural changes of proteins upon adsorption/embedding into different polyelectrolyte film architectures.<sup>17</sup> It has been shown in particular that films constructed by the alternate deposition of PLL and PGA are rich in  $\beta$ -structure,<sup>15</sup> similar to those of PLL/PGA complexes in solution.<sup>16</sup> In contrast with the complexes of PGA/PLL, those of PAA and PLL have no  $\beta$ -structure elements at all.<sup>18</sup>

The three-element system composed of PGA, PAA, and PLL was chosen to investigate the build-up and the properties of  $\{\text{PLL}/[c_1\text{PGA} + c_2\text{PAA}]\}_n$  multilayers constructed from a PLL polycation solution and a polyanion solution containing both PGA and PAA at the respective concentrations  $c_1$  and  $c_2$ . In the present paper, using the quartz crystal microbalance-dissipation (QCM-D) technique, we analyze the film build-up for various polyanion compositions. With FTIR spectroscopy in the attenuated total reflection mode (FTIR-ATR), it is demonstrated that the polyanion compositions of the films are different from those of the build-up solutions and that there is a preference toward the integration of PAA into the films. We also show that there is a deviation from linearity as regards the  $\beta$ -sheet content as a function of the polyanion composition, in contrast with what would be expected if PGA and PAA interacted with PLL in the multilayer absolutely independently.

## Materials and Methods

**Construction of the Polyelectrolyte Multilayers.** All experiments were performed in tris(hydroxymethyl)aminomethane (Tris, 10 mM, Gibco BRL, cat. no. 15504-020) and sodium chloride (NaCl, 0.15 M) buffer at pH 7.4. Millipore filtered water (Milli Q-plus system) or deuterium oxide (99.9% D, Aldrich, cat. no. 15,188-2) were used as solvents. Poly(ethyleneimine) (PEI, Aldrich, cat. no. 18,197-8, MW = 750 000  $\text{g}\cdot\text{mol}^{-1}$ ) was adsorbed first, for 10 min from a 5  $\text{mg}\cdot\text{mL}^{-1}$  solution onto the substrate. After 10 min of rinsing, polyanions were deposited by submerging the substrate for 10 min into a solution containing  $x \text{ mg}\cdot\text{mL}^{-1}$  of poly(L-glutamic acid) (PGA, Sigma, P-4886, MW = 17 000  $\text{g}\cdot\text{mol}^{-1}$ ) and  $(1 - x) \text{ mg}\cdot\text{mL}^{-1}$  of poly(L-aspartic acid) (PAA, Sigma, P-6762, MW = 35 400  $\text{g}\cdot\text{mol}^{-1}$ ). After another rinsing, the substrate was submerged for the same period of time into the polycation solution containing 1  $\text{mg}\cdot\text{mL}^{-1}$  of poly(L-lysine) (PLL, Sigma, P-2636, MW = 32 600  $\text{g}\cdot\text{mol}^{-1}$ ). This procedure was repeated until the architecture  $\text{PEI}/[x\text{PGA} + (1 - x)\text{PAA}]-\{\text{PLL}/[x\text{PGA} + (1 - x)\text{PAA}]\}_5$  was attained. Such a film will be denoted throughout the paper as  $\{\text{PLL}/[x\text{PGA} + (1 - x)\text{PAA}]\}_5$ .

**Quartz Crystal Microbalance.** The measurements were performed with a quartz crystal microbalance-dissipation (QCM-D) system from Q-Sense (Göteborg, Sweden). Briefly, in QCM-D the changes in the resonance frequency ( $-\Delta f$ ) of a quartz crystal are measured when material is adsorbed onto it from a solution. The crystal is excited at its fundamental

frequency (about 5 MHz), and observations are made at the 3rd, 5th, and 7th overtones ( $\nu = 3, 5$ , and 7) at 15, 25, and 35 MHz, respectively.<sup>19–22</sup> For rigid films,  $-\Delta f/\nu$  is independent of the overtone number  $\nu$ . According to the Sauerbrey relation,<sup>23</sup>  $-\Delta f/\nu$  can be related directly to the total mass of the film. This mass includes not only that of the deposited material but also that of the water bound to the layer.

