Electrochemical growth of composite hydroxyapatite coatings for controlled release

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Abstract

An electrochemical method was used to load ionic molecules into hydroxyapatite (HA) coatings during electrochemical synthesis. A metal cathode surface was first electrochemically coated with a thin layer of HA. Then, a second electrochemical coating step was conducted with non-reactive ionic molecules added to the synthesis solution. The loading and release from the HA films are driven in large part by the sign and valence of charge on the ionic additive. Cationic additives are attracted to the cathode surface and effectively loaded to form composite HA films, while anionic species are repelled from the cathode and not effectively incorporated. Divalent cations are released more slowly than monovalent cations. This simple method may be used to create drug eluting bioactive coatings for controlled release. The loading and release of streptomycin, a common antibiotic, was measured as a representative pharmaceutical example.

Keywords:

Hydroxyapatite, Electrochemical, Composite, Controlled release

1 **1. Introduction**

2 Hydroxyapatite (HA) is a crystalline form of calcium phosphate having the
3 same crystal structure as the inorganic component of bone and teeth tissue. Due
4 to its excellent biocompatibility and bioactivity, synthetic HA has been widely
5 used in orthopedic implant surgery to enhance osseointegration and accelerate

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6 bone growth [1, 2, 3, 4]. Unlike inactive materials, HA attaches directly to the
7 host bone and displays good fixation [5]. However, the poor mechanical proper-
8 ties of HA prevent it from being used alone in load bearing applications. Most
9 commonly, HA is employed as a thin coating on metallic orthopedic and den-
10 tal implants. Titanium-based alloys are relatively inert and provide mechanical
11 strength in implants, while the HA coating enhances bioactivity of the implant
12 surface. Many in vitro and in vivo studies have demonstrated that HA coatings
13 accelerate mineralization, enhance cell adhesion and proliferation, as well as pro-
14 mote osseointegration [6, 7, 8]. In addition, HA has been investigated as a drug
15 eluting coating to prevent bone infections following orthopedic surgery [9, 10].

16 It is often desirable to have composite coatings rather than pure HA in order
17 to improve the coating performance. Compounds can be incorporated into the HA
18 coating to improve chemical or mechanical properties, provide the coating surface
19 with additional functionality, or to allow the coating to act as a delivery vehicle
20 via elution from a composite HA matrix. For example, bone morphogenetic protein
21 has been incorporated into HA coatings and shown to enhance osseointegration
22 [11, 12, 13, 14]. Biomimetic composite coatings of HA with collagen fibrils were
23 created to study the interactions between HA nanocrystals with collagen and to
24 examine the ability to bind fibronectin [15]. A biocompatible composite of HA
25 with silver nanoparticles and chitosan was shown to provide corrosion protection
26 and simultaneously prevent microbial growth by the release of Ag^+ ions [16].

27 Coatings aimed at long-term use should have good chemical stability and
28 strong adhesion to the substrate. Many approaches, including plasma spraying,
29 sputter coating, electrophoretic deposition, electrochemical, hydrothermal, sol-gel
30 and biomimetic synthesis methods have been developed to fabricate HA coatings
31 on different substrates [17, 18, 19, 20, 21, 22, 23]. Electrochemical synthesis of
32 HA is wet chemical method that enables well crystallized coatings to be synthe-
33 sized on metallic substrates under mild conditions [24, 25, 26, 27]. In this method,
34 HA crystals grow from aqueous solution onto the surface of a metal cathode as
35 an electrical current is applied. The coating composition and morphology can be
36 adjusted by varying the applied electrical current, pH of the solution, temperature,
37 and concentrations of reagents. The electrochemical synthesis solution contains
38 dissolved calcium and phosphate ions, as well as a buffer to control pH and un-
39 reactive salt to increase electrical conductivity of the solution. When the electric
40 potential is applied, the local concentration of Ca^{2+} is enhanced near the cathode
41 surface due to electrostatic attraction. The applied voltage induces electrolysis
42 of water, which causes the formation of OH^- on the cathode surface. Due to the
43 local increase in pH and enhancement of Ca^{2+} concentration, HA nucleates and

grows selectively on the cathode surface. Recent reports demonstrate the formation of HA composite coatings through the electrochemical method. Carbon nanotubes added to the electrolyte solution can become incorporated into the coating to improve mechanical strength [28]. Added heparin has been electrochemically incorporated to reduce blood coagulation on the surface of the coating [29].

