Investigation of In-situ Immobilization of Contaminated Sediments using Alginate Gels for the Reduction of Ecological Risk

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Risk

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Investigation of In-situ Immobilization of Contaminated Sediments using Alginate Gels for the Reduction of Ecological Risk

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Abstract

Remediation methods for contaminated sediments include dredging, treatment and disposal and sediment isolation through capping. These methods are costly and adversely impact local ecosystems, in some cases causing major disturbances to the sediment bed and water column. A new remediation technique is proposed for slowly biodegradable contaminants using alginate hydrogels as a binding agent of sediment. The rational is that sediment particles bound in alginate hydrogel are less likely to be resuspended and the treatment is less disruptive than capping. Here we show addition of alginate increased the shear strength of sediment comprised of defined particle sizes. The increase in strength becomes more pronounced as the normal stress is increased. Alginate's ability to trap contaminants in its structure was found to be pH-dependent, with more effective contaminant sequestration at lower pH. Lab scale tests on contaminant transport from alginate bound sediment showed Naphthalene diffusion from sediment was reduced.
1. Introduction

An estimated 10% of all sediment in lakes, rivers and bays in the United States contain chemicals that can adversely affect organisms living in or on the contaminated sediment, including humans that eat contaminated fish and shellfish (McCauley, DeGraeve, & Linton, 2000). Commonly sediments are found to contain persistent organic pollutants and heavy metals. The most contaminated sediments are generally found in places of high ship traffic and industrial activities as well as places with limited flow such as are often found in harbors and canals (Daskalakis & O'Connor, 1995).

Current sediment remediation methods often involve dredging to remove the contamination or capping, whereby the contaminated sediment is isolated under a layer of clay, sand or gravel several inches to feet deep. Both of these methods are costly at large scales. In the case of dredging, the removed material is still hazardous and must be disposed of properly. Additionally, dredging has the potential to spread contamination due to sediment resuspension. Both methods are extremely damanging of the benthic environment (Ghosh, et al., 2008).

Hydrophobic contaminants accumulate in sediments because contaminants with low aqueous-solubility are transported through the environment adsorbed to particulates (McGechan & Lewis, 2002). These particulates have been shown to enhance the transport of low-solubility contaminants and plays an important role in the movements of metals (Ren & Packman, 2001). Once particulates are incorporated into benthic sediment, they can be resuspended through the shearing action of water on the sediment surface (Nearing & S.C., 1994). Finer particulates are easily resuspended and can be carried out of the sediment by flow (Ren & Packman, 2001).

An in-situ approach to prevent the resuspension of sediment while leaving the benthic environment largely intact is needed. It is proposed that contaminated sediment be bound using alginate. Alginate is a naturally occurring anionic polysaccharide found in the cell walls of brown algae.
It is a biocompatible hydrogel with an established industry and major suppliers located around the world (Saether, Gjertrud, Smidsrod, & Stokke, 2008) (Guide to seaweed industry). Industrial uses include thickening agents, stabilizing agents for products like ice cream and for the creation of dental molds (Guide to seaweed industry). Alginates are also used in the medical field as bandages that maintain moisture in the wound without encouraging bacterial growth. Additionally it is used in the biomedical and biotechnology fields to encapsulate cells (Yalpani, 1987) (Smidsrod & Gudmund, 1990).

Alginates are versatile due to their composition of linear copolymers, β-D-Mannuronic acid (M residues) and α-L-guluronic acid (G residues) which are 1,4 linked into blocks (see Figure 1A). M-blocks (two or more M residues) form straight chains while G-blocks (2 or more G residues) form a "zig-zag" chain (see Figure 1B) (Webber & Shull, 2004). Gels are formed via polyvalent cation cross-linking (commonly Ca$^{2+}$) (Chan, Jin, & Heng, 2002). Crosslinking follows the "egg-box model" where the cation is cradled in the G blocks (see Figure 1C) (Smidsrod & Gudmund, 1990). Therefore a higher ratio of G to M blocks generally results in stronger gels (Martinsen, Skjak-Braek, & Smidsrod, 1989).