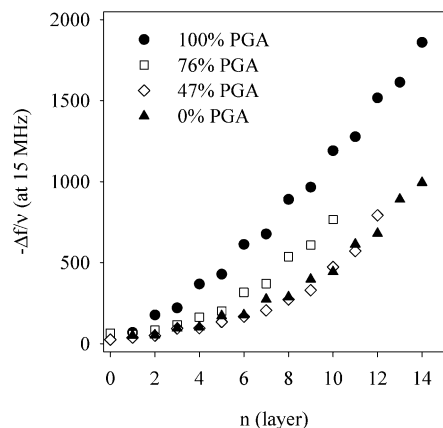
The details of the experimental setup have been described previously.<sup>24</sup> The crystal was coated with a  $\approx 100 \text{ nm}$  thick  $\text{SiO}_2$  film deposited by active sputter-coating. Before use, the crystals were cleaned at room temperature with Hellmanex 2% (v/v) during 45 min, with 0.1 M hydrochloric acid during 10 min, and finally with buffer. To build up the multilayers, 2 mL of the polyelectrolyte solutions or of the buffer was passed through a thermostated loop to set the temperature of the injected fluid to  $25.0 \pm 0.1^\circ\text{C}$  before it came into contact with the sensing crystal. The volume of the adsorption cell was 100  $\mu\text{L}$ , and the injection lasted for about 1 min. The polyelectrolyte solution or the buffer was then left at rest in the adsorption cell during 9 min before a new solution was injected. In all cases, this time was sufficient for steady-state frequency shifts to be reached.

**Infrared Spectroscopy in the Attenuated Total Reflection Mode.** For FTIR measurements, the  $\text{PEI}/[x\text{PGA} + (1 - x)\text{PAA}]-\{\text{PLL}/[x\text{PGA} + (1 - x)\text{PAA}]\}_5$  films were constructed on the surface of an internal reflective element, a ZnSe crystal in our case. To “clear” the amide I region of the strong water band at around 1643  $\text{cm}^{-1}$ ,  $\text{D}_2\text{O}$  (having the corresponding band at around 1209  $\text{cm}^{-1}$ ) was used as a solvent in all FTIR experiments. Otherwise, the films were built up as described above.

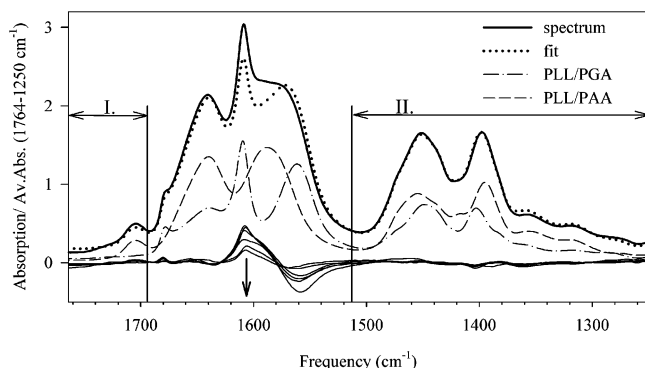
Each step of the film construction was monitored by FTIR spectroscopy with an Equinox 55 (Bruker, Karlsruhe, Germany) spectrometer. The films were constructed by allowing the polyelectrolytes to adsorb onto the ZnSe crystal from solutions circulating above the crystal surface. The crystal was constantly thermostated in the spectrometer. After a layer had been completed and rinsed, a single beam FTIR spectrum was recorded with a liquid nitrogen-cooled MCT detector, at a spectral resolution of 2  $\text{cm}^{-1}$  by averaging 512 scans. The absorption spectra of any desired layers were calculated from the single beam spectra recorded before (background) and after (sample) the layer(s) of interest. Data processing and spectrum analysis was performed by using the software SPSERV (Dr. Cs. Bagyinka, Biological Research Center, Szeged, Hungary).

