Virus deposition onto polyelectrolyte-coated surfaces: A study with bacteriophage MS2

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Abstract

Hypotheses
By selecting constituent polyelectrolytes and controlling conditions of their deposition, the resulting polyelectrolyte multilayers can be designed as surface coatings with controlled adhesive properties with respect to viruses. Charge and hydrophilicity of the polyelectrolyte multilayers govern virus adhesion.

Experiments
Four surfaces of different charges and hydrophobicities were designed using a layer-by-layer assembly of poly(styrene-4-sulfonate) and poly(dimethyl diallyl ammonium chloride). Contact angle measurements gave an estimate of MS2 hydrophilicity in terms of free energy of interfacial interaction in water. Experimental results on MS2 adhesion obtained using quartz crystal microbalance with dissipation monitoring were compared with predictions by the extended Derjaguin-Landau-Verwey-Overbeek (XDLVO) theory.

Findings
MS2 deposition onto polyelectrolyte multilayers occurred in two phases: an early phase defined by virus-surface interactions and a later phase with virus-virus interactions controlling deposition kinetics. Principal component analysis showed that the deposition rates in the two phases were independent one of another and that each was correlated to the depth of the secondary minimum of the corresponding XDLVO energy profile. Hydrophobic and electrostatic interactions governed the deposition process: short range hydrophilic repulsion prevented deposition into the primary minimum while electrostatic interactions defined the dependence of the deposition kinetics on the ionic strength. Different surfaces showed distinct kinetics of and capacities for MS2 deposition pointing to the potential of polyelectrolyte multilayers as easy-to-apply coatings for regulating virus adsorption, inactivating viruses via the virucidal action of cationic polyelectrolytes and reducing human exposure to viruses.

Keywords: polyelectrolyte multilayers; virus; bacteriophage MS2; deposition; XDLVO; QCM-D; adhesion; principal component analysis
1. Introduction

Virus deposition onto surfaces plays an important role in determining the likelihood of human exposure to viral pathogens. Environmental transmission via contaminated surfaces (fomites) can be a very important transfer pathway that complements or enhances direct person-to-person transfer, or transfer with contaminated food or water [1-4]. Having suitable non-pathogenic models of human pathogens would greatly facilitate studies of virus deposition onto surfaces. Bacteriophages (viruses that infect bacteria) span a broad range of physicochemical properties and offer a rich selection of potential surrogates for human viruses. Indeed, studies on virus transport and fate often employ bacteriophages as surrogates for human viruses [5]. Provided their physicochemical characteristics such as size, morphology, charge, and hydrophilicity match that of the target human virus, bacteriophages can serve as non-pathogenic model microorganisms.

Contact with contaminated surfaces is known to be an important transmission pathway for noroviruses [2]. Norovirus (NoV) recognized as the leading agent of gastroenteritis in humans, accounting for more than 90% of viral gastroenteritis and ~ 50% of all the outbreaks of gastroenteritis worldwide [6]. NoV is a non-enveloped, single-stranded RNA virus with a diameter in the 27 to 32 nm range. NoV was estimated to cause ~ 900,000 gastroenteritis cases and ~ 64,000 hospitalizations each year among children younger than 5 years old in high-income countries. For children in developing countries, the estimate is that NoV may be responsible for up to 1.1 million hospitalizations and 218,000 deaths every year [7]. A suitable surrogate for NoV is MS2 phage, which is similar to NoV in several key aspects: size, positive sense RNA genome, single-stranded RNA, ability to persist in the intestinal tract, and resistance to various disinfection treatments [8]. A member of the Leviviridae family, MS2 infects E. coli cells, has an icosahedral capsid and comprises 180 sequence-identical protein monomers.
MS2 is ~ 27 nm in diameter and has loops (~ 1 nm in size) of hydrophilic amino acids extruding from the capsid.

Numerous studies have been conducted on MS2 adsorption to different surfaces including soils [9-12], natural organic matter [13-15] and membrane filters [16-19]. Virus properties such as hydrophobicity [10, 11, 15], size and shape [9, 10, 15], and surface charge [16, 17] were shown to affect virus adsorption. Environmental conditions such as solution ionic strength and pH also impact virus adsorption: MS2 was reported to aggregate around its IEP and at pH ≤ 4.5 in 1 mM and 100 mM NaNO₃ [20, 21] and at pH ≤ 4 in 10 mM NaCl [15]. The surface charge of MS2 is believed to stem from the ionizable amino acids (AAs) located on the outer capsid surface [15]. The isoelectric point (IEP) of these AAs is 3.9, which falls within the range of IEP values (3.5 to 4) measured for MS2 [11, 15, 20] and is lower than the IEP (5.0 to 6.0 range) reported for norovirus-like particles [22, 23]. The difference in the IEP values indicates that surface characteristics of MS2 and norovirus are somewhat different and that results obtained with MS2 as a norovirus surrogate should be interpreted with caution.

Elucidating the relative importance of different interactions between a virus and a surface is for controlling virus deposition. The Derjaguin-Landau-Verwey-Overbeek (DLVO) [12-14] and, more recently extended DLVO (XDLVO) theories [11, 19, 24, 25] have been broadly applied to describe virus-surface interactions. The classical DLVO model showed only limited ability to explain experimental results from studies on virus adhesion [13-15]. For example, in a study on deposition kinetics of MS2 to coated surfaces [13] DLVO energy profiles exhibited no energy barrier in ionic strengths > 60 mM while the attachment efficiencies were low (~ 0.2). Non-DLVO interactions can be responsible for the observed discrepancies. The XDLVO approach builds on the DLVO theory and takes hydrophobic interactions into consideration. Hydrophobic forces were shown to make a significant contribution to the overall energy of interaction.
between phages (MS2, ΦX174) and clay surfaces [11]. Chattopadhyay et al. concluded that hydrophobic interactions controlled adsorption of T2, MS2 and ΦX174 phages to clays [10].

