

Layer-By-Layer Assembly of Gelatin

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ABSTRACT: Electrostatic assembly of one species can be realized using gelatin as a polyampholyte. Under suitable conditions where the electrostatic attraction and repulsion were both significant and in balance, linear growth of multilayers driven by electrostatic interactions was sustained over many successive assembly steps, and the maximum amount of adsorption of each layer was reached when the solution pH was around the isoelectric point. The rearrangement of the adsorbed chains after drying was confirmed by contact angle analysis. In addition with only one species involved, the assembled thin films should be chemically uniform rather than layered.

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INTRODUCTION

Gelatin is a polypeptide produced by acid or alkali denaturation of collagen and has been utilized extensively in food, pharmaceutical, and photographic industries.¹ It has many attractive properties, such as biodegradability, hydrophilicity, nontoxicity, and biocompatibility, and as a result it has been used in modification of various surfaces, such as the surfaces of electronic components,² functional membranes,³ and tissue engineering scaffolds.^{4–6} The adsorption behavior of gelatin is critical in these applications. On the other hand, gelatin is known as a typical example of polyampholytes with a nonuniform distribution of amino acids, containing both positively charged polar amino acids (arginine, lysine) and negatively charged amino acids (glutamate, aspartate) in addition to hydrophobic and hydrophilic neutral amino acids.^{7,8} Depending on the pH of the medium, gelatin can exhibit

properties of either a polyanion or a polycation, the net charge of the macromolecule being negative or positive, respectively. Therefore, its adsorption behavior is particularly interesting because the structure of the adsorbed layer varies with the pH, temperature, ionic strength, and concentration of the solution.^{7–9} A large amount of literature is available on the adsorption of gelatin.^{7–12} In one of the early studies, Curme and Natale¹⁰ investigated the influence of the ionizing groups on the adsorption of gelatin onto silver bromide sol and concluded that the gelatin adsorption reaches maximum around its isoelectric point (IEP). Kamiyama and Israelachvili studied gelatin adsorption on mica and showed that the primary driving force is electrostatic interactions.⁸ Recently, we demonstrated that gelatin can adsorb to either positively or negatively charged substrates depending on the pH of the gelatin solution.⁶ All these articles in the literature involved the adsorption of a single gelatin layer to a well-defined charged substrate.

Recently, layer-by-layer electrostatic deposition technique has attracted tremendous interest and has been used extensively in the con-

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struction of multilayer thin films from various charged species.^{13–15} The process involves at least two oppositely charged species assembled in an alternating fashion driven by electrostatic attraction and by repeating the cycle thin films with hundreds of layers can be assembled. Based on the same principle it is apparent that electrostatic assembly of species with same charge will fail. Drying after the deposition step can promote the adsorption driven by hydrophobic effect.¹⁶ However, for assemblies driven by electrostatic interactions, the effect diminishes quickly in several cycles.¹⁷ Other reports also concluded that extended growth of multilayers based on species of same charge is not possible.^{18,19}

In this study, using gelatin as the single charged species, we investigated its layer-by-layer assembly process and its dependence on the solution pH. We found that linear growth of gelatin multilayers driven by electrostatic interactions can be sustained over many successive assembly steps when the gelatin pH is close to its IEP.

EXPERIMENTAL

Materials

Poly(sodium 4-styrenesulfonate) (PSS) ($M_w = 7 \times 10^4$) and poly(diallyldimethylammonium chloride) (PDDA) ($M_w = 1 \times 10^5 \sim 2 \times 10^5$) were purchased from Aldrich. The gelatin used in this study from bovine skin was purchased from Sigma. All these chemicals were used as received without further purification. Water was purified using a Millipore Milli-Q system (18.2 M Ω).