## Results

**Build-up of the Multilayers.** The QCM-D measurements demonstrated that polyelectrolyte films can be constructed by the alternate dipping of a crystal into a cosolution of two different polyanions and into a solution of a polycation. Figure 1 reveals that, at any  $x$  in the range from 0 to 1  $\text{mg}\cdot\text{mL}^{-1}$ , a  $\text{PEI}/[x\text{PGA} + (1 - x)\text{PAA}]-\{\text{PLL}/[x\text{PGA} + (1 - x)\text{PAA}]\}_m$  film could be constructed and also that  $-\Delta f/\nu$  (for a given number  $n$  of layers) does not really depend on the composition of the  $[x\text{PGA} + (1 - x)\text{PAA}]$  polyanion mixtures for  $x < 0.5$ . At  $x > 0.5$ , the resonance frequency shifts increase with increasing amounts of PGA in the PGA/PAA polyanion mixtures used to construct the films. The QCM-D technique can additionally provide information on the nature of the adsorbed films. We found that, for all values of  $x$ , the evolution of  $-\Delta f/\nu$  with the number of deposited layers is fairly independent of the overtone number  $\nu$ . This is a sign of rigid films on the crystal surfaces and indicates that, to a first approximation,  $-\Delta f/\nu$  is proportional to the total mass of the films.<sup>23</sup>



**Figure 1.** Changes in the resonance frequency measured by QCM-D at the third overtone ( $\nu = 3$ ) as a function of the number  $n$  of deposited layers of PLL and  $[x\text{PGA} + (1-x)\text{PAA}]$ , with  $x$  corresponding to the PGA concentration of the polyanion build-up solutions. The even integers (with the exception of 0, which corresponds to the adsorption of PEI) correspond to the adsorption of PLL, and the odd integers, to the adsorption from the polyanion mixture.

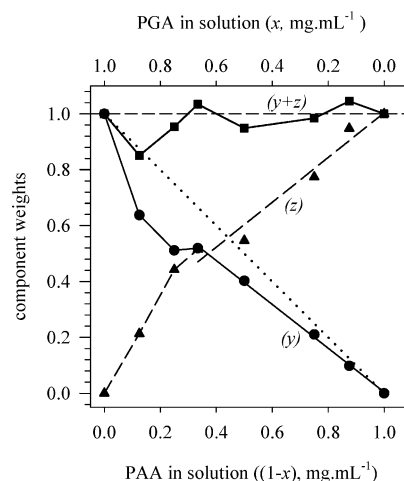


**Figure 2.** Infrared absorption spectra of  $\{\text{PLL}/[0.5\text{PGA} + 0.5\text{PAA}]\}_5$ ,  $\{\text{PLL}/\text{PGA}\}_5$ , and  $\{\text{PLL}/\text{PAA}\}_5$  films. Regions I and II were used to fit the film spectra by a linear combination of the  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$  spectra. The bottom curves indicate the differences between the measured film spectra and the best linear combination fits. From the top to the bottom (indicated by the arrow), the difference spectra correspond to  $\{\text{PLL}/[x\text{PGA} + (1-x)\text{PAA}]\}_5$  films with  $x = 0.87, 0.75, 0.66, 0.50, 0.25$ , and  $0.12$ , respectively.

### Secondary Structure of the Mixed Polyanion Multilayers.

The secondary structure of the poly(amino acid)-based polyelectrolyte films was determined by means of FTIR spectroscopy in the ATR mode. First, to determine the contributions of PGA and PAA to the films, we used a linear combination of the FTIR absorption spectra of “pure”  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$  multilayers to fit the spectrum of the actual film. These spectra were recorded as part of the  $[x\text{PGA} + (1-x)\text{PAA}]$  mixtures series, at  $x = 0$  and  $1 \text{ mg}\cdot\text{mL}^{-1}$  concentrations. This approach is based on the assumption that, due to their strong chemical similarity, there is not an appreciable interaction between PGA and PAA (the side chains differ only in their length by one  $\text{CH}_2$  group).

We did indeed find large frequency regions ( $1764\text{--}1694$  and  $1510\text{--}1250 \text{ cm}^{-1}$ ) where such linear combinations correctly approximate the spectra of all the  $\{\text{PLL}/[x\text{PGA} + (1-x)\text{PAA}]\}_5$  films (Figure 2). We refer to these as “insensitive” regions, in contrast to the region  $1694\text{--}1510 \text{ cm}^{-1}$ , where the spectrum depends on the composition of the polyanion build-up solution. We verified that in the insensitive regions the spectra of the  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$  films are linearly independent in a mathematical sense. In Figure 2, as an example,