In the case of MS2, specific surface features of this phage have been identified as responsible for its electrostatic interactions with other surfaces. Electrostatic interactions between MS2 and a charged surface are due to ionizable AAs on MS2 capsid [26]. The AAs include positively charged protonated amine and diaminooiminium groups (6 lysines and 4 arginine and α-NH$_3^+$ group of the protein), the negatively charged carboxylate groups (on the side chains of aspartate and glutamate) and the R-COO$^-$ group of the C-terminus of the protein [27].

Virus deposition onto a surface can be quantified using quartz crystal microbalance with dissipation monitoring (QCM-D) as long as the QCM sensor can be coated by a material that adequately represents the surface in question. By detecting shifts in the QCM sensor’s frequency, this technique can measure mass with nanogram sensitivity and, by measuring dissipation shifts, can offer insights into the viscoelastic behavior of the adsorbed layer [28, 29]. QCM-D has been used to study attachment of bacteriophages (MS2 [12, 13, 15], T4D [30]), norovirus virus-like particle (VLPs) [23, 31], adenovirus [32], airborne Vaccinia viruses [33] and pathogenic plant viruses [34, 35] to various surfaces such as bare QCM crystals [12, 23, 33], natural organic matter [13], polyelectrolyte multilayers [30, 32, 34], clays [12, 13], or self-assembled monolayers [15, 31, 34, 35]. Armanious et al. [15] studied differences in adsorption behaviors of MS2, fr, GA and Qβ bacteriophages and concluded that adsorption was mainly governed by electrostatic and hydrophobic interactions while van der Waals interaction was of secondary importance [15]. In many contexts, virus deposition occurs from a flowing solution, which further motivates the use of QCM-D in studying virus deposition and attachment.
Adsorption to polyelectrolytes is of special interest in light of the various uses of these materials as coatings for macroscopic and colloidal surfaces. Cationic polyelectrolytes such as PDAD, epichlorohydrin dimethylamine (epi-DMA), copolymers of acrylamide with various positive organic functional groups (e.g. with quaternary ammonium monomers) are used as flocculants in water and wastewater treatment [36]. An important emerging application of polyelectrolyte coatings is virus inactivation [37, 38]. Virucidal coatings with branched or linear N,N-dodecyl methylpolyethylenimines were shown to inactivate human and avian influenza viruses [39, 40] with kill-on-contact as one of inactivation mechanisms. Recently, the approach was applied to membrane filtration by Sinclair et al. who modified microfiltration membranes with polyethyleneimine to improve removal of infective MS2 [41, 42].

Polyelectrolyte multilayers (PEMs) [43] in particular are seeing increasing uses as coatings and thin films in separation applications. A monolayer may not provide homogeneous coverage especially for surfaces that are porous or have otherwise complex morphology; depositing several bilayers improves coating quality. The facility of polyelectrolyte removal from a surface makes PEMs promising as sacrificial layers for various uses including surface fouling control [44-47] and virus detection [48, 49]. Having multiple layers enables independent control over the PEM-substrate interaction (controlled by selecting an appropriate polyelectrolyte as the first layer) and the strategy for renewing the sacrificial coating (controlled by selecting polyelectrolyte constituents of the rest of the PEM) [46]. Further, PEMs feature excellent ion separation properties and are used to make membranes for pressure-driven separations and electrodialysis; several bilayers are normally required to achieve nanofiltration-level selectivity (i.e. selective removal of multivalent ions) [50, 51]. An important emerging application of PEMs is as hosts for other materials including functional nanoparticles [52], biocides [53] and viruses [54]. Multiple polyelectrolyte layers
are needed for efficient immobilization or embedding. In addition to their practical relevance, PEMs are excellent model surfaces wherein changes in thickness [55-57], surface morphology [58], charge [44, 48], hydrophobicity [44, 48, 58] and the degree of swelling [57] can be easily controlled by adjusting deposition conditions such as the number of deposition cycles [57, 59], polyelectrolyte type [57] – as well as the composition and ionic strength of the deposition solution [51, 56, 59, 60].

The present study was motivated by the need for easy-to-apply coatings to regulate virus adsorption and reduce human exposure to viruses. The central hypothesis of the work is that layer-by-layer deposition of polyelectrolyte multilayers is a suitable design approach for creating such coatings. We employed QCM-D to quantify MS2 deposition onto four different PEM surfaces of different charges and hydrophobicities. Poly(diallyldimethyl ammonium chloride) (PDAD) and poly(allylamine hydrochloride) (PSS) were selected to construct the PEMs because both polyelectrolytes have been studied extensively so that formation-structure-properties relationships for [PSS/PDAD]n multilayers are relatively well understood [3, 13-17]. In particular, separation properties (water and salt permeability coefficients) of [PSS/PDAD]-based nanofiltration films have been accurately measured [44]. The bacteriophage was deposited from solutions of low and high ionic strength (Ι_{M2S} = 10 mM or 100 mM) to probe the relative importance of acid-base (hydrophobic), electrostatic and van der Waals interactions between MS2 and PEM surfaces. Predictions by DLVO and XDLVO theories were evaluated based on a comparison with the QCM-D data. Principal component analyses identified correlations and general trends in the dependence of deposition kinetics on virus and surface properties.
2. Materials and Methods

All reagents were of high purity (>98%). Deionized water with a resistivity of 18.2 MΩ-cm was used to prepare all solutions. PDAD (MW ~ 70,000 Da) and PSS (sodium salt) (MW ~ 100,000 to 200,000 Da) were purchased from Aldrich. Procedures for the propagation, purification and quantification of bacteriophage MS2 are given in the Supplementary Material (SM).