The applied field during electrochemical synthesis results in a sharp gradient of positive versus negative charge in the Helmholtz layer immediately adjacent to the cathode surface. The local field strength in the Helmholtz layer can easily exceed 10^6 V/cm, and the high energy density of this layer is the basis for electrical storage in double layer capacitors [30, 31]. Our group recently reported that the strong local fields result in an extremely large quantity of trapped ionic charge in electrochemically grown HA coatings [32]. In the present study, we modify the electrochemical synthesis of HA to trap non-reactive ionic species into the coating during synthesis. Several cationic and anionic dyes are investigated as model ionic compounds with varying number of charged groups. Dyes were chosen primarily based on their electrostatic charge (sign and valence) to provide a range of ionic species that are easily detectable using UV/Vis spectroscopy. The molecular structure of the four dyes investigated are shown in Figure 1. Rhodamine 6g and Methyl Green are cationic dyes with net charge of +1 and +2, respectively. Methyl Orange and Allura Red are anionic dyes with net charge of -1 and -2, respectively. Therefore, the loading and release behaviors of the dyes in composite HA coatings could be systematically examined with the aim of developing a novel method to produce bioactive coatings that act as controlled release vehicles. As a proof of concept for biomedical applications, the loading and release of an antibiotic, streptomycin, was also examined. This novel method allows for the rapid low cost synthesis of drug eluting coatings under mild conditions.

2. Experimental procedure

NaCl (>99.0%), K_2HPO_4 (99.99%), $CaCl_2 \cdot 2H_2O$ (>99%), Methyl Green zinc chloride salt (~85%), and tris-(hydroxymethyl)aminomethane (>99.8%) were purchased from Sigma-Aldrich. Hydrochloric acid (37%) was purchased from Mallinckrodt Chemicals. Titanium foil (Ti, 0.89 mm thick), platinum foil (Pt, 0.127 mm thick) and Methyl Orange were obtained from Alfa Aesar. Rhodamine 6g was purchased from EMD Millipore. Allura Red AC was obtained from TCI America. Streptomycin sulfate (USP grade) and phosphate buffer solution (PBS) were obtained from Amresco. The PBS pH was 7.2-7.4, and it had the following

Figure 1: Molecular structures of (a) Rhodamine 6g, (b) Methyl Green, (c) Methyl Orange, and (d) Allura Red.

79 composition: NaCl (137 mM), KCl (2.7 mM), Na₂HPO₄ (10 mM), KH₂PO₄ (1.8
80 mM).

81 The surface of the Ti substrate was first seeded with HA crystals following
82 a previously reported electrochemical synthesis procedure [33, 21]. In brief, a
83 pre-cleaned Ti plate (12.5 x 12.5 x 0.89 mm) was used as the cathode while a
84 Pt foil (25 x 25 x 0.127 mm) was used as the anode for electrochemical deposi-
85 tion. An electrolyte solution was prepared by dissolving 138 mM NaCl, 50 mM
86 tris-(hydroxymethyl)aminomethane, 1.3 mM CaCl₂ and 0.84 mM K₂HPO₄ suc-
87 cessively in deionized water. The solution was buffered to pH=7.2 with 37% hy-
88 drochloric acid. Then the anode and cathode were immersed into the electrolyte
89 solution that was heated in an oil bath. When the temperature of the solution
90 reached ~95°C, a constant current density of 12.5 mA/cm² (relative to Pt elec-
91 trode area) was applied for 2 minutes.

92 To load ionic molecules, the sample seeded with HA crystals was removed
93 from the solution described above and placed into a second solution having iden-
94 tical concentrations of all reagents except that NaCl was omitted and either 1 mM
95 of dye or 10 mM of streptomycin were added. Then electrochemical deposition
96 was carried out for an additional 5 minutes using a constant current density of 12.5
97 mA/cm² while the solution was maintained at ~95°C and pH=7.2. To examine if
98 loading is possible through physical adsorption from the solution containing dye
99 or antibiotic, parallel experiments were conducted without applying electrical cur-
100 rent. In these control experiments, all experimental variables held constant except
101 electrical current was not applied.