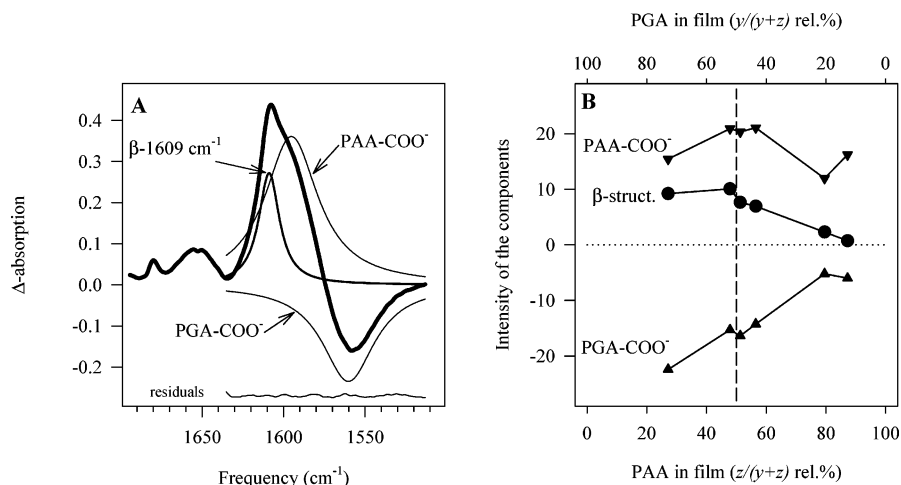
**Figure 3.** Weights  $y$  of  $\{\text{PLL}/\text{PGA}\}_5$  (●), and  $z$  of  $\{\text{PLL}/\text{PAA}\}_5$  (▲) spectra in  $\{\text{PLL}/[x\text{PGA} + (1-x)\text{PAA}]\}_5$  films as a function of the PGA concentration ( $x$ ) in the polyanion build-up solutions. The upper curve (■) is the sum of the two weights ( $y+z$ ). The dotted line represents the theoretical evolution of  $y$  with  $x$  if PGA and PAA were incorporated in the films at the same proportion as that in which they are present in the build-up solutions. Ideally,  $y+z$  should be equal to 1.0. In all cases, this sum lies between 0.85 and 1.05, and in most cases, it lies between 0.95 and 1.05. Hence, the error bars made in the linear combination procedure to yield the component weights of PGA and PAA in the film are of the order of  $\pm 5\%$ .

the FTIR-ATR spectrum of the  $\{\text{PLL}/[0.5\text{PGA} + 0.5\text{PAA}]\}_5$  film is depicted; the residuals (bottom curves) show the high-quality of the fits over the two “insensitive” regions. The weights  $y$  and  $z$  of the components  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$ , respectively, found by fitting the  $\{\text{PLL}/[x\text{PGA} + (1-x)\text{PAA}]\}_5$  spectra, allow an estimate of the actual proportions of PGA and PAA in the films. These weights are given in Figure 3 as a function of  $x$  (the concentration of PGA, expressed in  $\text{mg}\cdot\text{mL}^{-1}$ , in the polyanion solution). If the fits were perfect (i.e. the adsorptions of PGA and PAA onto PLL were completely independent of each other), the sums of the two weights should be exactly 1. This is almost always the case, as shown in the upper part of Figure 3.

The weights of the two components vary continuously with  $x$ , confirming the incorporation of both polyanions into each layer of the multilayer; that is, it is possible to construct multilayers with different PGA and PAA compositions by simply changing their proportions in the polyanion solution used for the build-up of the films. Nevertheless, it should be noted that the actual ratios of PGA and PAA in the films differ from those in their mixed solutions used to construct the films. The most important feature that emerges out of Figure 3 is that, for all values of  $x$ , the proportion of PGA incorporated in the multilayer always lies below the straight line of slope 1.0. This line would be expected if PGA and PAA were incorporated in the film in the same proportions as those in which they are present in the polyanion build-up solutions. This clearly shows the preference toward the incorporation of PAA over PGA. This preference is especially pronounced in the composition range rich in PGA (for  $x > 0.7$ ). The preference toward PAA incorporation is also supported by the fact that the straight lines fitted for the weights  $y$  and  $z$  for  $0.7 > x > 0$  cross each other at about  $x = 0.65$ , instead of at the theoretical value  $x = 0.5$ .