Quartz Crystal Microbalance

AT-cut quartz crystals with a parent frequency of 9 MHz were purchased from Beijing Zi Wei Xing Microelectronics Co. The crystals (9-mm diameter) were coated on both sides with silver electrodes 4.5-mm in diameter. The frequency was monitored by a Protek frequency counter (Model C3100). The amount of gelatin adsorbed, Δm , was calculated from the frequency decrease of the quartz crystal microbalance (QCM), Δf , using Sauerbrey's equation²⁰ as follows:

$$-\Delta f = \frac{2f_0^2}{A\sqrt{\rho_q\mu_q}} \times \Delta m$$

where f_0 is the parent frequency of the QCM (9×10^6 Hz), A the electrode area (0.159 cm²), ρ_q

the density of the quartz (2.648 g/cm³), and μ_q the shear modulus (2.947×10^{11} dyn/cm²). This equation is reliable when the QCM measurement is carried out in air,²¹ which was the case in this study.

Gelatin Adsorption to the QCM Electrodes

To maintain a consistent surface condition, the QCM electrodes were first primed with three PDDA/PSS bilayers with a PSS outermost layer before gelatin deposition, following a procedure reported in the literature.²² Then the QCM electrodes were immersed into an aqueous gelatin solution of different pH and 1.0 mg/mL concentration for 20 min, taken out, rinsed with water for several tens of seconds, and dried with a stream of nitrogen. We studied the adsorption kinetics and found that in general the amount of gelatin adsorbed reaches a plateau within 5 min at various pH. Therefore, we chose 20 min deposition time for all the gelatin adsorption experiments. The gelatin adsorption cycle was repeated until the desired number of layers was reached. Three replicate electrodes were used for each pH value. Each experiment was performed at least twice, and the mean value and the standard deviation were reported. All experiments were carried out at room temperature.

Surface Analysis

Water contact angles were measured at room temperature using a sessile drop technique on a KRUSS DSA1 v 1.80 Drop Shape Analyzer using Milli-Q water as the probe fluid. The gelatin films were dried under vacuum at room temperature for 24 h before the contact angles were measured. Surface morphology was assessed using a Seiko SPA300 atomic force microscope (AFM) operating in the tapping mode.

RESULTS AND DISCUSSION

Figure 1 shows the frequency shift of the QCM crystal against the number of gelatin assembly cycles at different pH. The resonance frequency decreases with the increasing assembly step, indicating stepwise gelatin deposition. The amount of adsorption for each step is strongly dependent on the solution pH (details will be discussed later). Because gelatin is a weak amphoteric polyelectrolyte, the local charge density

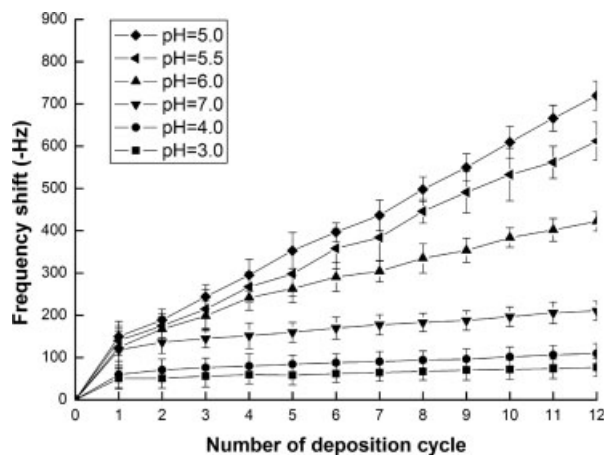


Figure 1. The gelatin assembly at different pH monitored by QCM. The error bars represent the standard deviations.

