Analysing Chem/Bio-Markers in Saliva using a Portable Optical Detection Platform

Simon Coleman

Andrew Kavanagh, Dylan Orpen, Jung Ho Kim, King Tong Lau and Dermot Diamond

CLARITY: Centre for Sensor Web Technologies, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland
Overview

- **Treatment and monitoring of Bipolar Disorder**
  - Lithium therapy for mood stabilisation
  - Non-invasive sampling of patient

- **Chemo/Bio-markers for Bipolar patient monitoring**
  - Fluorescent detection of Lithium
  - Colourimetric determination of α-amylase

- **Portable analysis of Bipolar disorder using multi-analyte platform**

- **Detection of Chemo/Bio-markers using mobile platform**

- **Conclusions and future applications**
Treatment and monitoring of Bipolar Disorder

Lithium treatment for mood stabilisation

- Current treatment of Bipolar disorder involves administration of Lithium in the form of lithium carbonate tablets.

- Typical serum (blood) levels range between 0.6 – 1.3mmol\(^1\) with toxic levels above 1.5mmol\(^1\)

→ narrow therapeutic/toxic range

Two issues from current methods of monitoring dosage:

1. Blood typically drawn ONCE weekly to determine lithium levels in patient.
   - Risk of overdose/intoxication event being missed by infrequent monitoring

2. Invasive nature of blood drawing procedure.
   - Discomfort associated with blood sampling

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Bipolar Disorder
Non-invasive sampling of patient

• Invasive nature of drawing blood limits number of samples and decreases patient compliance.

• Interest in alternatives to Blood analysis sought for conditions requiring frequent analysis such as Diabetes.

• Saliva chosen due to ease of accessibility, low sample preparation requirements and minimal invasiveness.

Bio/Chemo-markers for Bipolar Patient monitoring

Chemical marker: Lithium

- Uncommon element in body resulting in minimal interference due to alternate sources of lithium.

- Readily found in bodily fluids during treatment; urine, saliva, blood and sweat. *Typically found to be in higher concentration in saliva than blood*.

Biomarker: α-amylase

- Present in blood (Pancreatic) and saliva (salivary)

- Salivary α-amylase found to exhibit sensitivity to stress.

- Proposed use as stress monitor in conjunction with other methods of mood analysis (psychiatric assessment, vocal pattern analysis).

Lithium (top): http://www.sciencephoto.com/image/111204/530wm/C0046993-Lithium_Carbonate-SPL.jpg <accessed 14/06/12>

Amylase (bottom): http://www.salimetrics.com/my-spit-research/analytes/a-amylase.php <accessed 11/06/12>
Fluorescent Detection of Lithium

- Highly sensitive method using ‘dark background’ and lithium specific probe dye.
- 1,4-Dihydroxyanthraquinone (Quinizarin) found to preferentially complex with Lithium
- Complex absorption at 605nm and corresponding emission at 624nm

Quinizarin dye and Lithium complex formation
Fluorescent Detection of Lithium

Excitation spectrum of 1mM Quinizarin-lithium complex

Emission spectrum of 1mM Quinizarin-lithium complex
Colorimetric determination of α-amylase

- Exploitation of α-amylase enzymatic break down of starch.
- Water insoluble starch polymer with attached blue dye is added to aqueous saliva sample.
- As the starch is broken down, the dye is released into the water resulting in colour change.
- Measurement of absorption at 620nm allows for the determination of amylase concentration in saliva.

Colour change in α-amylase concentrations in response to stress
Colorimetric determination of α-amylase

Characteristic absorption spectra of released blue due following breakdown of starch polymer backbone.

Absorption changes related to varying levels of amylase present in saliva throughout a day (healthy individual)
Portable analysis of Bipolar disorder using multi-analyte platform
Portable analysis of Bipolar disorder using multi-analyte platform

• **Wireless microcontroller (Wixel©) with dual LED system and individual photodiode detectors optimized for each target marker.**

• **Automated Software programme:**
  - ‘li’ (lithium) and ‘aa’ (α-amylase) commands initiate custom measurement routine.
  - 180s settling time hard coded to device for lithium analysis.
  - 20 individual measurements printed to screen
  - Device calculates average and prints to screen.
Portable analysis of Bipolar disorder using multi-analyte platform

**Fluorescence:**
- Lithium detection
  - Ex: 605nm
  - Em: 624nm

**Absorbance:**
- α-amylase detection
  - Abs: 620nm

Blue dye released by starch breakdown
Determination of Chemo/Bio-markers using mobile platform

- Standard addition technique to determination of lithium levels:
  - Increasing addition of stock solution containing lithium of known concentration added to saliva sample containing unknown lithium concentration.
  - Validation versus standard method (ICP-AES)

Standard addition curve for the determination of unknown saliva lithium concentration

<table>
<thead>
<tr>
<th>sample</th>
<th>concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known saliva concentration</td>
<td>1.00</td>
</tr>
<tr>
<td>Portable platform measurement</td>
<td>1.06</td>
</tr>
<tr>
<td>ICP-AES measurement</td>
<td>0.93</td>
</tr>
<tr>
<td>standard deviation</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Determination of Chemo/Bio-markers using mobile platform

- Calibration curve for determination of α-amylase:
  - Calibration curve constructed using human amylase standard
  - Device optimised for determination of alpha-amylase levels within specific range (0.1 U/mL – 1.2 U/mL)
  - Clinical trials in progress.

(a) UV-Vis spectra of amylase standards. (b) Calibration curve for validation using UV-Vis
Conclusions and future applications

• Use of Non-invasive sample for *increased frequency of patient monitoring*.

• Reliable Portable optical detection of both chemical and biological markers for eventual **Reduction in requirement for hospital visits** with patients able to determine levels at home.

• **Customisable** nature of device allows for the potential of tailoring the system (LEDs and detectors) to alternative targets that can be detected optically allowing for use with other disorders.
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