In the spectrum region sensitive to the film composition ( $1694\text{--}1510 \text{ cm}^{-1}$  in Figure 2), however, there are large deviations from the simple linear combinations that would be expected if the PGA and PAA chains interacted independently

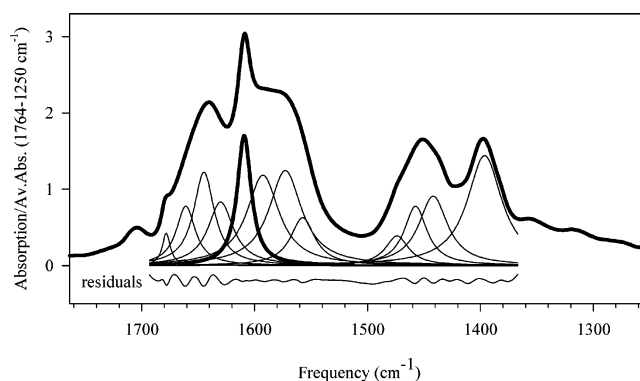




**Figure 4.** (A) Decomposition of the secondary structure-sensitive region of a  $\{\text{PLL}/[x\text{PGA} + (1-x)\text{PAA}]\}_5$  film residual spectrum, obtained by fitting with the sum of the pure  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$  absorption spectra as shown in Figure 2. The thick line shows the residual spectrum of a film made from the  $x = 0.5$  solution. Thin lines indicate the component bands. (B) Evolution of the intensity component bands in the residual spectra as a function of the PGA/PAA composition in the polyelectrolyte films. The dashed line indicates the composition, which is at the border of the two regimes. To the left of this composition, the  $\beta$ -structure prevails; to the right, it disappears. For details, see the text.

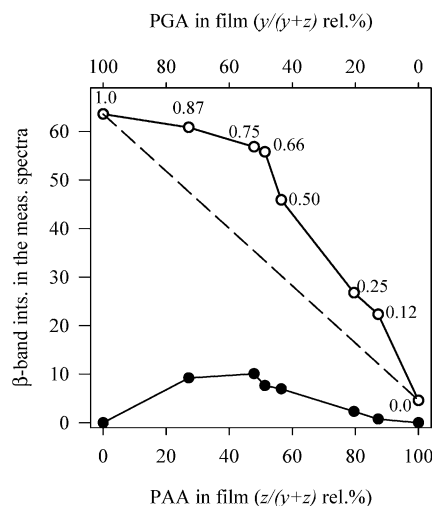
with PLL in the film. These deviations decrease with decrease of the PGA content in the polyelectrolyte films; they are depicted at the bottom of Figure 2. The origin of these deviations can be understood by decomposing the residual spectra into their component bands assuming Lorentzian shapes (Figure 4A). The decomposed ( $1640\text{--}1510\text{ cm}^{-1}$ ) residual spectra gave a negative band at around  $1560\text{ cm}^{-1}$ , a positive one at around  $1595\text{ cm}^{-1}$ , and a narrow positive band at around  $1609\text{ cm}^{-1}$ . By comparison with the  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$  film spectra, the bands at around  $1560\text{ cm}^{-1}$  and at  $1595\text{ cm}^{-1}$  can be assigned to the  $\text{COO}^-$  groups of PGA and of PAA, respectively,<sup>25</sup> and the band at around  $1609\text{ cm}^{-1}$  to  $\beta$ -structures similar to those found in denatured and aggregated proteins.<sup>26,27</sup> The presence of two other bands in the residual spectrum may be noted, one broad band at around  $1650\text{ cm}^{-1}$ , corresponding to  $\alpha$ -helices and random and turn structures, and another band at around  $1680\text{ cm}^{-1}$ , which, when simultaneously present with the band at  $1609\text{ cm}^{-1}$ , is also typical of  $\beta$ -structures.<sup>27</sup> The contribution of the band centered at around  $1609\text{ cm}^{-1}$  is always positive (Figure 4B), indicating that the  $\beta$ -sheet contents are, for all film compositions, “above” the PGA/PAA stoichiometry. In Figure 4B, the evolutions of the intensities of the two other component bands of the residual spectra at around  $1560$  and  $1595\text{ cm}^{-1}$  are also depicted as a function of the polyanion composition of the films. The positive contribution of the  $1595\text{ cm}^{-1}$  PAA- $\text{COO}^-$ -related band indicates that the simultaneous presence of PGA and PAA in the films induces an increased dissociation of the carboxylic groups of the PAA side chains. The negative band at  $1560\text{ cm}^{-1}$ , assigned to PGA- $\text{COO}^-$ , shows that the enhanced PAA integration into the bilayers was at the cost of that of PGA. Both component bands diminish in absolute value with decreasing concentrations of PGA in the films, and this phenomenon therefore correlates with the decrease in the amount of the “additional”  $\beta$ -sheets in the same films. An advantage of this approach is that the fit is simple: only three component bands are to be taken into account.