and the net charge of which are decided by the solution pH, the strong dependence of the adsorption on the pH indicates that the major driving force for the gelatin adsorption is electrostatic interactions rather than hydrophobic effect. This also agrees with the finding reported in the literature.⁸ Yet, when the solution pH is near the isoelectric point (IEP) of the gelatin, as we see in Figure 1, pronounced and linear growth of gelatin is maintained over many assembly steps (we only show data of up to 12 steps), even though the molecules to adsorb carry a net charge of the same type as the substrate. This can be attributed to the amphoteric nature of the gelatin molecule. A typical polyelectrolyte used in layer-by-layer electrostatic assembly studies carry only one type of charge, the charge density of which may be regulated by solution pH if it is a weak polyelectrolyte, which can adsorb to an oppositely charged substrate, but then the only electrostatic interaction between the adsorbed chains and the chains in the solution for next deposition is repulsion. Drying after the deposition step was found to promote the adsorption of an additional amount of the same polyelectrolyte on reimmersion, which has been attributed to a reorganization of the last adsorbed layer, but the effect diminishes quickly in several cycles,¹⁷ and the maximal amount of total adsorption thus is no more than twice that of a single layer.¹⁸

For a polyampholyte such as gelatin, however, at a pH below or above the IEP, even though the whole molecule carries a net charge, there are simultaneously local positively charged groups

as well as negatively charged groups on the gelatin chain. Hence, both electrostatic attraction and repulsion are involved in the adsorption process, and on adsorption and drying a rearrangement of the charged groups on the adsorbed chains probably leads to a favorable surface structure, so that the electrostatic attraction between this newly formed surface and the polyelectrolyte chains in solution dominates, resulting in the adsorption of the next layer. The process repeats and the multilayer growth is sustained.

Based on this speculation, there need to be significant amount of both positive and negative charges on the chain simultaneously, otherwise the polyampholyte becomes either a polycation or a polyanion, and the assembly process quickly ends. This indicates that the pH window suitable for the multilayer assembly is very narrow and should center around the IEP of the polyampholyte, because by changing the pH by 1 unit, the amount of the charges changes by a factor of 10, which can quickly destroy the balance between the numbers of opposite charges. In our study, the IEP of the gelatin was ~ 5 (without the presence of additional salt, the pH of the gelatin solution drifts and we were not able to determine the IEP very accurately). As can be seen in Figure 1, maximal amount of a single layer adsorbed is observed at pH 5 \sim 5.5, which is just around the IEP, and a lower but still significant amount is found at pH 6, while at other pH farther from the IEP the amount adsorbed for each layer decreases quickly. The farther the pH from the IEP, the less amount of adsorption. We will now examine the influence of pH in more detail.

Below the IEP, the gelatin molecules carry a net positive charge, and are expected to strongly adsorb onto the negatively charged PSS surface, and this type of adsorption should lead to gelatin chains with a relatively flattened configuration.⁸ The lower the pH, the more positive charges on each gelatin chain, and the flatter the configuration and less the amount of adsorption. In this configuration, most of the positive charges of the gelatin molecule should combine with the negative charges of the substrate surface, leaving the relatively few negative groups with the rest of the positive groups of the gelatin on the surface, and when subsequent gelatin molecules with net positive charges adsorb, they can still bind to the negative charges on the surface. However, the repulsion between the posi-

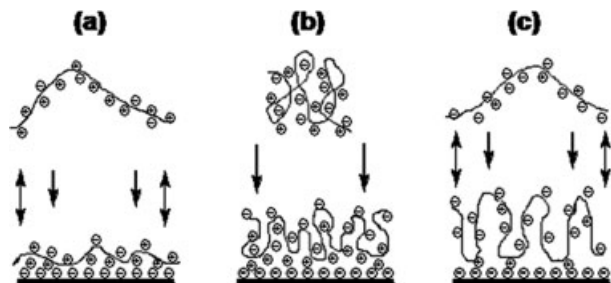


Figure 2. Schematic illustration of possible configurations of gelatin molecules adsorbed and in solution at different pH, and the electrostatic interactions involved in the adsorption. (a) $\text{pH} < \text{IEP}$. (b) $\text{pH} \sim \text{IEP}$. (c) $\text{pH} > \text{IEP}$.

tive charges on the gelatin segments and on the surface is more significant. These two factors lead to a much lower amount of gelatin adsorption for the second and the subsequent layers. Figure 2(a) illustrates the configuration of a gelatin molecule adsorbed from the aqueous solution at a pH below the IEP to the negatively charged QCM electrode. At $\text{pH} = 4$, the average frequency shift for each layer was 4.5 ± 2.4 Hz, and the thickness was calculated to be 0.12 nm assuming a gelatin density of 1.0 g/cm^3 .