102 To measure controlled release, samples were placed in 1.5 ml of phosphate
103 buffer solution at 37°C. After a predetermined period of time, the buffer solution
104 was collected for analysis and replaced with fresh solution. All of the collected
105 samples were placed in a dark area prior to analysis. The amounts of Rhodamine
106 6g, Methyl Green, Methyl Orange and Allura Red were quantified by detecting the
107 absorbance at different wavelengths, namely 527, 633, 464, 504 nm, respectively,
108 using a UV/VIS spectrometer (Lambda 900, Perkin Elemer, Inc.) The concentra-
109 tion of streptomycin released was analyzed by adding ferric ammonium sulfate
110 and detected at 524 nm [34]. Trace amounts of HA can be released into buffer so-
111 lution and affect the absorption spectra [35]. Therefore, HA blank samples were
112 prepared following the same procedure except the ionic dye or antibiotic was not
113 added to the solution. Spectra were measured relative to solutions collected from
114 the blank HA samples in parallel experiments. After 24 hours of controlled re-
115 lease measurement, the HA samples were taken out and dried until the weight did
116 not change. The residual amount of HA was tested by dissolving loaded and blank

117 samples into 2 ml of 0.5 M HCl.

118 The crystal structure was determined by X-ray diffraction (XRD) using a
119 Philips PW3020 with Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$). Four as-synthesized
120 Rhodamine 6g loaded HA samples were removed from the substrate using a
121 blade, and the powders were analyzed by XRD. The morphology of the sam-
122 ples was examined using a field emission scanning electron microscope (SEM,
123 Zeiss-Leo DSM982). All images were acquired using an accelerating voltage of
124 3 kV and a secondary electron detector. The chemical composition was analyzed
125 by an energy dispersive x-ray (EDX) spectrometer (Phoenix EDAX) under 15
126 kV accelerating voltage. For elemental analysis, samples were weighed with a
127 PerkinElmer Model AD-6 Autobalance and then compositions were determined
128 with a PerkinElmer 2400 Series II Analyzer. The purity of the samples was fur-
129 ther characterized by Raman spectroscopy. The Raman spectra were collected in
130 a back-scattering geometry using a 660 nm diode laser (Cobolt Flamenco 100)
131 providing ~35 mW of power at the sample. Scattered light was dispersed by
132 a triple monochromator (Princeton Instruments Trivista 555, with 300/300/600
133 gr/mm gratings blazed at 750 nm) in subtractive mode. Light was collected with
134 a TE cooled CCD camera (Princeton Instruments, Pixis 400BR Excelon). Each
135 spectrum was obtained from 10 scans with exposure time up to 120 s. Three
136 spectra were obtained for each spot and the average intensity was used in plots.

137 3. Results and Discussion

138 Electrochemical loading of the HA coatings was first attempted in a single
139 step. As an example, Fig. 2a shows an SEM image of a sample on Ti prepared by
140 a single 5 minute electrochemical synthesis of HA from the solution containing 1
141 mM Rhodamine 6g. After deposition, spheres of 2–8 μm in diameter are observed
142 along with smaller aggregated particles. The spheres appear smooth and amor-
143 phous, indicating that they are not comprised of crystalline HA. On the surface of
144 the spheres and in the interstitial regions appear aggregated crystalline particles
145 having a morphology consistent with that of HA based on previous examples of
146 electrochemical synthesis [33, 21]. The carbon peak in the EDX spectrum of this
147 sample (Fig. S1 of supplementary data) is much stronger than the peaks of Ca,
148 P, and O, indicating a significant fraction of the deposited material is comprised
149 of organic material. The SEM and EDX data suggest that the observed spherical
150 particles are comprised of deposited Rhodamine 6g. To confirm that Rhodamine
151 6g is deposited as large spherical particles during the electrochemical synthesis,
152 a control experiment was carried out by using 1 mM of Rhodamine 6g alone as

the electrolyte solution and applying the same current density for 5 minutes. Fig. 2b shows that large spherical particles are deposited in this control experiment, and none of the smaller crystalline particles are present. The results shown in Fig. 2 indicate that Rhodamine 6g is effectively deposited onto the Ti surface during the electrochemical process. However, it is clear that the deposition of the dye competes with HA. The dye can therefore disrupt the nucleation and growth of HA onto the Ti surface.