Another approach with the aim of investigating how the secondary structure of these polyelectrolyte multilayers changes as a function of the PGA/PAA concentration is to decompose their entire measured FTIR spectra directly into component bands. Such a decomposition is shown in Figure 5 for a  $\{\text{PLL}/[0.5\text{PGA} + 0.5\text{PAA}]\}_5$  film where each component band was again assumed to be a Lorentzian curve.



**Figure 5.** Decomposition of the infrared spectra of polyelectrolyte films made from PGA-PAA mixtures. Each spectrum was normalized with its own integral between  $1764$  and  $1250\text{ cm}^{-1}$ . Here, as an example, the fit of the  $\{\text{PLL}/[0.5\text{PGA} + 0.5\text{PAA}]\}_5$  film (upper curve with thicker line) is shown. Initial values of the frequencies were determined from the second derivative of the spectra. A detailed description of the fitting procedure is given in ref 17. The component bands were all assumed to be Lorentzian curves. The component band drawn with the thicker line represents the  $\beta$ -structure-related component at around  $1609\text{ cm}^{-1}$ . The intensity (total area) of this component is plotted in Figure 6 as a function of the PGA concentration in the polyelectrolyte films.

The results of such fits are not necessarily unequivocal, since the set of Lorentzian components is not an orthogonal system. However, these spectra featured a narrow, well-separated band at around  $1609\text{ cm}^{-1}$  (assigned to intermolecular  $\beta$ -structures).<sup>27</sup> Its contribution can therefore be determined with confidence, and attention was focused only on this band. The intensity of the  $1609\text{ cm}^{-1}$  band is displayed in Figure 6 as a function of the PGA proportion in the polyelectrolyte films. It can be seen that the  $\beta$ -structure content of the films varies continuously, but not linearly with the proportion of PGA in the film, between the high value characteristic of  $\{\text{PLL}/\text{PGA}\}_5$  films and zero for  $\{\text{PLL}/\text{PAA}\}_5$ . For all films, the  $\beta$ -sheet content is larger than would be expected if PGA and PAA interacted independently with PLL. The  $\beta$ -structure content, as a function of PAA concentration in the films, can be divided into two regions: (i) in the interval from about 100% to 50% of PGA in the films, the secondary structure hardly changes, whereas, (ii) between 50% and 0% PGA, the  $\beta$ -structures disappear in a roughly linear manner.



**Figure 6.** Intensities of the  $1609\text{ cm}^{-1}$  intermolecular  $\beta$ -structure-related component band (○) calculated from the measured spectra (as shown in Figure 5) of the  $\{\text{PLL}/[x\text{PGA} + (1-x)\text{PAA}]\}_5$  polyelectrolyte films, and intensities of the same band calculated from the residual spectra (●) (as shown in Figure 4) obtained after the subtraction of the linear combination of  $\{\text{PLL}/\text{PGA}\}_5$  and  $\{\text{PLL}/\text{PAA}\}_5$  from the film spectra. The dashed line corresponds to the expected  $\beta$ -sheet content if PGA and PAA interacted independently with PLL in the films. The numbers at the data points indicate the original PGA concentrations ( $x$ ) in the build-up solutions.

## Discussion

With the use of two independent techniques, QCM-D and FTIR spectroscopy, it has been found that, through the alternation of mixed solutions of PGA and PAA polyanions and a solution of the polycation PLL, stable polyelectrolyte multilayers can be constructed. QCM-D experiments revealed that the mass built into the multilayer is a nonlinear function of the compositions of the polyanion build-up solutions. Clearly, there is a change in the film build-up at around the  $\{\text{PLL}/[0.5\text{PGA} + 0.5\text{PAA}]\}_5$  architecture. Below  $x = 0.5$ , the total mass of the multilayers does not depend on the PGA/PAA ratio in the build-up solution. Above  $x = 0.5$ , the total mass increases strongly with increasing PGA content in the polyanion solution.