At a pH above the IEP, the gelatin molecule carries a net negative charge, and the molecule adopts a similar extended configuration in the solution due to the electrostatic repulsion between the negatively charged segments. When the gelatin molecules first adsorb, even though both the molecules and the QCM electrode carry a net negative charge, there is still considerable adsorption of gelatin on the electrode, presumably a result of the local positive charges on the gelatin molecules binding to the negative charges on the QCM electrode surface. In addition, the electrostatic repulsion between the negative charges on the gelatin segments and on the surface results in a thicker, or more expanded adsorbed layer,⁸ and the amount of adsorption is higher than that for pH below the IEP. After the first layer is adsorbed on the QCM electrode, the subsequent adsorption is much less, because of much fewer binding pairs and significant repulsion between the like charges on the surface and on the gelatin molecules. This is schematically depicted in Figure 2(c). At $\text{pH} = 6$, the average frequency shift for each layer was 27.0 ± 9.9 Hz, corresponding to a layer thickness of 0.73 nm.

At the IEP, the gelatin molecule carries equal amount of positively and negatively charged

groups, and the net charge of the molecule is zero. The equal number of oppositely charged groups results in electrostatic attraction between the chain segments in solution.⁸ When adsorbed to the negatively charged electrode surface, the attraction between the surface and the positively charged amino groups of the gelatin molecule causes the gelatin coil to adsorb, while the repulsion between the negative charges on the surface and the carboxylate groups causes the adsorbed chain to extend into the solution away from the surface. The balance between these two effects results in maximal adsorption at the IEP. The carboxylate groups on the loop and tail segments provide sufficient negative charges for the new surface that support subsequent gelatin adsorption. This process is repeated and stepwise gelatin adsorption is sustained. Figure 2(b) illustrates the adsorption of gelatin at the IEP and the interactions involved in the process. From Figure 1, we observe a linear frequency shift with the increase in the assembly step, and when the pH of the gelatin solution is 5, the amount of the adsorption is the highest, with an average frequency shift of -56 ± 7 Hz for each layer adsorbed, which corresponds to a 1.5-nm layer thickness. For pH 5.5, the average frequency shift and layer thickness are 43 ± 12 Hz and 1.2 nm, respectively.

Then the surface morphology of the gelatin multilayers assembled at different pH was assessed using atomic force microscopy (AFM). Figure 3 shows the AFM topography images of the surfaces of four gelatin layers deposited at pH 3, 5, and 7. It can be seen that in all cases the gelatin molecules cover the surfaces completely and evenly. The RMS roughness is 1.2, 2.3, and 2.0 nm, respectively. This result is consistent with our above argument about the dependence of the conformation of the gelatin chains deposited upon solution pH. In addition, after the first several layers, the surface morphology remained pretty much the same with the increase of the number of gelatin layers.

Because only one species is involved in the assembly, the "multilayers" thus prepared are uniform down to molecular level rather than layered, and they are same as a thin film spin-cast from the gelatin solution in terms of composition. However, their surfaces can be rather different. Contact angle measurement is a surface sensitive technique and can assess the outermost layer properties. Figure 4 plots the water

