Creatinine Detection using Molecular Imprinted Polymer and Functionalized Nanodiamond as Biosensors

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Introduction

- Creatinine (Cr) is a waste product of creatine created by the wear and tear of the body, especially the muscular system, and it's also a contributing factor of kidney failure.
- High levels of creatinine concentrations in urine and/or blood samples can be an indicator of renal disfunction.
- Using the molecular imprinting(MIP) method with nanodiamonds is an effective way of detecting creatinine because of nitrogen impurities within the nanodiamond structure that cause fluorescence.
- MIPs are polymers that have been processed using the molecular imprinting technique which leaves complementary cavities in the polymer matrix with an affinity for a chosen template molecule.
- Nanodiamonds have excellent mechanical and optical properties, high surface areas and are non-toxic, which makes them suitable for biomedical applications. When diamonds are reduced to the nanometer scale, nitrogen-vacancy defects are closer to the surface where they can interact with adsorbates to modify the near-surface band structure.

Methods and Materials

- Hydrogels were formed by co-polymerization of acrylamide and bis-acrylamide.
- To prepare the polymer 15% of T the monomer, and 5% of C crosslinker, was diluted in 7mL of DI water. In addition, 200µm of creatinine, and 200µm of fluorescent nanodiamond were also added.
- MIP and NIP samples were prepared identically except no creatinine was added to the NIP sample.
- The initial thickness of each sample was 0.75 mm.

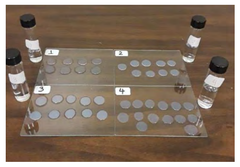
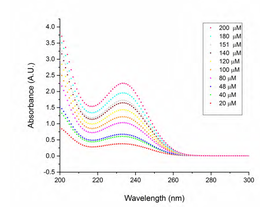


Figure 1.
After the hydrogel polymerized the two samples were cut into circular shapes 1 cm in diameter.

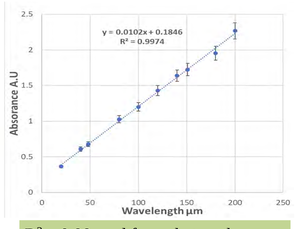
Results

Absorbance Measurements

- Ten different concentrations of creatinine were prepared in DI water and tested for absorbance using UV vis spectrometer.
- Calibration curve used to determine the concentration of any unknown samples.



The highest concentration had the highest absorbance and the lowest concentration had the lowest absorbance, which follows Beers-Lambert Law

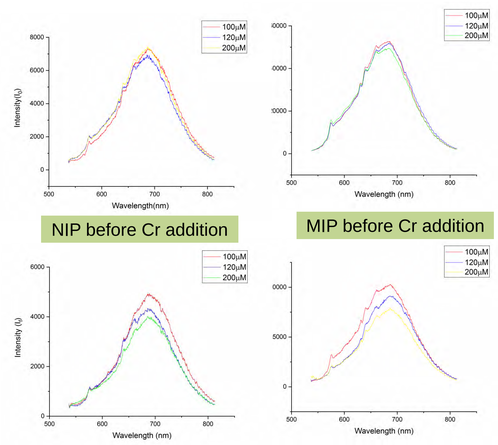


$R^2 = 0.99$, and from that we know our data is precise and the line passes mostly all dots; no outliers.

Results continued

Fluorescence Measurements

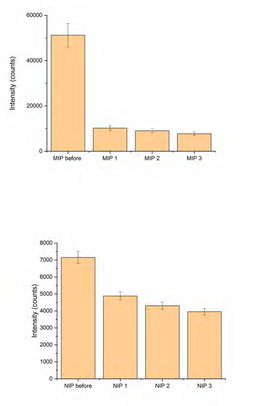
- To observe fluorescence of our samples we used photoluminescence spectroscopy.
- The imprinting factor was found from the change in the peak emission intensity of nanodiamond around (700 nm) before and after the creatinine quencher was added.



NIP before Cr addition | MIP before Cr addition

NIP after Cr addition | MIP after Cr addition

- From the data we find that creatinine does in fact quench fluorescence of nanodiamond due to the relationship between creatinine and the nitrogen impurities within the nanodiamond.

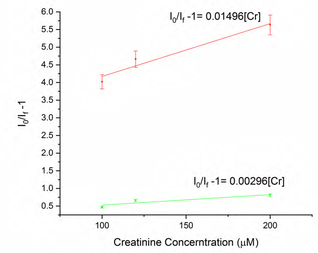


- Creatinine quenches Nanodiamond fluorescence at concentrations of 100µM, 120µM, and 200µM for both Molecularly Imprinted Polymer and Non-Imprinting Polymer.
- Molecularly imprinted polymer samples quenched 5 times as much as the Non-Imprinting.
- These results demonstrate that the polyacrylamide hydrogel can be effectively used as an imprinting platform for creatinine.

Discussion

- From this Stern-Volmer plot we determined the imprinting factor.
- After rebinding steps, an imprinting factor of 5.05 ± 2.15 was found.
- The imprinting factor is defined as the fluorescence quenching ratio of molecularly imprinted polymer divided by the quenching ratio of non-imprinted polymer after binding with creatinine.

$$\text{Imprinting Factor} = \frac{Quenching\ ratio\ of\ MIP}{Quenching\ ratio\ of\ NIP}$$



Red Line: Molecularly Imprinted Polymer
Green Line: Non-Imprinted Polymer

Conclusion

- Quenching of nanodiamond fluorescence from the molecularly imprinted polymer was substantially higher than that of the non-imprinted polymer, yielding an imprinting factor of 5.05.
- The relationship between the nitrogen impurities within the Nanodiamond and creatinine allows the creatinine to quench nanodiamond fluorescence.
- This indicates that the molecularly imprinted polymer has a higher affinity for creatinine than the non-imprinted polymer.
- This supports that molecularly imprinted polymers when combined with fluorescent nanodiamond is an effective biosensor to detect creatinine.

Future Strategies

- In future work we aim to optimize T and C concentrations to control hydrogel functionality.
- Using a Carboxylated Nanodiamond that will likely interact with creatinine more efficiently, and increase imprinting factor more than 50%.

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National Science Foundation
WHERE DISCOVERIES BEGIN

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