Figure 2: SEM images of samples on titanium after a single 5 minute electrochemical stage. (a) Codeposition of HA and Rhodamine 6g. (b) Deposition of Rhodamine 6g alone.

In order to ensure that a bioactive HA surface is first covering the Ti prior to loading the ionic additive, a two-stage synthesis procedure was developed. In the first stage, a standard electrochemical reaction to synthesize HA is carried out for 2 minutes. It was found that the short 2 minute reaction enabled a uniform coating of HA nanocrystals to be deposited onto the surface, as shown in Fig. 3a. The morphology of the coating is similar to other reports of electrochemical growth of HA under slightly longer reaction times [33, 21]. The HA nanocrystals appear rod-shaped with the long axis associated with the crystallographic c-axis [20]. In the second stage, a 5 minute reaction was carried out with 1mM Rhodamine 6g added to the electrolyte solution. Sodium chloride was not added to the electrolyte solution during the second stage in order to reduce competitive adsorption of the non-reactive salt. Fig. 3b shows the composite HA/Rhodamine 6g layer deposited using this two stage approach. The rod shaped HA crystals are visible, along with smooth amorphous material interspersed between the HA crystals. The material that appears amorphous is presumed to be Rhodamine 6g, as the coating is visibly deep red in color. The XRD spectrum from this sample confirms that the only

176 crystalline phase detected is HA according to PDF Card no. 9-432 (Fig. S2 of
177 supplementary data).

178 In comparing Fig. 2 and Fig. 3, it can be seen that the two stage approach
179 results in a more uniform distribution of the Rhodamine 6g into the coating. The
180 HA crystal growth in the first stage is due to the standard electrochemical crystal-
181 lization mechanism [26]. Crystal nucleation and growth is understood to be due to
182 enhancement in local concentration of calcium ions from electrostatic attraction to
183 the cathode combined with the local increase in pH due to water splitting. As a re-
184 sult of the combined effects of concentration and pH changes, calcium phosphate
185 becomes locally supersaturated and nucleates selectively as HA on the cathode
186 surface. In the second electrochemical stage, the HA crystals on the cathode sur-
187 face act as seeds to promote additional crystal growth. The cationic dye is also
188 attracted to the cathode as a result of electrostatic attraction. It is believed that
189 dye adsorption competes with HA crystal growth in the second stage, resulting in
190 a composite film. Since the surface is uniformly coated with HA in the first stage,
191 this two stage approach ensures that a bioactive HA layer will remain after release
192 of the dye.

Figure 3: SEM images of coatings on titanium formed through the two stage electrochemical synthesis method. (a) The HA layer produced in the first stage (b) The composite HA/Rhodamine 6g layer after the second stage

193 Fig. 4 shows the EDX patterns of all composite coatings. As expected, strong
194 peaks from the oxygen, phosphorus and calcium atoms of HA are detected. Strong
195 carbon peaks are observed from samples containing cationic dye and strepto-
196 mycin, as seen in Fig. 4a, 4b, and 4e. The carbon peaks indicate that cationic
197 organic molecules are loaded into the HA coatings. In contrast, the carbon peak is
198 nearly undetectable for the samples formed with anionic dyes, as seen in Fig. 4c

and 4d, indicating little incorporation of the anionic organic compounds. In addition, it should be noted that additional peaks that do not correspond to either HA or the organic compound are detected from the Methyl Green and streptomycin samples. For Methyl Green, the additional peak is attributed to electrochemically reduced zinc from the Methyl Green zinc chloride salt. The ZnCl_2 from the Methyl Green salt is dissolved into the electrolyte solution and likely some of the Zn^{2+} is electrochemically reduced to elemental Zn^0 at the electrode surface. With the streptomycin sample, an additional peak is observed that is attributed to sulfur. The presence of sulfur is not unexpected since streptomycin was added as the sulfate salt.