2.1. Preparation and characterization of polyelectrolyte-coated surfaces

2.1.1 PEM deposition

PEMs were assembled on a gold QCM-D crystal in situ in the QCM-D chamber. Prior to the assembly, the crystal was cleaned following the cleaning procedure provided by Biolin Scientific. It was first soaked in a 5:1:1 mixture of DI water, hydrogen peroxide (30%) and ammonia (25%) at 75 °C for 5 min. It was rinsed with DI water and then dried out with N₂, followed by 20 min ozone cleaning to remove hydrocarbon contaminants. The PE solutions were diluted to a repeat unit concentration of 0.02 M with the ionic strength \( (I_{\text{LBL}}) \) adjusted to 10 or 100 mM NaCl. (Repeat units of PSS and PDAD are \( \text{C}_8\text{H}_7\text{SO}_3\text{Na} \) (Mw=206.2 g/mol) and \( \text{C}_8\text{H}_{16}\text{NCl} \) (Mw = 161.7 g/mol), respectively.) The pH of PSS and PDAD solutions was adjusted to 6.4 and 4.6, respectively, using 1 mM HCl and 1 mM NaOH solutions. After the crystals were cleaned, they were mounted within QCM-D modules. Resonance frequencies in air were established for each crystal, followed by a stable baseline for at least 5 min. This step was repeated in either 10 or 100 mM NaCl solution to establish a stable baseline in wet condition. To build up PEMs on a crystal, the PSS solution was first deposited for 5 min, followed by rinsing with NaCl solution to remove unbound PE chains. The PDAD solution was then deposited on top of the PSS layer, followed by 5 min NaCl rinsing. This cycle was repeated 4 or 4.5 times to create positive or negative PEMs [61].
2.1.2 Measurements of the $\zeta$-potential and hydrophobicity

The zeta potentials of PEM surfaces were measured using an electrokinetic analyzer (EKA, Anton Paar) in 10 mM KCl (see SM, section S2, for details). Every measurement was repeated at least two times. Contact angles of DI water, glycerol, and diiodomethane on PEM surfaces and MS2 lawns were determined and used to calculate the free energy of cohesion in water, $\Delta G_{sws}$, which was used as a measure of hydrophobicity (see SM, section S2). The PEM-coated surfaces were dried in room temperature overnight prior to the contact angle measurement. The bacteriophage lawn was prepared according to the procedure reported earlier [48, 62]. Contact angles were measured based on droplet shapes, 5 s after droplet deposition on a surface, using a ramé-hart goniometer and DROPimage Advanced software.

2.1.3 AFM characterization of PEM morphology

The surface roughness of PEM-coated surfaces was measured using AFM (Cypher) with the samples exposed to 10 mM NaCl or 100 mM NaCl solutions. The measured area for each sample was 2 $\mu$m x 2 $\mu$m. Statistical analysis of the AFM data rendered three metrics of surface morphology: average roughness, root-mean-square (RMS) roughness and surface area difference. For each [PSS/PDAD]$_4$ and [PSS/PDAD]$_{4.5}$ coating, the measurements were performed with all four combinations of the two ionic strengths (i.e. $I_{LbL}$ and the ionic strength of the deposition solution).

2.2 QCM-D experiments

MS2 suspensions used in deposition tests were prepared by diluting the purified MS2 stock (see SM, section S1) by either 10 mM or 100 mM NaCl solutions to a final concentration of $(1.24 \pm 0.07) \times 10^{10}$ PFU/mL. The pH of the virus suspension
was adjusted to 7 where MS2 was shown to exist as individual virions, and not as aggregates [62]. Both PEM assembly onto the QCM-D sensor and MS2 deposition onto PEM-coated surface were carried out in situ within QCM-D modules at 25 °C and in a continuous flow mode at a flow rate of 0.15 mL/min using a digital peristaltic pump (IPC, 4 channels, ISMATEC). Values of QCM frequency and dissipation were recorded every 1 min. The frequency shifts acquired for a clean and PEM-coated sensor were fitted into the Sauerbrey equation [63] to determine the mass change: $\Delta m = -C \Delta f / n$, where $C$ is the mass sensitivity constant ($C = -17.7$ ng·Hz$^{-1}$·cm$^{-2}$), $n$ is the overtone number ($n = 3$), and $\Delta f$ is the frequency shift (Hz). The frequency shift was obtained by subtracting the frequency of the clean sensor from the frequency of the PEM-coated sensor. The mass of MS2 deposited onto the PEM-coated sensor was calculated as the difference between the frequencies of the PEM-coated sensor before and after 1 h of MS2 deposition.

2.3. Nomenclature
In what follows, we use the following scheme to describe different MS2 deposition scenarios: $X$-[PSS/PDAD]$_n$-$Y$. In this notation, $X$ is the ionic strength ($I_{LbL}$, mM) of the NaCl solution from which the PEM was deposited, $n$ is the total number of bilayers deposited, and $Y$ is the ionic strength ($I_{MS2}$, mM) of the MS2 suspension. For example, 100-[PSS/PDAD]$_4$-10 denotes an experiment where MS2 bacteriophages deposit from 10 mM NaCl solution onto a 4-bilayer PDAD-terminated polyelectrolyte coating LbL-assembled from 100 mM NaCl solution.

3. Results and Discussion
Both PSS and PDAD are strong polyelectrolytes and a change in pH does not affect their ionization. In this study, the pH of the virus deposition solution was maintained constant (pH 7). The ionic strength of the solution was adjusted to either 10 mM or 100 mM and this change entailed variations in both electrostatic and acid-base interactions [64] between the virus and the PEM-modified surface.
3.1 Characteristics of MS2 and polyelectrolyte multilayer coatings

The 10-[PSS/PDAD]₄ multilayer was hydrophobic ($\Delta G_{\text{SWS}} = -16.3 \text{ mJ/m}^2$) and positively charged ($\zeta = 6.7 \text{ mV}$) (Table 1). Depositing the same sequence of polyelectrolytes from a solution with $I_{\text{BL}} = 100 \text{ mM}$ led to a film (100-[PSS/PDAD]₄) that was slightly hydrophilic ($\Delta G_{\text{SWS}} = 7.6 \text{ mJ/m}^2$) and had a higher positive surface charge (27.5 mV). We attribute the higher hydrophilicity to a larger excess of electrical charges when depositing polyelectrolytes from a higher ionic strength electrolyte. In a high ionic strength solution, more polyelectrolyte segments pair with salt counterions and additional waters of hydration, resulting in higher hydrophilicity. Swelling was found to correlate with the PEM's hydrophilicity [57, 59, 65] and thus we expect 100-[PSS/PDAD]₄ film to be more swollen. The 10-[PSS/PDAD]₄.₅ multilayer was highly hydrophilic ($\Delta G_{\text{SWS}} = 45.2 \text{ mJ/m}^2$) and negatively charged ($\zeta = -5.7 \text{ mV}$). Depositing the same sequence of polyelectrolytes from a solution with $I_{\text{BL}} = 100 \text{ mM}$ led to a film (100-[PSS/PDAD]₄.₅) that had higher negative charge (-17.8 mV) while the hydrophilicity remained statistically the same.