With alginate being readily available and naturally occurring, it may be a unique, environmentally benign and sustainable solution to binding toxic sediments together as microbial biodegradation occurs. Calcium alginate treatment may actually promote biodegradation because oxygen and nutrients readily diffuse through the hydrogel (Yabur, Bashan, & Hernández-Carmona, 2007) while predators of bacteria including benthic ciliates are excluded (Matz & Kjelleberg, 2005). The alginate will serve as a substrate for bacteria as well and ultimately break down, leaving a less toxic sediment behind without entirely destroying the ecosystem that is present.

The aim of this investigation is to determine the suitability of sediment binding with alginate as a method of in-situ sediment remediation. To be a viable treatment, alginate should minimize sediment resuspension by reducing the rate of leaching from the sediment. This investigation shows that the
addition of alginate to sediment both increases the mechanical strength and decreases the amount of leached organics. Increased strength prevents mixing and shifting of sediment while decreased leaching is derived from favorable partitioning into the gel and reduced diffusion out of the gel.

2. Methods

2.1 Hardness testing

Hardness measurements were conducted using two indentors were made to have the type OO and OOO geometries. Additionally, these indentors were of an overall size to impart the required force of 113 gf (1.111N) (CCSi, 2006) by gravity. To use, the indentor was attached to a ringstand. A marking at one end was then aligned with the clamp and the whole assemblage lowered until the indentor tip made contact with the sample’s surface. The indentor was then released from the clamp and the degree of penetration measured with calipers as the distance between the clamp and marking on the indentor. The penetration can be converted to a Shor Hardness using the following:

\[ \text{Shor Hardness} = -40P + 100 \]  

(1)

Where \( P \) is the penetration in millimeters (CCSi, 2006).

2.2 Alginate Gel Preparation

2% w/v Alginate solutions were made using alginic acid labeled as low and medium viscosity from MP Biomedical (Santa Ana, California; 100g). Alginate solutions are then poured into the bottom of a 100mm diameter petri dish until filled, then Spectrapor 45 mm diameter (molecular weight cutoff 3500) dialysis tubing was spread over the alginate and held down using a petri dish cover whose center was removed, leaving an approximately 0.5 cm wide ring around the edge. The dish assembly was clamped together and immersed in a bath of 0.1M CaCl\(_2\). After curing overnight, the dialysis tubing was removed, the gels were flipped, and the clamped dishes were again immersed without the dialysis.
tubing for a total of 24 hours. After the 24 hour cure time, gels were placed under the durometer and tested multiple times across their surface following the method described above. Gels made in this manner were of uniform thickness with a slightly smaller diameter than the petri dish.

2.3 Determination of G/M ratio

Small amounts of the alginic acid was dissolved in deuterium oxide and analyzed in a Bruker 400 NMR Spectrometer according to the method described by Grasdalen et al (Grasdalen, 1983). In addition, to facilitate dissolving, samples were spun in the NMR prior to analysis.

2.4 Alginate Molecular Weight Determination

A 2% w/v alginate stock solution was prepared in 0.1M NaCl then diluted in 0.1M NaCl to obtain alginate concentrations of 0.5, 0.25, and 0.1 % w/v. The viscosity of each was measured using a Brookfield DV-E viscometer equipped with the SC4-13R(P)/18 small sample adapter/spindle set. Measurements were conducted with a spindle speed of 50 RPM at a temperature of 24 C. Average molecular weight \( M_w \) of the alginates were found using the Mark-Houwink equation:

\[
[\eta] = k' \times M_w^a
\]

where \([\eta]\) is intrinsic viscosity and \(k'\) and \(a\) are constants with published values of \(2.10 \times 10^{-3}\) and 0.97 respectively (Rinaudo & Graebling, 1986) (Lapasin & Pricl, 1999). Intrinsic viscosity was taken as the y-intercept of the Huggins/Kramer plot.