Elemental analysis was used to obtain a more detailed examination of the chemical composition of the composite coatings, as shown in Table 1. The carbon and hydrogen content correlates to amount of the organic compound loaded into the coating. The highest carbon and hydrogen content was obtained with Rhodamine 6g loaded in a single step. Although the loading is very high, deposition of Rhodamine 6g in a single step disrupts the formation of HA on the surface, as illustrated in Fig. 2a. The seed crystals formed in the first stage provide sites for additional growth of HA in the second stage electrochemical reaction [23]. For the dyes loaded in the two stage process, the carbon content obtained from elemental analysis follows the same trend found with EDX. The cationic dyes have much higher loading than the anionic dyes, with the divalent cation Methyl Green having a slightly higher loading than the monovalent cation Rhodamine 6g. The carbon and hydrogen content of the two anionic dyes, Methyl Orange and Allura Red, are similar to each other and much lower than that obtained with the cationic dyes. Table 1 also shows the elemental analysis of a control sample of HA formed in the same two stage synthesis except that no dye was added in the second stage. Surprisingly, HA has measurable carbon content without the addition of organic additives. The samples formed with anionic dyes have similar carbon content as the control HA sample. The results indicate that the measured carbon content from Methyl Orange and Allura Red samples comes from the HA itself rather than any significant loading of the anionic dyes. The carbon measured from the HA control sample shown in Table 1 likely is the result of carbonate incorporated into the apatite crystal structure. Previous studies have shown that carbonate ions may be included into electrochemically synthesized HA from CO_2 dissolved in the synthesis solution from air [25, 36]. Carbonate may be avoided by conducting the electrochemical synthesis in a N_2 atmosphere, but all reactions in the present study were carried out in air.

Additional analysis of the chemical composition of the composite films were

Figure 4: EDX spectra of composite coatings formed by two stage electrochemical deposition of HA with (a) Rhodamine 6g, (b) Methyl Green, (c) Methyl Orange, (d) Allura Red, and (e) Streptomycin

Sample	Carbon [wt%]	Hydrogen [wt%]
Rhodamine 6g (one step)	37.7	4.5
Methyl Green	17.7	2.6
Rhodamine 6g	13.5	1.5
Methyl Orange	4.1	1.0
Allura Red	4.3	1.2
HA	4.3	1.0

Table 1: Weight percent of carbon and hydrogen from elemental analysis.

carried out using Raman spectroscopy. The Raman spectra were obtained for two samples. The first was the sample loaded with Rhodamine 6g following the two stage synthesis method. The second sample was a control of HA synthesized following the same two stage procedure except that no organic additive was added in the second stage. Raman spectra for the two samples are shown in Fig. 5. For the HA control sample shown in Fig. 5a, peaks located at 960, 1045, and 1056 cm^{-1} are ascribed to stretching modes of phosphate groups. In addition, peaks assigned to the bending mode of phosphate groups appear in the range of 400 to 600 cm^{-1} . Additional peaks near 620, 648, 1300, and 1412 cm^{-1} have been ascribed in previous studies to carbonate incorporated into HA [37]. The Raman spectrum in Fig. 5a provides supporting evidence for the presence of carbonate that is likely responsible for the carbon content measured from elemental analysis of the HA control sample shown in Table 1. The Raman spectrum in Fig. 5b shows a number of intense peaks that can be ascribed to Rhodamine 6g for the sample loaded with dye. The Raman spectrum confirms the loading of Rhodamine 6g into the sample, as is consistent with EDX and elemental analysis.

The total amount of Rhodamine 6g in the composite coating, as well as the release rate into PBS was determined by UV/Vis spectroscopy. A control sample was formed using the identical two-stage procedure except that electrical current was not applied in the second stage in order to test for physically adsorbed dye. The total amount of Rhodamine 6g loaded was determined from UV/Vis spectroscopy after completely dissolving both the dye and HA in acidic solution. Fig. 6a shows that the total amount of Rhodamine 6g is 20 times larger in the sample formed with electrochemical loading than in the control sample formed by physical adsorption of the dye from solution. Fig. 6b shows the cumulative release of dye into PBS at 37°C versus time. For the physically adsorbed Rhodamine 6g, over 90% is released in the first hour and ~100% is released within the first 4 hours. The electrochemically loaded sample shows a burst release, with nearly

Figure 5: Raman spectra of HA coatings formed by two stage electrochemical synthesis of (a) control sample without organic additive and (b) sample loaded with Rhodamine 6g.