As shown in Table 1, for each of the four types of PEM (i.e., 10-[PSS/PDAD]₄, 100-[PSS/PDAD]₄, 10-[PSS/PDAD]₄.₅, and 100-[PSS/PDAD]₄.₅), the same value of zeta potential was used when evaluate electrostatic interactions in solutions of different ionic strengths. The $\zeta$-potential, which was measured in 10 mM KCl (see section 2.1.2), approximated the surface potential, $\psi_s$, while the dependence of the energy of electrostatic interaction, $E^{EL}_{\text{SLV}}$, on the ionic strength was taken into account via the terms that include the ionic strength-dependent Debye length, $\kappa^{-1}$ (see eq. S3 in SM). The surface potential was assumed to not depend on the ionic strength (i.e. no specific adsorption of ions onto the surface) so that an increase the concentration of salt in the continuous phase impacted electrostatic interactions only via the compression of the electrical double layer.

For both polyanion- and polycation-terminated films, their surface roughness was higher when they were deposited from the background solution of higher ionic
strength (\(I_{LbL} = 100\) mM). The changes in roughness were due to the increased shielding, lower polyelectrolyte charge density [66] and fewer cross-links between constituent polyelectrolytes leading to “loopier” [67, 68], thicker, and rougher PEM films [59, 69]. McAloney et al. [68] studied effects of increased NaCl concentration on morphology of [PSS/PDAD]_{10} and reported that above 10 mM NaCl, PEM roughness increased almost linearly with increasing NaCl concentration. A study on [PAH/PSP]_{n} by Apaydin et al. [67] showed that roughness of the PEM significantly increased with ionic strength above 200 mM. Both studies argued that above a critical salt concentration, polyelectrolytes transit from extended rod to coil configuration, resulting in a thicker film and a rougher surface.

Zeta potential of MS2 was taken to be -35 mV, which is the value reported by Armanious et al. for MS2 at pH 7 [15]. In consistence with previous reports [70], MS2 was estimated to be hydrophilic (\(\Delta G_{sws} = 48.0\) mJ/m\(^2\); Table S2). Because of the effect of residual PEG on the hydrophobicity [62] the value likely overestimates \(\Delta G_{sws}\) of a pristine MS2. As an accurate characteristic of the virus suspensions employed in QCM-D tests, however, this estimate is useful in that it allows for quantitative comparison of XDLVO predictions and QCM-D results.
Table 1: Zeta potentials, hydrophobicity, and morphological characteristics of [PSS/PDAD]$_4$ and [PSS/PDAD]$_{4.5}$ surface coatings assembled from solutions of different background electrolyte concentrations (10 mM or 100 mM NaCl), rinsed, and exposed to either 10mM NaCl or 100 mM NaCl solutions.

* Measured in 10 mM KCl (see section 2.1.2).
** Root mean square roughness is the standard deviation of the average roughness.
*** Surface area difference is the difference between the three-dimensional surface area and its two-dimensional projection or "footprint".

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<th>$I_{LBL}$ (mM)</th>
<th>10</th>
<th>100</th>
<th>$I_{MS2}$ (mM)</th>
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<td><strong>[PSS/PDAD]$_4$</strong></td>
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<td>27.5 ± 1</td>
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<tr>
<td>Free energy of interfacial interaction, $\Delta G_{sws}$ (mJ/m$^2$)</td>
<td>-16.3 ± 2.1</td>
<td>6.9 ± 14.4</td>
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<td>RMS roughness of the PEM surface** (nm)</td>
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<td>3.3</td>
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<td>Surface area difference** (%)</td>
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<td>0.5</td>
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<td><strong>[PSS/PDAD]$_{4.5}$</strong></td>
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<td>Zeta potential of the PEM, $\zeta$ (mV)</td>
<td>-5.7 ± 2</td>
<td>-17.8 ± 0</td>
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<td>Free energy of interfacial interaction, $\Delta G_{sws}$ (mJ/m$^2$)</td>
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<td>44.3 ± 10.2</td>
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<td>Root-mean-square roughness of the PEM surface* (nm)</td>
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<td>Surface area difference*** (%)</td>
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3.2 XDLVO energy profiles for MS2-interactions with PEM surfaces

MS2 deposition onto polyelectrolyte multilayers was modelled as occurring in two phases: an early phase (corresponding to as-prepared PEM surface) defined by virus-PEM interactions and a later phase (corresponding to MS2-covered PEM surface) with virus-virus interactions controlling deposition kinetics.

Figure 1 shows DLVO and XDLVO energy profiles for MS2 interaction with the positively charged 10-[PSS/PDAD]₄ and 100-[PSS/PDAD]₄ surfaces during phase 1 of the deposition process with the assumption that the surfaces are smooth. The calculated energy barrier ($E_{\text{max}}$) was high (> 200 kT) for all combinations of the surface type and the ionic strength of the solution ($I_{\text{MS2}}$) preventing MS2 deposition into the primary minima. The secondary minima ($E_{\text{min}}$) in 10 mM solution were – 3.1 kT and – 10.6 kT for 10-[PSS/PDAD]₄ and 100-[PSS/PDAD]₄, respectively; in the case of 100 mM solution the secondary minima were shallow, with a magnitude of less than 1 kT for both the PEMs.