2.5 Sediment-gel Shear Testing

Sediment was collected approximately 100 yards downstream from the outlet of Ball Pond in New Fairfield, Connecticut and dried before being sieved into three size classes: 1.18 to 3.36 mm, 0.850 to 1 mm, and 74 to 177 µm. The sediment and 4 inch sections of 3 inch diameter PVC pipe were
autoclaved then capped with a Petri dish cover and the seam wrapped with parafilm. Sediment and alginate were added and mixed in the mold. A layer of 0.1M CaCl$_2$ salt solution was added over the sediment-alginate mixture. Because alginate contracts due to gelling, the sediment-gel mixture will pull away from walls of the mold. At this point, the sediment-gel was extruded into a CaCl$_2$ bath and left to cure for two days. Once the sediment-gels were cured, they were cut to size and fitted into the test chamber of a Humboldt Direct Residual Shear tester. Loose sediment was used as a control. Shear strength of pure alginate gels was not tested due to extreme difficulty in creating useable gels of appropriate size. Samples were tested while submerged with shear stress rates of 0.025 inches per minute with normal loads of 1, 2, and 4 tons per square foot (tsf). These loads were chosen to correspond to typical hydrostatic pressure at estuary depths. The most common type of estuary is a drowned river valley estuary which is typically 10 m deep increasing to 20-30 m deep at the mouth. Examples of this type of estuary include the Chesapeake and Delaware Bays (Perillo, 1996).

### 2.6 Solute Leaching from Gels

To measure leaching of solutes from treated sediment, gels were prepared as described above with 0.001% w/v methylene blue or 17.5 mg/L naphthalene added separately. Once cured, gels were cut into squares measuring 2.5 cm per side. Solutions of distilled water, half-strength and full-strength seawater were prepared and used as the receiving solutions for the analytes. Full-strength and half-strength seawater solutions were prepared using Instant Ocean™ following package instructions. A block of gel was pinned to the bottom of a container with 400 mL of background solution. The solution was constantly stirred and samples taken periodically. Distribution coefficients were calculated using the following equation:

$$k_d = \frac{[\text{gel}]}{[\text{solution}]}$$
where [solution] is the concentration of analyte in the background solutions and [gel] and the calculated concentration of analyte left in the gel.

A second set of similar tests were run with buffered background solutions. Tests involving methylene blue were buffered at a pH of 5.8 with Bis-Tris. Tests involving naphthalene were buffered at the same pH with an acetate buffer. Analyte concentrations were measured using a Genesys 10S UV-Vis spectrophotometer set to 400 nm for methylene blue and 221 nm for naphthalene (Wu & Chern, 2006) (Naphthalene). Sediment gels for diffusion tests were made in a similar manner as described above for gel diffusion. Enough alginate was added to the sediment to completely fill the pores before immersion in the CaCl₂ bath. Sediment gel diffusion tests were conducted only in buffered solutions as described above.

2.7 Analyte Adsorption

Sorption of methylene blue and naphthalene to sediments were measured with batch iotherms. Approximately 1, 2, and 3 grams of sediment were massed into polypropylene centrifuge tubes in the case of Methylene Blue or glass vials in the case of Naphthalene. Then 40 mL of Methylene Blue solution or Naphthalene solution were added and each tube was sealed and shaken for 2 hours. Next the supernatant was sampled and filtered through a Fisherbrand PTFE 0.45μm syringe filter to remove particulates and absorbance was measured with the UV-Vis spectrophotometer. The adsorbed concentration was calculated from the difference between the initial and final concentration per mass of sediment used. This was then plotted against the final concentration and the slope of a best fit line taken as $K_d$, the sorption distribution coefficient (Wilhem, 2004). Additionally, in the case of methylene blue, changes in adsorption over time were examined as well. A similar method as described above was used with 50 mL of solution used instead of 40 mL. Samples of 10 mL were drawn periodically and
treated as described before with $K_d$ found for each time. Naphthalene was not tested in this manner because it was found not to adsorb significantly after 2 hours.

3. Results and Discussion

3.1 Gel Characterization

*As can be seen in Table 1,* the medium viscosity alginate creates a gel that is more rigid than the low viscosity alginate and thus was expected to have a higher G/M ratio. Contrary to expectations, the low viscosity alginate has a G/M ratio that is nearly twice as high as that of the medium viscosity alginate. Additionally, the low viscosity alginate's GGG-block frequency is nearly twice that found in the medium viscosity alginate. This would indicate that the low viscosity alginate has nearly twice as many potential cross-linking sites as the medium viscosity alginate and so should produce stronger gels. *An examination of Table 1 reveals a possible explanation.* Low viscosity alginates have nearly twice the frequency of MG-blocks, which are or flexible than MM or GG-blocks (Kong, Smith, & Mooney, 2003), when compared to medium viscosity alginate. This results in a more flexible chain compared to medium viscosity alginate resulting in softer gels. Chain length is an important factor in the gel's hardness.