265 20% released within the first hour. After the first hour, the release is much slower
 266 with only ~27% released after 24 hours. Fig. 6c shows a representative image
 267 of two samples after being submerged for 24 hours in PBS at 37°C. The sample
 268 with electrochemically loaded Rhodamine 6g remains bright red in appearance,
 269 while the sample that had physically adsorbed dye is colorless. The electrochem-
 270 ical loading method results in the crystallization of additional HA in addition to
 271 the electrostatic attraction of the cationic Rhodamine 6g to the negative cathode
 272 surface. As a result, it is likely that a portion of the dye is embedded in the HA
 273 matrix as well as being physically deposited on the surface. In drug delivery
 274 applications, such an embedded active ingredient would allow sustained continu-
 275 ous release by diffusion through the HA matrix. In addition, the HA matrix may
 276 undergo a dissolution or dissolution-recrystallization processes depending on the
 277 pH, ionic strength, and composition of the surrounding medium that would enable
 278 one to control the release rate.

279 Rhodamine 6g is a cationic dye, as illustrated in the molecular structure shown
 280 in Fig. 1a. It is hypothesized that the loading of Rhodamine 6g is driven by
 281 electrostatic attraction of the cationic dye to the negatively charged anode surface
 282 during the electrochemical reaction. To explore this hypothesis, the two stage
 283 electrochemical loading process described above was repeated on a series of dyes
 284 having varying ionic charge. Rhodamine 6g has a single positive charge, Methyl
 285 Green has two positive charges, Methyl Orange has a single negative charge, and
 286 Allura Red has two negative charges. The total loading obtained with all four dyes

Figure 6: Comparison of electrochemically loaded versus physically adsorbed Rhodamine 6g (a) The total amount loaded (b) The percent released versus time into PBS at 37°C (c) Photograph of representative samples after 24 hours of immersion in PBS at 37°C. The red colored sample is electrochemically loaded and the colorless sample has physically adsorbed Rhodamine 6g.

is illustrated in Fig. 7a, as measured by UV/Vis spectroscopy after dissolving the coatings in acidic solution. It is apparent that the ionic charge on the dye plays a major role in determining the effectiveness of electrochemical loading. The amount of Methyl Green, which has two positive charges, is ~25% higher than that obtained with Rhodamine 6g. As expected, there was very little loading for both anionic dyes. Images of the samples taken with electron microscopy are consistent with the measured dye loading (Fig. S3 supplementary data). In the case of electrochemical loading of the cationic dye Methyl Green, SEM images show rod-shaped HA crystals with a significant amount of smooth amorphous material between the crystals, indicating that a composite layer is formed. On the other hand, very little amorphous looking material appeared on the SEM images of the samples formed with anionic dyes. With Methyl Orange, there was sparse coverage of amorphous particles on the surface of the HA coating. For Allura Red, the rod shaped HA crystals were visible with almost no visible evidence of amorphous material.

Figure 7: Loading and release of various ionic dyes from composite HA films. (a) total amount loaded and (b) release kinetics into PBS at 37°C.

The release of the various dyes over time into PBS solution at 37°C was measured, as shown in Fig. 7b. Distinct differences in release rates are observed between cationic and anionic dyes. Even though very little anionic dye was incorporated into the films, some release of Allura Red and Methyl Orange was detected in the first hour. After the initial burst release, no additional release of anionic dyes was detected in 24 hours. In contrast, Fig. 7b shows that the cationic dyes Rhodamine 6g and Methyl Green have a long extended release following the initial burst release in the first hour. The cationic dyes are attracted to the negatively charged cathode, and incorporated into the growing HA film during

311 electrochemical synthesis. Release of these molecules therefore must occur in
312 part through diffusion through the composite film rather than only from the more
313 rapid dissolution from the surface of the film. In addition, it is known that the
314 surface of the HA layer formed in the first stage of the process is rich in nega-
315 tively charge PO_4^{3-} and OH^- groups [32]. Therefore, it is expected that some of
316 the cationic dye is strongly bound to the film through ion pairing with anionic
317 species. Any ion paired dyes would be released through a slower ion exchange
318 mechanism, which is supported by the release results. After 24 hours, only 4% of
319 the divalent Methyl Green is released compared to 27% of the monovalent Rho-
320 damine 6g. The ion exchange process would be much slower with divalent cations
321 in comparison to monovalent cations. The combined effects of both diffusion and
322 ion exchange likely govern the release of the cationic dyes from the composite
323 film.