Figure 2 shows DLVO and XDLVO energy profiles for MS2 interaction with the negatively charged 10-[PSS/PDAD]₄.₅ and 100-[PSS/PDAD]₄.₅ surfaces during phase 1 of the deposition process with the assumption that the surfaces are smooth. For all scenarios, $E_{\text{max}}$ was very high (> 650 kT) preventing MS2 deposition into the primary minima. The secondary minima, $E_{\text{min}}$, were shallow ($\leq$ 0.6 kT). XDLVO profiles were also calculated while taking surface roughness into account (see SM, Figures S1 and S2). Roughness had only a very minor effect on the energy profiles and, specifically, on the values of $E_{\text{max}}$ and $E_{\text{min}}$. XDLVO energy profiles for phase 2 (see SM, Figures S3 and S4) were calculated using the same approach as for phase 1 except that the electrical charge and surface energy parameters of the collector surface were assumed to be those of the MS2 phage.
Figure 1: DLVO and XDLVO energy profiles of MS2 interaction with as-prepared (i.e. MS2-free) surfaces of 10-[PSS/PDAD]$_4$ (a, b) and 100-[PSS/PDAD]$_4$ (c, d) in 10 mM NaCl (a, c) and 100 mM NaCl (b, d) solutions. The profiles correspond to early stages of the phase 1 of MS2 deposition (see Figure 3). In the Figure legends, the acronyms EL, LW and AB refer to electrostatic, van der Waals, and acid-base interactions, respectively.
Figure 2: DLVO and XDLVO energy profiles of MS2 interaction with as-prepared (i.e. MS2-free) surfaces of 10-[PSS/PDAD]_{4.5-10} (a, b) and 100-[PSS/PDAD]_{4.5-10} (c, d) in 10 mM NaCl (a, c) and 100 mM NaCl (b, d) solutions. The profiles correspond to early stages of phase 1 of the MS2 deposition (see Figure 4). In the Figure legends, the acronyms EL, LW and AB refer to electrostatic, van der Waals, and acid-base interactions, respectively.
3.3 QCM-D measurements of MS2 deposition onto polyelectrolyte-coated surfaces

In QCM-D tests, changes in both QCM frequency and dissipation were recorded. Frequency data translated into mass deposition rate, $m(t)$, are shown in Figures 3 and 4. Dissipation data, $D(t)$, and a summary of deposition kinetic parameters (deposition rate $dm/dt$ and lag time $t_1$) can be found in the SM file (Figures S5 and S6, Table S2). Lag time is operationally defined as the time interval between the start of the deposition test and the moment when mass deposition is detected by QCM.

3.3.1 Deposition of MS2 bacteriophage onto positively-charged PEM surfaces

At pH 7, MS2 is negatively charged (-37.9 mV) due to the carboxylate and α-COO⁻ groups while [PSS/PDAD]₄ carries positive charge due to ammonium groups of its terminating PDAD layer. Therefore, electrostatic interactions between MS2 and [PSS/PDAD]₄ are favorable.

Figure 3 presents the deposition kinetics of MS2 on the positively-charged surfaces of the 10-[PSS/PDAD]₄ (Figure 3a) and 100-[PSS/PDAD]₄ (Figure 3b) from solutions of different ionic strengths, $I_{MS2}$. In each of the four scenarios, deposition occurred in two distinct phases – an early stage (phase 1) with fast deposition kinetics and a later stage (phase 2) where the deposition was much slower or not detectable. Similar two-phase kinetics was reported by Guleryuz et al. who studied deposition of silica nanoparticles onto oppositely charged alumina coated surfaces [71]. For both phase 1 and phase 2, deposition data were fit using linear regression $m = (dm/dt)t - const$ to estimate the deposition rates in phase 1 and 2 ($dm/dt_1$ and $dm/dt_2$, respectively) as well as the deposition lag time ($t_1 = const \cdot (dt/dm)_1$). The following specific trends could be identified:
a) The largest deposited mass during phase 1 was measured on the 100-[PSS/PDAD]_{4-10}, where the total XDLVO energy profile of MS2-surface interactions had the deepest secondary minimum (\(-10.6 \text{kT}\), Figure 1c and Table S2) across all conditions considered in this study. Indeed, the 100-[PSS/PDAD]_{4} surface had the highest positive charge while the low ionic strength (\(I_{MS2} = 10 \text{mM}\)) offered minimal screening of electrostatic attraction between the negatively charged MS2 and the surface. Comparison with the 10-[PSS/PDAD]_{4-10} scenario indicates that hydrophilic repulsion due to higher hydrophilicity of 100-[PSS/PDAD]_{4} (6.7 mJ/cm\(^2\) for 100-[PSS/PDAD]_{4} vs -16.3 mJ/cm\(^2\) for 10-[PSS/PDAD]_{4}) was outweighed by the electrostatic attraction due to a higher charge (+27.5 mV vs +6.7 mV). Given the significant (10.6 kT) depth of the secondary minimum, the deposition of MS2 was limited by its diffusion towards the surface (i.e. in the direction normal to the flow lines in the QCM-D channel).

b) In tests with low ionic strength (\(I_{MS2} = 10 \text{mM}\)), no deposition was observed during phase 2. It appears that the deposition of the negatively charged virus onto the PEM surface during phase 1 overcompensated the positive charge on 10-[PSS/PDAD]_{4} and 100-[PSS/PDAD]_{4} surfaces flipping electrostatic interactions between the virus and the surface to be repulsive. In consistence with this reasoning, at the higher ionic strength (\(I_{MS2} = 100 \text{mM}\)) the deposition on both 10-[PSS/PDAD]_{4} and 100-[PSS/PDAD]_{4} continued, albeit at a slower pace than during phase 1. Repulsive electrostatic interactions between the virus and the virus-coated surface are effectively screened in 100 mM NaCl electrolyte making a multilayer deposition possible. This interpretation is consistent with conclusions reached in earlier studies with inorganic colloids [71, 72].

c) The plateau values of \(\sim 560 \text{ng/cm}^2\) and \(\sim 1220 \text{ng/cm}^2\) (60 min into QCM-D test) observed during MS2 deposition from 10 mM NaCl (10-[PSS/PDAD]_{4-10} and 100-[PSS/PDAD]_{4-10} experiments, respectively) are lower than \(\sim 2100 \text{ng/cm}^2\) reported by Armanious et al. for deposition of MS2 (also PEG-purified) onto positively charged poly-L-lysine surfaces (also at \(\sim 60 \text{min into QCM-D}\).
test and from 10 mM NaCl electrolyte.) Although MS2’s electrostatic interactions with both [PSS/PDAD]$_4$ and poly-L-lysine are favorable, the strength of these interactions differs for the two surfaces. We infer that LbL deposition of polyelectrolyte coatings onto surfaces can be used to not only flip interactions from attractive to repulsive (or vice versa) but also to selectively alter the strength of such interactions.

