Applications of Fluorescent Biosensors for Non-Invasive Glucose Monitoring

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Overview

• Background
  • Diabetes – Side Effects
  • Monitoring Devices

• Project Goal

• Boronic Acids (BAs) for Sugar Recognition

• Direct Sensing

• Indirect Sensing

• Conclusions

• Future Work
Importance of Saccharide Sensing

- Disease: Diabetes and the consequential side effects
  - Stroke
  - Excessive Thirst
  - Weight Gain
  - Blindness
  - Heart Attack
  - Kidney Damage
  - Difficultly Passing Urine
  - Foot Ulcers
  - Peripheral Nerve Damage

- Monitoring glucose levels to prolong life expectancy
- Currently no noninvasive, continuous monitoring systems available
- Demonstrates a need for real-time, non-invasive monitoring
Current Monitoring Methods

Implanted Wearable Devices

- Real-time monitoring
- Continuous
- Coupled to insulin pump
- Elimates injections via syringe

Disadvantages:
- Invasive

Finger Pricking Method

Advantages:
- Minimally Invasive

Disadvantages:
- Not continuous
- Insulin injections required
- Miss episodes of hyper- and hypoglycaemia

https://www.accu-chek.co.uk/gb/products/
Contact Lenses – The Answer!

Electrochemical sensor in a wearable platform

Battery Powered
Interference from Electroactive Species in Ocular fluid
Use of Enzymes

Realistically....Not a Real Working Device

- Attached to a BASi Epsilon- EC Potentiostat +400 mV
- Sensing platform proposes glucose monitoring between 0.5-50 mM
- Ocular glucose range is 0.05-0.5 mM and up to 5 mM in diabetics
- Major shortcomings to meet immediate expectations

The Solution!
Boronic Acids (BAs) and Sugars

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{B}^{-} & \quad \text{OH}^{-} \\
\text{pK}_a & = 9 \\
\text{R} & \quad \text{R} \\
\text{OH} & \quad \text{OH} \\
\text{O} & \quad \text{O} \\
\text{B}^{-} & \quad \text{B}^{-} \\
\text{pK}_a & = 6 \\
\text{R} & \quad \text{R} \\
\text{OH} & \quad \text{OH} \\
= & \text{Diol/Sugar}
\end{align*}
\]
Direct vs. Indirect Sensing

Direct Sensing

Indirect Sensing

Fluorescence Decrease

Fluorescence Increase

Fluorophore
Direct Sensing
Addition of OH- ions/glucose
Addition of water/removal of glucose

(i) Addition of OH- ions/glucose
(ii) Addition of water/removal of glucose
Synthesis of COOHBA Sensors

(i) Anhydrous dimethylformamide, N\textsubscript{2}, 80 °C for 48h.

Successful synthesis of novel BA sensors were confirmed by NMR.
Fluorescence Results

**m-COOHBA** 0.5 mM in pH 7.4 phosphate buffer; Excitation 390 nm; Emission 465 nm

**o-COOHBA** 0.5 mM in pH 7.4 phosphate buffer; Excitation 380 nm; Emission 485 nm

Glucose Concentration (mM) vs. Fluorescence Intensity (F. U.)

- **m-COOHBA**:
  - Glucose Concentration: 0 to 50 mM
  - Fluorescence Intensity: 1500 to 7500 F. U.
  - 78% decrease

- **o-COOHBA**:
  - Glucose Concentration: 0 to 50 mM
  - Fluorescence Intensity: 4500 to 8500 F. U.
  - 40% decrease

Wavelength (nm) vs. Fluorescence Intensity (F. U.)

- **m-COOHBA**
- **o-COOHBA**
**pK$_a$ Investigation – Glucose Sensing pH Range**

Glucose response for m-COOHBA and o-COOHBA (0.5 mM) in different pH buffer solutions ranging from pH 5-11.

Glucose concentration vs. $F/F_0$ for different pH values:
- **pH 5.3**
- **pH 7.4**
- **pH 11.8**

Chemical structures of m-COOHBA and o-COOHBA:
Indirect Sensing
Two-Component Sensing

- \( \text{B} \text{HO} \text{HO} \text{HO} \text{HO} \text{HO} \text{HO} \)
Indirect Sensing – Sensor Synthesis

\[
\begin{align*}
2 \text{Br} & \text{B} & \text{OH} + \text{N} & \text{2} & \text{Br} & \text{N} & \text{2} & \text{Br}
\text{an. MeCN} & \rightarrow & \text{DBA1} & \\
\text{N}_2 & 70 \, ^\circ\text{C} & 30\% \\
\end{align*}
\]

\[
\begin{align*}
2 \text{Br} & \text{B} & \text{OH} + \text{N} & \text{2} & \text{Br} & \text{N} & \text{2} & \text{Br}
\text{an. THF} & \rightarrow & \text{DBA2} & \\
\text{N}_2 & 70 \, ^\circ\text{C} & 30\% \\
\end{align*}
\]
Two-Component Sensing

Non-Fluorescent

Fluorescent
Two-Component Sensing – Fluorescence Quenching

Excitation and emission spectra of 4 µM 7HC in pH 8.12 buffer solution with increasing DBA1 concentrations up to 0.5 mM (125 eq.); Medium sensitivity; 2.5 nm bandwidth

Fluorescence OFF
Two-Component Sensing – Fluorescence Quenching

Excitation and emission spectra of 4 µM 7HC in pH 8.88 buffer solution with increasing DBA1 concentrations up to 0.8 mM (200 eq.); Medium sensitivity; 2.5 nm bandwidth