Table 1 shows the calculated average molecular weight ($M_w$) of the two alginates. Medium viscosity alginate chains are nearly twice as long, allowing for greater entanglement and thus more interactions between chains. Most likely, the combination of all the above effects are responsible for low viscosity alginate being weaker than medium viscosity alginate, despite the higher frequency of GG and GGG-blocks.

3.2 Effect of Alginate on Sediment Shear Strength
The addition of alginate has increased the strength sediment and increased its resistance to shear. Peak shear was taken at the highest load exhibited, either at the end of the shear test (in the case of gelled sediments), or at a plateau in the load versus displacement plot (in the case of loose sediment) as shown in Figure 2. Figure 3 demonstrates that while both alginates increased peak shear, the medium viscosity alginate appears to have a larger impact on peak shear strength. In the case of both alginates, the effect of their addition is larger at lower normal stresses. Loose sediment demonstrated complete shearing shown by the plateauing of the load exerted vs displacement plot. The load does not return to zero due to friction between the halves of the sample caused by the normal load. In contrast, gelled sediment did not exhibit this behavior. The displacement could not be extended further due to limitations in the equipment. Figure 3 also shows that increased normal load lead to increased peak shear. This suggests that deeper sediments could be even better candidates for this technique. Overall, the increased shear strength of the sediment-alginate composite suggests a reduced propensity for contaminant resuspension, even in the presence of flowing water.

### 3.3 Contaminant Diffusion from Pure Gels

Figure 4 shows the change in concentration of methylene blue and naphthalene out of the alginate gels over time through diffusion. In each case, there is a clear separation of the equilibrium concentration dependent on background ionic concentration (no ionic concentration for distilled water and high ionic concentration in the case of full sea water). It was thought that the higher concentrations of ions in the receiving solution (DI water, half or full seawater) would cause Ca\(^{2+}\) ions, employed in cross-linking, to diffuse out or exchange of the gels at a faster rate, especially since the Instant Ocean is largely sodium chloride. This is not true however as the final concentration and not the rate of diffusion is affected. For each receiving solution, the final concentration is reached at approximately the same time.
The pH was then thought to be the controlling factor and is important in the consideration to use alginate in remediation. Estuaries have variable pHs resulting from the mixing of salt and freshwater. Lower pHs are found closer to sources of freshwater (rivers, streams) while salt water has higher pHs (8.0-8.6 typically). Other factors contributing to pH are bacterial and algal activity, and the natural constituents of runoff entering the estuary. Human factors can include sewage overflow and industrial outflows (EPA, 2006). These results show that the use of alginate should be limited to systems or areas of lower pH. Systems or areas with higher pH would appear to partially or even completely negate the benefits of alginate's ability to limit diffusion of contaminants.

The pH was monitored during the diffusion tests for low viscosity alginates. At the start of testing, the pH of the distilled water was 5.73 and 5.71 for naphthalene and methylene blue respectively. At the end of testing, the pH was 6.52 and 6.83 respectively. The final pH for full sea water was found to be at 8.13 and 8.38 for naphthalene and methylene blue respectively having started at a pH of 8.95 and 9.02. Half sea water had a pH similar to that of full sea water at 7.83 and 8.16 for naphthalene and methylene blue respectively from a starting pH of 8.92 and 8.96 respectively. This demonstrates a possible correlation between the pH of the receiving solution, and the final concentration, and was investigated by repeating the tests under buffered conditions. Figure 5 shows that once all receiving solutions were buffered to the same low pH the final concentration reached was statistically identical, achieving similar equilibrium levels seen in the unbuffered DI water tests, which had similar pHs. This demonstrates that pH is a controlling factor for diffusion of the analytes from the gels and not the ionic strength as expected, at the time scales tested. This is in agreement with the literature where the link between pH and diffusion of macromolecules from alginate has been demonstrated; that lower pHs result in less degradation and swelling of gels. (Gombotz & Fong Wee, 1998) (Sugawara, Imai, & Masaki, 1994) Less swelling results in lower free volume in the gel and
restricted molecular movement (in this case methylene blue and naphthalene) within the gel (Sugawara, Imai, & Masaki, 1994).