d) Phase 1 deposition kinetics depended on ionic strength $I_{MS2}$ for the highly charged 100-[PSS/PDAD]$_4$, but not for the weakly charged 10-[PSS/PDAD]$_4$. The difference is due to the screening of the attractive surface-virus electrostatic interactions, which were stronger for 100-[PSS/PDAD]$_4$.

e) Pair comparisons of 10-[PSS/PDAD]$_4$-10 vs 100-[PSS/PDAD]$_4$-10 and 10-[PSS/PDAD]$_4$-100 vs 100-[PSS/PDAD]$_4$-100 show that significantly more deposition occurred onto the 100-[PSS/PDAD]$_4$ than on 10-[PSS/PDAD]$_4$ surfaces. The trend could be due to the higher roughness of 100-[PSS/PDAD]$_4$; the roughness exceeds the distance where the XDLVO barrier is 3 kT (see SM, Fig. S7) and thus can facilitate physical entrapment of a fraction of viruses that approach the surface.

f) The changes in dissipation (see SM; Figures S5 and S6) correlated with changes in the deposited mass indicating that the adsorbed layer was “soft” and exhibited a viscoelastic behavior.

g) Ionic strength of the MS2 deposition solution ($I_{MS2}$) compressed electric double layer and screened electrostatic repulsion to determine the total mass gain over the long term; as expected, virus-virus interactions govern the kinetics of virus mass accumulation for times significantly exceeding the time it take for the first layer of viruses to cover the surface.
Figure 3: Kinetics of MS2 deposition onto (a) 10-[PSS/PDAD]4, and (b) 100-[PSS/PDAD]4 surfaces from solutions of different ionic strengths ($I_{MS2} = 10$ mM or 100 mM NaCl). Corresponding dissipation values are given in SM (Figure S5). Red and blue symbols and trend lines correspond to deposition phase 1 while yellow and green symbols and trend lines – to deposition phase 2.
Figure 4: Kinetics of MS2 deposition onto (a) 10-[PSS/PDAD]4.5 and (b) 100-[PSS/PDAD]4.5 surfaces from solutions of different ionic strengths ($I_{MS2} = 10$ mM or 100 mM NaCl). Corresponding dissipation values are given in SM (Figure S6). Red and blue symbols and trend lines correspond to deposition phase 1 while yellow and green symbols and trend lines – to deposition phase 2.
3.3.2 Deposition of MS2 bacteriophage onto negatively-charged PEM surfaces

At pH 7, MS2 is negatively charged (-37.9 mV) due to the carboxylate groups and α-COO- groups while [PSS/PDAD]4.5 carries negative charge due to sulfonate groups of its terminating PSS layer. Therefore, electrostatic interactions between MS2 and [PSS/PDAD]4.5 are unfavorable. Figure 4 shows QCM-D frequency changes in 1 h of MS2 deposition onto the negatively-charged surfaces of 10-[PSS/PDAD]4.5 (Figure 4a) and 100-[PSS/PDAD]4.5 (Figure 4b) from solutions of 10 mM NaCl and 100 mM NaCl. Three specific trends could be identified:

a) Deposition onto the negatively-charged surfaces was significantly (~3 to 5 times in terms of ng/cm²) weaker than onto the positively-charged ones, as expected based on the unfavorable MS2-PEM electrostatic interactions and the higher hydrophilicity of [PSS/PDAD]4.5 surfaces (Table 1). This was consistent with the XDLVO predictions of very shallow secondary minima (≤ 0.6 kT, Figure 2 and Table S2) for MS2-[PSS/PDAD]4.5 interactions. The difference was less pronounced in tests with MS2 deposition from 100 mM NaCl where repulsive electrostatic interactions are screened more effectively.

b) The increase in adsorbed mass on [PSS/PDAD]4.5 was slower and the transition between phase 1 and phase 2 was much less abrupt than for the positively charged [PSS/PDAD]4 surfaces. The more gradual transition can be explained by the lack of charge reversal due to the surface coverage by MS2.

c) MS2 deposition in 10 mM NaCl onto 10-[PSS/PDAD]4.5 was negligible. This was not the case with the 100-[PSS/PDAD]4.5 surface, which could be due to the low roughness of the 10-[PSS/PDAD]4.5-10 surface (Table 1) and a lower chance of physical entrapment of MS2 by protruding polyelectrolytes loops. Although the principal component analysis (section 3.4) showed that deposition rate in phase 1 was independent of the surface roughness across the entire dataset, the above argument is based on a pair-wise comparison of a subset (2 of 8 surfaces) and may still be valid.
3.3.3 Comparison of QCM-D data with DLVO and XDLVO predictions

DLVO predicted overall attractive MS2 interactions with both 10-[PSS/PDA]₄ and 100-[PSS/PDA]₄ surfaces in both 10 and 100 mM NaCl solutions. This was consistent with the general trend in QCM-D results (significant deposition on all positively charged surfaces) but could not explain differences between 10-[PSS/PDA]₄ and 100-[PSS/PDA]₄. Further, DLVO profiles also showed attractive interactions of MS2 with both 10-[PSS/PDA]₄,₅ and 100-[PSS/PDA]₄,₅ in both 10 and 100 mM NaCl solutions. This was not consistent with QCM-D observations.

Acid-base interactions accounted for in XDLVO give rise to energy barriers and secondary minima. XDLVO predicted deeper secondary minima at low ionic strength and for 100-[PSS/PDA]₄ pointing to more deposition in these cases. XDLVO profiles also showed high energy barriers and shallow secondary minima for MS2 interactions with 10-[PSS/PDA]₄,₅ and 100-[PSS/PDA]₄,₅, predicting unfavorable conditions for adsorption. This was consistent with results of QCM-D tests wherein minimal virus deposition was observed in phase 1. In sum, we conclude that XDLVO is better suited than DLVO to describe virus-surface interactions attraction. The primary energy barriers and secondary minima predicted by XDLVO qualitatively explain QCM-D results.