FTIR experiments confirmed the incorporation of both polyanions into the polyelectrolyte multilayers. In addition, they permitted determination of the polyanion compositions and the secondary structures of the films. The relative amounts of PGA and PAA incorporated in the films were not a linear function of their concentrations in the build-up solutions. It seems that, when small amounts of PAA are introduced into the solutions, the PGA-dominated films incorporate higher than proportional amounts of PAA (Figure 3). At the same time, however, the secondary structures of the films did not change appreciably and the intermolecular  $\beta$ -structures, characteristic of pure PLL/PGA films, prevailed (Figure 6). The amount of PAA present in the films has to reach a critical value, above which the organization of the polyelectrolyte films seems to be markedly different from that in the previous regime. This critical PAA proportion is around 50% (Figure 6); above it the relative amounts of PGA and PAA in the films are linear functions of their concentrations in the build-up solutions (Figure 3) and the content of intermolecular  $\beta$ -structures in the films decreases linearly (Figure 6).

The very different natures of the QCM-D and the FTIR measurements do not facilitate direct comparisons. Nevertheless, it is noteworthy that both techniques point to the same critical PGA/PAA ratio at which changes take place in the evolution

of the multilayer build-up and film structure. It is even more remarkable that while the mass of the film changes strongly in the range 100–50% PGA content, in the same range the  $\beta$ -structure content is virtually constant. This latter phenomenon is possible only if PAA molecules (which alone do not form  $\beta$ -structures with PLL) are forced to do so when they are incorporated in PGA-rich  $\beta$ -sheets. This hypothesis seems to be supported by the fact that the incorporation of PAA chains into the films leads to an increase in the dissociation of their carboxylic groups, while the dissociation of those of the PGA chains decreases (Figure 4). Such a situation is in accordance with the conclusions of Domard and Rinaudo, who suggested that the interactions between PGA and PLL chains induce the formation of  $\beta$ -sheets where the positive charges of PLL are stabilized by the negative charges of the carbonyl groups of PGA.<sup>28</sup> Thus, the acidic groups involved in the maintenance of the  $\beta$ -structure should be in dissociated forms. Accordingly, the increased amount of  $\text{PAA-COO}^-$  groups and the nonchanging level of the  $\beta$ -structure content indicate that, indeed, PAA is incorporated into the PGA/PLL  $\beta$ -sheets at PGA contents of 100–50% in the films. The PGA content is then high enough to “saturate” the film with  $\beta$ -sheets. When the PAA content exceeds that of PGA in the film, PLL/PGA can no longer form a continuous network. The PLL/PGA matrix probably breaks into isolated domains, and on the further decrease of PGA in the films there is a linear (simple dilution-like) disappearance of the PLL/PGA domains and the  $\beta$ -structures with them (Figures 3 and 6). It is reassuring that the existence and the disappearance of the additional  $\beta$ -structure content calculated from the residual spectra (i.e. from the deviation from linearity, due to the interacting PAA and PGA) follow the same trend as the difference between the top curve in Figure 6 and the theoretical straight line corresponding to the  $\beta$ -sheet content if PGA and PAA interacted independently.

In conclusion, the present study has demonstrated that it is possible to build up stable polyelectrolyte films by using solutions containing simultaneously two polyanions and a “pure” polycation solution. We found that the proportions of the two polyanions in the film and the  $\beta$ -sheet content vary continuously with the composition of the polyanion solution used for the film build-up. In this way, the film properties can be tuned simply by changing the composition of the polyanion solution. However, the two polyanions are not incorporated into the film in the same proportion as that in which they are present in the solution. Cooperative effects must exist between the polyanions, leading to larger  $\beta$ -sheet contents than expected from the composition of the film on the assumption that the polyanions interacted independently with the polycation. This work shows that the self-assembling capacity of polyelectrolyte multilayers that they acquire from the presence of more than one polyanion in the build-up solutions could lead to properties which might not be attained by multilayers constructed only from one type of polyanion and one polycation. The introduction of different combinations for both the polyanions and the polycations may open up new possibilities in the design of polyelectrolyte multilayers with tailored features.

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