Table 3 shows calculated distribution coefficients of naphthalene and methylene blue between the alginates and receiving solutions. Both alginates behave similarly with respect to naphthalene. As the pH increased (from distilled water to full-strength seawater) the distribution coefficient decreased showing less naphthalene being trapped in the gel. Methylene blue also exhibits this behavior but has a significant difference between the alginates. Medium viscosity alginate exhibits much lower coefficients with no methylene blue being retained by the gel in the full-strength sea water (and highest pH). Methylene blue's positive charge may allow it to interact with the alginate's G-blocks much as Ca\(^{2+}\) does. More of the methylene blue is retained by the low viscosity alginates as it has a higher frequency of G-blocks.

### 3.4 Contaminant Diffusion from Sediment-gels

The addition of sediment to the alginate adds the possibility of interaction between the surface of cleaned sediment and the analytes. Table 2 lists calculated sorption coefficients for different analyte-sediment size combinations. Methylene blue is a positively charged molecule and as such is expected to interact with the negatively charged organic matter in the sediment. This is demonstrated best with the smallest particle size, the 74-177 µm sediment. This can be seen in Figure 6. At such small sizes, the surface area per volume is high, resulting in more adsorption then with larger particle sizes (Kehew, 2006) Figure 6 shows the change in \(K_d\) over time, demonstrating that two hours are sufficient for maximum adsorption. At the start of the diffusion tests, the sediment will have achieved maximum adsorption, similar to actual situations where the contaminant has been resting for a period of time. Due to naphthalene's lack of charge, it is not expected to interact with the sediment. This is
demonstrated in Table 1. After 2 hours, calculated sorption coefficients are zero or negligible in the case of the 74-177 µm sediment.

Diffusion of methylene blue out of the sediment gels was negligible, as seen in 7. The diffusion of naphthalene is slower than when compared to diffusion in unsedimented gels. In gels without sediment, an equilibrium concentration is established within 50 minutes. In comparison, naphthalene concentrations take nearly twice as long to equilibrate in tests with gelled sediment. This is attributed to the added tortuosity and lower effective porosity (ie. the gel portion of the gel-sediment composite) of a gel containing sediment since the naphthalene cannot diffuse through the sediment itself. This is most evident between 1.18-3.36mm and 0.85-1mm sediment gels. The distribution of sediment size in the 1.18-3.36mm range favors the larger size resulting in a larger size difference between the 1.18-3.36mm and 0.85-1mm sediment then between the latter and the 74-177µm. Since tortuosity increases with decreasing sediment size at a maximum fill fraction, there is a greater difference in the tortuosity of the 1.18-3.36mm sediment and the 0.85-1mm sediment.

A comparison of both gels reveals little difference between the release of naphthalene. This is likely due to the addition of sediment. It is possible that the alginate has only minimal interactions with the sediment and only encapsulates the particles. It has been noted that when handling sediment-gels, some particles of sediment would freely dislodge from the alginate matrix. This was most apparent with sediment in the 1.18-3.36mm range and would result in the creation of additional surface area for leaching.
4. Conclusion:

Alginate encapsulation is a possible alternative to capping for submerged sediment remediation. Addition of alginate to sediment increases the sediment's resistance to shearing, limiting the removal of sediment. This is most important for contaminants that adsorb strongly to the sediment as their movement through the environment will be dependent on the sediment's transport. This is demonstrated by methylene blue not leaching from the alginate in the presence of sediment. In the case of contaminants that interact with sediment weakly or not at all, alginate hinders leaching by trapping a portion of the contaminant. At lower pHs there is favorable partitioning of both methylene blue and naphthalene into the gels. This partitioning would keep more contaminant in contact with sediment microbes for more complete biological breakdown. If there is no biological breakdown of the contaminant, the alginate can possibly release it at a rate that is harmless to the environment. Future work necessary to advance this method is to control the degradation of the alginate. Additionally, the affect of alginate addition on the oxygen levels in the sediment will need to be investigated, as alginate is a potential food source for microbes and could cause a 'bloom' and anoxic conditions.
Works Cited


Appendix A: Tables:

Table 1: Characteristics of the gels that were used.