3.4. Principal component analysis

Principal component analysis was carried out on QCM-D experimental data (Figures 3 and 4, Table S2) and XDLVO predictions (Figures 1 and 2, Table S2) to reveal correlations and trends that may not be obvious based solely on pair-wise comparisons of data subsets. The primary objective was to discern correlations between PEM surface properties (ζ, ΔGₛₜₜₛ) and deposition conditions (Iₘₛ₂) on the one hand and MS2 deposition kinetics (dm/dt, t₁) on the other hand. The secondary objective was to
assess the predictive ability of the XDLVO model applied to MS2 deposition onto PEMs. The analysis was performed using XLSTAT 2018 statistical software.

The PCA data matrix included the following active variables: deposition rates during phases 1 and 2 \((dm/dt)_1\) and \((dm/dt)_2\), deposition lag time \((t_1)\), zeta potential \((\zeta)\), RMS roughness \((\rho)\), and the free energy of interfacial interaction \((\Delta G_{sws})\) of PEM surfaces. The secondary minima in XDLVO total energy of virus-surface interactions during phase 1 \((E_{min}^{(1)})\) and phase 2 \((E_{min}^{(2)})\) were used as supplementary variables.

Figure 5 presents factor loadings and factor scores computed by PCA. Nearly 80% of variability is captured by the analysis based on the two principal components, PC1 and PC2. The factor loadings graph (Figure 5a) reveals the following correlations:

1) The deposition rate in each of the two phases is correlated to the depth of the secondary minimum of the corresponding XDLVO total energy of interaction: \((dm/dt)_1\) correlates with \(E_{min1}\) while \((dm/dt)_2\) correlates with \(E_{min2}\). This result points to the predictive ability of the XDLVO model to describe deposition kinetics.

2) Deposition rates during phase 1 \((dm/dt)_1\) and phase 2 \((dm/dt)_2\) are nearly independent. The result is consistent with the premise that phase 1 deposition is governed by virus-surface interactions while phase 2 deposition – by virus-virus interactions.

3) The deposition lag time, \(t_1\), is countercorrelated with the deposition rate \((dm/dt)_1\). This can be rationalized in terms of the overall capacity of the surface for virus adsorption: the slower the deposition rate the longer it should take to saturate the capacity and to complete phase 1.
Figure 5: Principal component analysis (PCA): factor loadings (a) and factor scores (b). In Figure 5b, a simplified notation is used for compactness: [PEM]_4 and [PEM]_{4.5} stand for [PSS/PDAD]_4 and [PSS/PDAD]_{4.5}, correspondingly.
4) In accordance with XDLVO predictions (see SM, Figures S1 and S2), the deposition rate during phase 1 \( ((dm/dt)_1) \) is nearly independent of roughness.

Figure 5b presents factor scores for PEMs. Several general observations could be made:

1) The first principal component (PC1) separates the positively-charged [PSS/PDAD]4 surfaces (negative PC1 domain) with high \((dm/dt)_1\) and \(\zeta\) from the negatively-charged [PSS/PDAD]4.5 surfaces (positive PC1 domain) with high \(t_1\) and \(\Delta G_{sws}\).

2) The second principal component (PC2 axis) separates surfaces based on ionic strength of the MS2 deposition solution \( (I_{MS2}) \). PEMs exposed to \( I_{MS2} = 100 \) mM NaCl (domain of positive and near-neutral PC2 values) are characterized by larger \((dm/dt)_2\) than PEMs exposed to \( I_{MS2} = 10 \) mM NaCl (negative PC2 domain).

3) The surfaces are grouped largely by the surface charge (and hydrophilicity, which is correlated with negative charge in the present case). Less hydrophilic and positively charged surfaces were characterized by much faster deposition kinetics during phase 1.

3.5. Roles of electrostatic and acid-base interactions

For the PEMs selected in this study, hydrophobicity and negative charge are counter correlated (Figure 5a): regardless of the ionic strength of the PEM deposition solution \( (I_{LbL}) \), positively charged [PSS/PDAD]4 surfaces are more hydrophobic than the negatively charged [PSS/PDAD]4.5 surfaces. Indeed, as shown in Table 1, both [PSS/PDAD]4.5 are strongly hydrophilic \( (\Delta G_{sws} = 44.3 \text{ and } 45.2 \text{ mJ/m}^2) \) while [PSS/PDAD]4 are either hydrophobic (-16.3 mJ/m²) or only slightly hydrophilic (6.7 mJ/m²). Because PEM hydrophobicity and negative charge both deter attachment of the negatively charged MS2, the present selection of PEM surfaces is not optimal for separating the effects of these two PEM properties on MS2 deposition. Limited conclusions, however, can be drawn. First,
the correlation (observed for both phases of deposition) between deposition rate and the secondary minimum in the XDLVO energy profile, shows that accounting for acid-base interactions explains the observed deposition kinetics. Second, XDLVO predictions indicate that the depth of the minima is defined primarily by the acid-base and electrostatic interactions, with van der Waals forces playing a relatively minor role. Third, when the deposition occurs from high ionic strengths, the screening of electrostatic interactions makes the role of acid-base interactions more prominent emphasizing the importance of hydrophilicity as a surface design criterion.

3.6. Limitations of the modeling approach

The modeling approach employed in this work considers MS2 virion as a sphere; while partly justified given MS2’s icosahedral shape, the approximation does not account for surface protrusions on the MS2 surface (see below). The surface of the collector is assumed to be either flat or with uniform roughness, measured as described in section 2.1.3 and accounted for in XDLVO as described in section 3.2. The modeling approach does not account for non-XDLVO interactions such as those due to hydration and steric forces. Accounting for these additional colloidal forces can improve model predictions [73, 74].