Fluorescence OFF
Two-Component Sensing – Fluorescence Recovery

Excitation and emission spectra of 7HC (4 µM) and DBA2 (700 µM) (1:175 eq.) in pH 8.12 buffer solution with increasing concentrations of glucose up to 5 mM; Medium sensitivity; 2.5 nm bandwidth

Fluorescence Intensity (F. U.) vs. Wavelength (nm)

Fluorescence Intensity (F. U.) vs. Glucose Concentration (mM)

Fluorescence ON
Excitation and emission spectra of 4 µM 7HC in pH 7.4 with minimal MeOH (40 µL) with increasing DBA2 concentrations up to 0.3 mM (75 eq.); Medium sensitivity; 2.5 nm bandwidth
Two-Component Sensing – Fluorescence Quenching

Fluorescence Intensity (F. U.)

Wavelength (nm)

370 nm

453 nm

98%

Excitation and emission spectra of 4 µM 7HC in pH 7.4:MeOH (1:1) (pH 8.6) with increasing DBA2 concentrations up to 1.2 mM (300 eq.); Medium sensitivity; 2.5 nm bandwidth

Fluorescence OFF

7HC

+ DBA2

Fluorescence OFF
Two-Component Sensing – Fluorescence Recovery

Excitation and emission spectra of 7HC (4 μM) and DBA2 (80 μM) (1:20 eq.) in pH 7.4:MeOH (1:1) (pH 8.6) with increasing concentrations of glucose up to 100 mM; Medium sensitivity; 2.5 nm bandwidth

Fluorescence ON
Conclusions and Future Work

Conclusions

• Novel BAs are capable of direct and indirect glucose sensing
• -COOH substituent for desired anchoring possibilities
• Two-Component Sensing depends on the pKₐ of the fluorophore and hence, the pH of the buffer solution

Future Work

• Immobilisation of the sensors on to a lens-like platform
• The incorporation of the two component sensing system in to ionogels
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Thank You for Your Attention!
PDMS Lens Fabrication

1. Vacuum 1h; Oven 60 °C 12h
2. Oxygen Plasma
3. 1.2 mm
4. 18 mm
5. PDMS “Lenses”
6. Doped in o-COOHBA EtOH:DI H₂O (1:1)
7. λ<sub>ex</sub> = 380 nm
8. λ<sub>em</sub> = 485 nm
Functionalised PDMS Lens Fluorescence Studies

Glucose Concentration
0 mM
\[\downarrow\] 5 mM

Glucose Concentration
2 mM
\[\downarrow\] 5 mM

Wavelength (nm)

Fluorescence Intensity

380 420 460 500 540 580 620 660 700

Fluorescence Intensity

0 250 500 750 1000 1250 1500 1750 2000

Lens

\(\alpha\)-COOHBA

B–OH

Br–N

\(\alpha\)-COOHBA

Glucose
$^1$H NMR of 1,3-bis(2-boronobenzyl)-5-bromo-1,3-dinium bromide
(HO)₂B + \( \text{Br} \) + \( \text{OH} \) \( \rightarrow \) anhydrous THF \( \xrightarrow{75 \, ^\circ \text{C}} \) \( \text{N}_2 \) \( \xrightarrow{5 \, \text{Days}} \) \( \text{Br} \)-\( \text{N}^+ \)-\( \text{N}^+ \)-\( \text{Br} \)-\( \text{OH} \)
Direct vs. Indirect Sensing

**Direct Sensing**

Fluorescein

(i) Addition of -OH ions or Diol/Sugar
(ii) Addition of Water

Fluorescence ON

Fluorescence OFF

**Indirect Sensing**

Fluorescein BA Derivative

72%

ON

OFF

Glucose

67%

Diol/Sugar

Fluorescein BA-Sugar Bound Derivative
Two-Component Sensing – Fluorescence Quenching

**Excitation and Emission Spectra**

- **Fluorophore:** 7-Hydroxycoumarin (7HC)
- **DBA1**

- **Excitation and emission spectra of** 7 µM 7HC in pH 8.12 buffer solution with increasing DBA1 concentrations up to 7 mM (1000 eq.); Medium 2.5 nm

- **Fluorescence OFF**

- **Fluorescence Quenching**
  - **367 nm:** 80%
  - **454 nm:** 24%

**Graphs:**
- **Wavelength (nm):** 250 to 430, 390 to 600
- **Fluorescence Intensity (F. U.):** 0 to 9000
- **DBA1 Equivalence:** 0 to 1500

**Chemical Structures:**
- 7-Hydroxycoumarin (7HC)
- DBA1

**Formulas:**
- **7-Hydroxycoumarin (7HC):** HO-\(\text{B}\)-\(\text{B}\)-\(\text{O}\)
- **DBA1:** \(\text{N}^+\)-\(\text{N}^+\)-\(\text{B}^-\)-\(\text{Br}^-\)-\(\text{Br}^-\)-\(\text{B}^-\)
Fluorescence Intensity (F. U.) vs. DBA1 Equivalence

- No Glucose
- 20mM Glucose
- 100mM Glucose

Chemical Structures:
- 7HC
- DBA1
- Glucose
Two-Component Sensing

Non-Fluorescent
Two-Component Sensing

Non-Fluorescent

Fluorescent
Two-Component Sensing

Chemical structures of fluorescent and non-fluorescent compounds.
Two-Component Sensing
Two-Component Sensing

Non-Fluorescent

Fluorescent