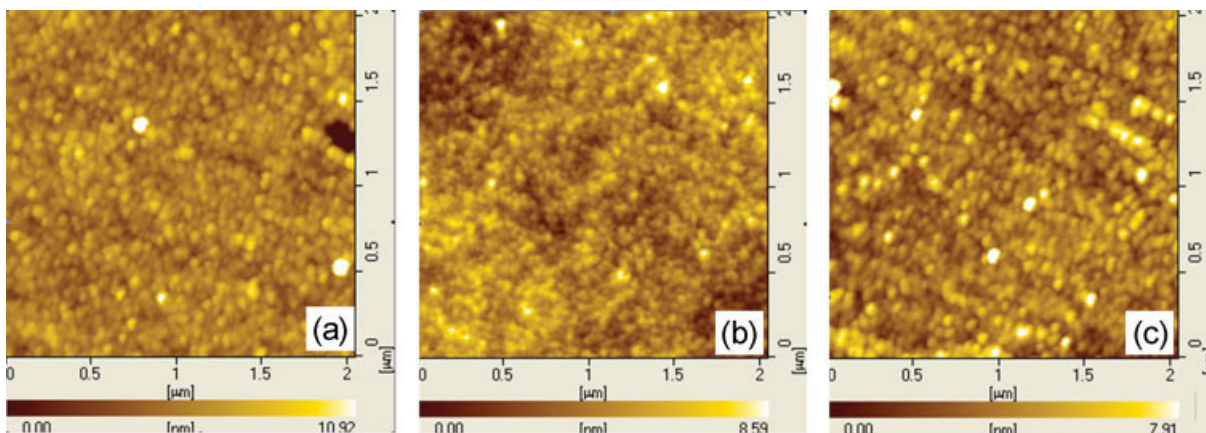


Figure 3. AFM height images of gelatin multilayers assembled at pH = 3 (a), pH = 5 (b), and pH = 7 (c).

contact angles of different gelatin “multilayer” surfaces, which shows that depending on the pH at which the multilayer is assembled, the contact angle can change from 30 to 75°. While the water contact angle for a spin-cast gelatin thin film was $\sim 39^\circ$, the surfaces of the gelatin “multilayers” can be more hydrophobic. This indicates that compared with the surface of a spin-cast gelatin film, the groups at the surface of the “multilayer” films rearrange on adsorption and drying, where more hydrophilic ionic groups can bind with the opposite charges on the substrate, leaving the more hydrophobic segments on the new surface. The effect is most pronounced at the IEP, probably because at the IEP the negative and positive groups are more balanced, and more ionic groups form “internal” pairs, leaving the least amount of the free ionic groups at the

new surface. This is in agreement with our discussion above on the assembly mechanism.

CONCLUSIONS

In this work, we study layer-by-layer assembly of gelatin and the influence of pH to the process. We demonstrate that layer-by-layer electrostatic assembly based on a single species is possible and extended multilayer growth can be realized using an amphoteric material such as gelatin, by tuning the balance between the electrostatic attraction and repulsion and rearranging the charged groups at the surface. This adds more flexibility to the layer-by-layer assembly technique and smears the distinction between multilayered films and traditional homogeneous cast films. In addition, since gelatin is a polypeptide, the unique assembly behavior it exhibits as a polyampholyte may provide new insight into protein assemblies in living systems.

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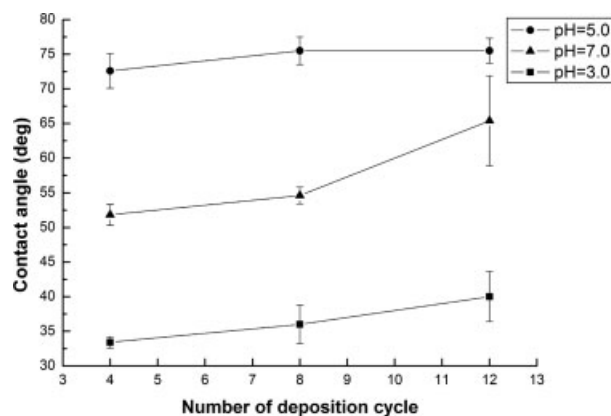


Figure 4. Water contact angle (sessile drop) on the surface of gelatin multilayers with different number of assembled layers.

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