Steric forces can be either repulsive (for hydrophilic surfaces) or attractive (in the case of intermolecular bridging). In the absence of multivalent cations in the solution, we do not expect bridging to be of concern. Repulsive steric interactions, however, are very likely as both MS2 and PEM coating have complex morphology deviating from the assumption embedded in XDLVO. MS2’s repulsive steric interactions with other surfaces are through two relatively hydrophilic polypeptide structures on MS2 surface: a long hairpin $\beta$A-$\beta$B loop ($\sim 1$ nm) and short $\alpha$A-$\alpha$B loop [27]. This steric hindrance was thought to be responsible for low adsorption of MS2 to surfaces and was evoked to explain discrepancies between experimental results and predictions of DLVO theory [13, 15, 27]. Mylon et al. [75] attributed stability of MS2 suspensions in a high ionic
strength solution (> 1 M) to steric hindrance caused by MS2 loops. Steric interactions were also invoked in a study of MS2 adsorption on quartz granular media by Penrod et al. [27]. Steric barriers have also been reported as playing a role in PEM formation and PEM-adsorbate interactions [59, 76]. A study by Lowack et al. [76] of the [PAH/PSS] bilayer found that 50%-70% of the functional groups of PAH were not bound by PSS and that only 40%-80% of PSS were bound to PAH. This indicates that there are coexisting negative and positive segments of polyelectrolytes on the PEM surface. The tails and loops of the top polyelectrolyte layer can protrude to the bulk solution as far as 4 nm to create a steric barrier and thus control the amount of adsorbed polyelectrolytes. This was consistent with the finding that about 2/3 of the functional groups of the top adsorption layer are not bound with the underneath layer [77]. Because steric forces are not accounted for by the XDLVO theory, their role in MS2 and PEMs interactions (during phase 1) and in virion-virion interactions (during phase 2) was not estimated in the present work.

Another major assumption of the XDLVO modeling adopted in this work is that the virion and collector present solid, impermeable surfaces. Thus, the model does not account for the soft nature of MS2 and PEM surfaces. Both the simplified treatment of the electrokinetic properties of MS2 and PEM coating and the possible role of non-XDLVO forces (steric interactions, specifically) not accounted for by the model make the interpretation of the experimental data in terms of XDLVO predictions tentative.

The “soft” nature of virions is known to partly define its electrokinetic properties and, by extension, its interactions with other surfaces. The “softness” of MS2 is due to the finite permeability of the virion [78] and a possible layer of macromolecular adsorbates on its surface [62, 79]. The collector surface coated by a PEM is a “soft” surface as well. A recent study on the deposition of colloids with grafted poly(acrylic acid) brush on a polyelectrolyte-coated substrate showed that whether the rigidity of the surface is a good approximation depends on the ionic strength of the deposition solution [80]. The theory developed by
Ohshima [81-83] has been used to describe the finite permeability of and charge distribution within “soft” layers on the interacting surfaces but the approach could not fully explain differences between theory and experiments [84, 85].

3.7. Implications for virus control

Preventing virus adhesion, inactivating viruses on contact, and removing viruses from surfaces can be of great practical importance in settings such as hospitals wherein microbiological safety is of utmost concern and surface disinfection is a regular practice. PEM coatings can be applied to surfaces of different morphologies, charges and energies for applications ranging from virus detection to minimizing human exposure to viruses. Polyelectrolytes can be sprayed over large surface areas [86], bridge surface pores smaller than several tens of nm and give a conformant coating for larger surface features. Given the broad range of polyelectrolytes available, non-toxic, biodegradable and inexpensive coatings can be created with desired antiadhesive or virucidal properties.

Strategies for virus removal can be designed to weaken favorable electrostatic interactions between a virus and a surface. Specifically, MS2 could be detached from a PEM’s surface by either deprotonating the weak amino acids of MS2 and thus disrupting the electrostatic interactions between MS2 and PEM, or by screening favorable electrostatic interactions using a high ionic strength cleaning solution. An alternative approach can be based on designing the coating to be sacrificial – as long as one of the constituents of a PEM film is a weak polyelectrolyte, the multilayer can be removed from the surface by dissolving it at high pH [46, 87-89]. Viruses attached to such surfaces would be removed together with the sacrificial layer. In addition to surface cleaning such sacrificial coatings can find analytical uses in workplace safety monitoring and environmental forensics among other applications. Finally, the virucidal function of cationic polyelectrolytes can be brought to bear to coat surfaces in settings such as hospitals where environmental transfer is likely and of especial concern.
4. Conclusions

The premise of the paper is that understanding the relationship between virus deposition conditions and properties of the collector surface on the one hand and the deposition kinetics on the other hand can guide the design of specialty surfaces for regulating virus adsorption. Versatile, easy-to-apply and scalable coatings based on polyelectrolyte multilayers provide a suitable framework for exploring this premise. In this study, deposition of bacteriophage MS2 onto positively- and negatively-charged polyelectrolyte-coated surfaces was studied using QCM-D experiments and XDLVO modeling. The polyelectrolyte multilayers were terminated with a cationic (PDAD) or an anionic (PSS) polyelectrolyte and deposited from two different solutions (10 mM and 100 mM NaCl) yielding the total of four different surfaces. MS2 deposition on these films occurred in two distinct phases: the early phase 1 when the surface is relatively virus-free and phase 2 when the surface charge is affected by the virus accumulation on the surface. The overarching observation is that differently prepared surfaces showed distinct kinetics of and capacities for MS2 deposition supporting the hypothesis that polyelectrolyte multilayers can be designed to have tailored adhesive properties with respect to viruses.

Comparison of QCM-D observations with XDLVO predictions showed that the deposition rate correlated with the depth of the secondary minimum in the XDLVO energy profile. The depth of the minima is defined primarily by the acid-base and electrostatic interactions supporting the hypothesis that charge and hydrophilicity of the polyelectrolyte multilayers control virus adhesion. When the deposition occurs from high ionic strengths, the screening of electrostatic interactions makes the role of acid-base interactions more prominent emphasizing the importance of hydrophilicity as a surface design criterion.

Although not a part of the present study, detachment experiments can be used to corroborate the reversibility of virus-surface association via shallow secondary
minima and further explore the relative importance of electrostatic and hydrophobic virus-surface interactions. A more discriminating analysis of the relative importance of charge and hydrophilicity should be possible with a larger selection of surfaces covering a broader range and different combination of charge and surface energy values.

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