Detection of Emerging Contaminants in Water by a Displacement Assay Based on High-Performance Affinity Chromatography

Ryan Matsuda1, So-Hwang Kye1, Christopher White II1, Elliott Rodriguez1, Donald Jobe1, Daniel Snow2, David S. Hage1

1 Department of Chemistry (University of Nebraska-Lincoln, Lincoln, NE)
2 Water Sciences Laboratory/Nebraska Water Center, a part of the Daugherty Water for Food Institute, and School of Natural Resources (University of Nebraska-Lincoln, Lincoln, NE)

Introduction
In a recent survey conducted by the World Health Organization, pharmaceutical and related contaminants were discovered in recycled or reclaimed water. This is a global concern as water is one of world’s most essential natural resources, and the use of recycled and reclaimed water for irrigation is a common practice in food industry around the world. This is a major issue in Nebraska, because Nebraska ranks 2nd in the US for using recycled or reclaimed water for irrigation.

Goal of Study
The purpose of this study was to explore the use of affinity sorbents and high-performance affinity chromatography (HPAC) as a tool for rapidly screening for common emerging contaminants found in water. HPAC is chromatographic technique that utilizes a biologically-related binding agent as the stationary phase to retain chemicals. A displacement assay based on HPAC was constructed by using a fluorescent labeled analog of warfarin, phenytoin and an affinity column containing immobilized bovine serum albumin (BSA). BSA is a serum transport protein found in cattle that has a series of sites that are capable of binding to various pharmaceuticals and hormones, as well as some pesticides (as shown in Fig. 1).

Warfarin, carbamazepine (widely found in wastewater), and L-tryptophan were used as the model chemicals and binding probes to develop and test this assay. All of these compounds were found to displace labeled phenytoin when applied to the BSA column and provided a signal within a few minutes of sample application. This approach was examined in this study as a possible a screening tool that could be used for detection of emerging contaminants in reclaimed and recycled water.

Labeling Phenyltoin with Fluorescein
Figure 2. Reaction involved in synthesis of fluorescein labeled phenytoin.

Labeled phenytoin was synthesized by combining 20 µmol 3-amino-5,5-diphenylisoxazolidine-2,4-dione (ADPH) with 9 µmol 5-hydroxyisouminodefluorine (NHS-fluorescein) in dimethyl sulfoxide (DMSO) and triethylamine. This reaction is shown in Fig. 2. The mixture was allowed to react in the dark and in an ice bath for 4 hours. DMSO and triethylamine were then removed from the final product by using a vacuum oven at 60 °C and 25 mmHg.

Displacement Assay Format
Labeled phenytoin was first applied to a column containing immobilized BSA and allowed to bind to the BSA, as depicted in step 1 of Fig. 3. Following the injection of the labeled phenytoin, the various model analytes were injected onto the column. This resulted in displacement of some of the labeled phenytoin, as depicted in step 2. Changes in the displaced peak area were found to be correlated with the concentration of the applied analyte, as shown in Fig. 4.

Calibration Plots
Calibration curves were obtained for the displacement assay by using a set of standards ranging from 0.1-10 µM for warfarin and carbamazepine, as shown in Fig. 5(a-b). The linear range (0.1-1 µM) for the calibration curves for carbamazepine and warfarin is shown in the insets of Fig. 5a and b. A similar calibration curve was also developed for L-tryptophan. The estimated limits of detection for warfarin and carbamazepine were 0.15 µM and 0.09 µM respectively.

Spiked Samples
Spiked samples were prepared by adding in a known concentration of either carbamazepine or warfarin to a 20 mL sample of tap water. The samples were then injected onto the BSA column containing the labeled phenytoin. The displayed peak area was used with the linear calibration plots to determine the concentration of the spiked samples, as shown in Table 1.

<table>
<thead>
<tr>
<th>Actual (µM)</th>
<th>Detected Carbamazepine (µM)</th>
<th>Detected Warfarin (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>0.10 (±0.03)</td>
<td>0.08 (±0.06)</td>
</tr>
<tr>
<td>0.25</td>
<td>0.25 (±0.08)</td>
<td>0.24 (±0.07)</td>
</tr>
<tr>
<td>0.49</td>
<td>0.49 (±0.15)</td>
<td>0.49 (±0.02)</td>
</tr>
<tr>
<td>0.73</td>
<td>0.75 (±0.21)</td>
<td>0.73 (±0.12)</td>
</tr>
<tr>
<td>1.0</td>
<td>1.04 (±0.12)</td>
<td>1.12 (±0.04)</td>
</tr>
</tbody>
</table>

Table 1. Results from spiked sample experiments for carbamazepine and warfarin

Conclusion
This study examined the development of a displacement assay for the detection of pharmaceutical agents and other chemicals contaminants in water samples. The detection range for the different analytes was in the nM to µM range, with detection limits in the low nM range. The displacement assay also demonstrated comparable results to actual concentrations for spiked samples containing either warfarin or carbamazepine. Although this particular study looked at carbamazepine, warfarin and L-tryptophan as model analytes, the same approach could be extended to other compounds that bind to BSA. The information provided by this study indicates that this approach can be used as a potential screening tool to detect emerging contaminants in water and may thus be developed into a screening tool for irrigation water quality. Future work will consider the use of this approach with methods such as LC/MS/MS.

Acknowledgments
This project was funded by the NIH (R01 GM044831), NSF (CHE – 1156560), and the USGS (104b grant). Special thanks to Dr. Martha Morton for her assistance with NMR spectroscopy and the NCMS.

References