324 One potential application of the electrochemical loading process is in fab-
325 ricating drug eluting coatings for controlled release applications. Streptomycin
326 sulfate was chosen as a model drug compound to examine electrochemical load-
327 ing and release from composite HA films. Streptomycin is a common antibiotic
328 in the aminoglycoside family commonly used to treat infections of aerobic gram-
329 negative bacteria [38]. The ionic charge of the streptomycin molecule is pH de-
330 pendent, as it has four primary amino groups, a carboxylic acid group, and one
331 secondary amino group. The amino groups are partially neutralized in the sulfate
332 salt, giving an overall cationic charge on the molecule. The two-stage electro-
333 chemical synthesis procedure was used to load streptomycin sulfate into compos-
334 ite HA films. In the second stage, 10 mM of streptomycin sulfate was used in
335 the electrochemical synthesis solution. The concentration of streptomycin sul-
336 fate was 10 times higher than that used for the dyes due to the lower signal from
337 the colorimetric detection of streptomycin. Fig. 8a shows the total amount of
338 streptomycin loaded into the film compared to streptomycin physically adsorbed
339 onto the HA film in a parallel control experiment. The electrochemical process
340 resulted in ~21% higher loading of streptomycin compared to physical adsorp-
341 tion. The release of loaded streptomycin into PBS at 37°C is shown in Fig. 8b.
342 The release profile for both the electrochemically loaded and physically adsorbed
343 streptomycin are similar, with error bars overlapping for most time points mea-
344 sured. However, after 24 hours there is slightly lower fraction released from the
345 electrochemically loaded sample (76%) compared to the sample with physically
346 adsorbed streptomycin (82%). Unlike the dye molecules examined, significant
347 loading of streptomycin is obtained by simple physical adsorption and there are no
348 significant differences in release rates from physically adsorbed samples in com-

349 parison to electrochemically loaded samples. The higher concentration of strep-
 350 tomycin used during loading may be responsible in part for the increased physical
 351 adsorption in comparison to the dyes. In addition, properties of the molecular
 352 structure other than ionic charge may be important in determining the effective-
 353 ness of physical adsorption as a route to load the HA films.

Figure 8: Loading and release of electrochemically deposited streptomycin in comparison to physically adsorbed streptomycin. (a) total amount of streptomycin loaded and (b) release kinetics of streptomycin into PBS at 37°C.

354 In the electrochemical coating process, it is important to note that the local
 355 pH near the cathode surface is significantly different from the bulk pH of the so-
 356 lution. The electrolysis of water causes the continuous formation of OH^- anions
 357 during the electrochemical reaction. High local pH is required in order to form
 358 the HA crystal phase at the cathode surface. Otherwise, other phases of calcium
 359 phosphate would form, such as dicalcium phosphate $[\text{CaHPO}_4]$ or octacalcium
 360 phosphate $[\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4 \cdot 5\text{H}_2\text{O}]$ [39]. Mobility data show that Rhodamine
 361 6g is fully ionized in the pH range of 4 to 10 [40]. Therefore, Rhodamine 6g
 362 is expected to maintain its positive charge in the high pH environment near the
 363 cathode surface. Streptomycin, on the other hand has a pK_a value of 8.7 [41]. Al-
 364 though streptomycin carries a positive charge in the electrolyte solution ($\text{pH}=7.2$),
 365 the charge likely becomes neutralized at higher pH near the cathode surface. As a
 366 result, electrochemical loading may be less effective with streptomycin than Rho-
 367 damine 6g.

368 4. Conclusions

369 A novel and simple electrochemical approach to incorporate ionic molecules
370 into hydroxyapatite coatings was illustrated. The well established electrochemi-
371 cal coating of HA onto a cathode surface was modified to trap nonreactive ionic
372 species into the HA film. Cationic species are attracted to the cathode and ef-
373 fectively incorporated into the HA film, while anionic species are repelled from
374 the cathode and are not loaded into the films. Some of the cationic additive likely
375 forms ion pairs with anionic species in the HA film. The release from the resulting
376 composite films appears to be governed by a combination of diffusion through the
377 film and ion exchange.

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