<table>
<thead>
<tr>
<th>Gel Characteristics</th>
<th>Low Viscosity Alginate</th>
<th>Medium Viscosity Alginate</th>
</tr>
</thead>
<tbody>
<tr>
<td>OOO Hardness</td>
<td>31.65</td>
<td>52.5</td>
</tr>
<tr>
<td>G/M Ratio</td>
<td>0.633</td>
<td>0.372</td>
</tr>
<tr>
<td>MM-block Frequency</td>
<td>0.385</td>
<td>0.583</td>
</tr>
<tr>
<td>MG-block Frequency</td>
<td>0.248</td>
<td>0.122</td>
</tr>
<tr>
<td>GG-block Frequency</td>
<td>0.153</td>
<td>0.140</td>
</tr>
<tr>
<td>GGG-block Frequency</td>
<td>0.034</td>
<td>0.014</td>
</tr>
<tr>
<td>M_w</td>
<td>237,455</td>
<td>473,760</td>
</tr>
</tbody>
</table>

Table 2: Adsorption behavior of the sediment and analytes used

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Sorption Coefficients (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methylene Blue</td>
</tr>
<tr>
<td>1.18-3.36 mm</td>
<td>98.02</td>
</tr>
<tr>
<td>0.850-1.0 mm</td>
<td>77.91</td>
</tr>
<tr>
<td>74-177 µm</td>
<td>437.19</td>
</tr>
</tbody>
</table>
Table 3: Distribution Coefficients of Naphthalene and Methylene Blue in unbuffered systems.

<table>
<thead>
<tr>
<th></th>
<th>Low Viscosity Alginate</th>
<th>Medium Viscosity Alginate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naphthalene</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled Water</td>
<td>129.55</td>
<td>155.26</td>
</tr>
<tr>
<td>Half-Strength Sea Water</td>
<td>15.78</td>
<td>48.26</td>
</tr>
<tr>
<td>Full-Strength Sea Water</td>
<td>10.11</td>
<td>17.79</td>
</tr>
<tr>
<td><strong>Methylene Blue</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled Water</td>
<td>54077.77</td>
<td>35.55</td>
</tr>
<tr>
<td>Half-Strength Sea Water</td>
<td>44.53</td>
<td>11.98</td>
</tr>
<tr>
<td>Full-Strength Sea Water</td>
<td>44.53</td>
<td>0</td>
</tr>
</tbody>
</table>
Appendix B: Figures:

Figure 1: The different residues of Alginate arranged in MM and GG blocks are shown in A; the mechanism for cross linking where cations fit between the chains in the egg-box model shown in B; and the points of interaction between calcium and alginate shown in C.
Figure 2: Example of shear curves. Arrows highlight where peak shear values are taken for the different curve shapes encountered.
Figure 3: Peak shear of A) Sediment without alginate, B) Sediment gelled with low viscosity alginate and C) Sediment gelled with medium viscosity alginate at different applied normal stresses.
Figure 4: Change in concentration of Naphthalene released from A: low viscosity alginate and B: medium viscosity as well as Methylene Blue released from C: low viscosity alginate and D: medium viscosity alginate into unbuffered solutions of DI water, Half and Full seawater at 24°C
Figure 5: Change in concentration of Naphthalene released from A: low viscosity alginate and B: medium viscosity alginate as well as Methylene Blue released from C: low viscosity alginate and D: medium viscosity alginate into buffered solutions of DI water, Half and Full seawater at a pH 5.8 and 24°C.
Figure 6: Sorption coefficient over time for Methylene Blue to sediment
Figure 7: Normalized leaching from Sediment-gels. Low viscosity gel data is on the left while medium viscosity gel data is on the right. Methylene Blue diffusion is graphed with a solid line while naphthalene diffusion is graphed as points only. Sediment size is in decreasing order from the top with 1.18-3.36mm sediment in A and D; 0.850-1mm sediment in B and E; and 74-177 µm in C and F. Solution are buffered to a pH of 5.8 and a temperature of 24°C.