PAPER-BASED POTENTIOMETRIC SENSOR
FOR MONITORING GALACTOSE LEVELS

by BSc. Julio Cesar Zuaznabar

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Tarragona
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Galactosemia

\[
\text{β-D-Galactose} \quad \leftrightarrow \quad \text{α-D-Glucose}
\]

Leloir pathway

Gal-1-P-uridylyltransferase.
Galactokinase
UDP-D-galactose 4-epimerase

High galactose levels in blood (> 1 mmol/L) damages some organs.

New born screening is mandatory

Diagnosis and treatment should be started ASAP.
Bacteria-based assays

E. Coli K12

Bacteriophage C21

Blood spot

Diameter vs [Gal]

Enzyme-based assays

• Require special reagents
• Involve multisteps
• Sample preparation procedures
• Time-consuming (days to hours)

NEW APPROACHES
• Faster
• Reagentless
• Portable
• Easy-to-use
Electrochemical assays

AMPEROMETRIC ($\hat{e} \rightarrow I \propto [X]$)

76 articles at WOS trying to eliminate the interferences

POTENTIOMETRIC ($\mu \rightarrow \Delta E \propto \log[X]$)

1 article at WOS

- ISE kingdom
- Difficult to measure neutral molecules
- Redox interferences
- Noble metals as electrode material
Paper as substrate for electrodes

- Porosity
- High surface-to-volume ratio
- Capillary action
- Compatibility with biological entities
- Low-cost of manufacturing

To develop a paper-based potentiometric sensor to determine galactose levels.
To immobilize galactose oxidase on the surface of platinized paper by electrostatic interactions and crosslinking.

To assess the effect of enzyme loading on the sensitivity of the sensors.

To evaluate the influence of pH on the potentiometric response of the sensor.

To eliminate the effect of redox species on the performance of the sensor.

To determine the analytical performance of the sensor.
Working principle

Galactose + Galactonic Acid

O2 + H2O2 → O2 + H2O

GALOx

Nafion®

Platinized paper
Electrode Fabrication

Platinized paper → Mask strips

Sandwiching

→ Nafion®

→ GALOx

→ Nafion®

→ GALOx

→ PEI

→ Nafion®
Potentiometric responses of sensors

EMF changes with additions of galactose.

Potentiometric responses of sensors

EMF changes with additions of galactose.

0 4 8 12 16 20
240
280
320
360
400
440

EMF (mV)
Time (min)

Sandwiching
Crosslinking

13
The linear range is within the clinically relevant range.

**Analytical performance of the sensor**

<table>
<thead>
<tr>
<th></th>
<th>Sandwiching</th>
<th>Crosslinking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (mV/dec)</td>
<td>-33±4</td>
<td>-57±3</td>
</tr>
<tr>
<td>LOD (mol/L)</td>
<td>0.5·10⁻³</td>
<td>0.5·10⁻³</td>
</tr>
<tr>
<td>Linear range (mol/L)</td>
<td>1·10⁻³ - 1·10⁻²</td>
<td>1·10⁻³ - 1·10⁻²</td>
</tr>
<tr>
<td>Time of response (min)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
The amount of enzyme loading and the working pH affect the sensitivity of the sensor.

**Enzyme loading**

**Working pH**
Effect of interferences

Ascorbate causes interference.

\[
\bar{\mu}_t = \bar{\mu} + \mu
\]
To use MnO$_2$ nanoparticles as external oxidizing layer.
Synthesis of MnO$_2$ nanoparticles

TEM image of MnO$_2$ ~ 50 nm

MnO$_4^-$

Mn$^{2+}$

MnO$_2$ NP
MnO$_2$ NPs as oxidizing layer

There are no interferences.

Glucose
Citrate
Lactate
Ascorbate
Uricate
Galactose

EMF (mV) vs. Time (min)
Analytical performance of the sensor with MnO₂ NPs

The linear range is within the clinically relevant range.

Analytical parameters of the sensor

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sandwiching</th>
<th>Crosslinking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (mV/dec)</td>
<td>-23±4</td>
<td>-41±3</td>
</tr>
<tr>
<td>LOD (mol/L)</td>
<td>0.5⋅10⁻³</td>
<td>0.5⋅10⁻³</td>
</tr>
<tr>
<td>Linear range (mol/L)</td>
<td>1⋅10⁻³ - 1⋅10⁻²</td>
<td>1⋅10⁻³ - 1⋅10⁻²</td>
</tr>
<tr>
<td>Time of response (min)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>
Analytical performance of the sensor with MnO₂ NPs

Validation of the sensor

<table>
<thead>
<tr>
<th>C(X)_{added} mol/L</th>
<th>C(X)_{found} mol/L</th>
<th>% error</th>
<th>C(X)_{found} mol/L</th>
<th>% error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.20 \cdot 10^{-3}</td>
<td>1.27 \cdot 10^{-3}</td>
<td>6</td>
<td>1.23 \cdot 10^{-3}</td>
<td>3</td>
</tr>
<tr>
<td>1.80 \cdot 10^{-3}</td>
<td>1.71 \cdot 10^{-3}</td>
<td>5</td>
<td>1.74 \cdot 10^{-3}</td>
<td>3</td>
</tr>
<tr>
<td>2.10 \cdot 10^{-3}</td>
<td>2.01 \cdot 10^{-3}</td>
<td>5</td>
<td>2.04 \cdot 10^{-3}</td>
<td>3</td>
</tr>
<tr>
<td>2.40 \cdot 10^{-3}</td>
<td>2.34 \cdot 10^{-3}</td>
<td>3</td>
<td>2.36 \cdot 10^{-3}</td>
<td>2</td>
</tr>
<tr>
<td>3.00 \cdot 10^{-3}</td>
<td>3.17 \cdot 10^{-3}</td>
<td>6</td>
<td>3.05 \cdot 10^{-3}</td>
<td>2</td>
</tr>
</tbody>
</table>
Conclusions

• Paper-based potentiometric galactose sensors based on platinum as transducer were prepared by immobilizing galactose oxidase through two different methods.

• MnO$_2$ particles were employed to remove the effect of common interferences presented in real samples.

• The sensors showed linear response in the range of 1.10$^{-3}$ to 1.10$^{-2}$ mol/L with an average sensitivity of 23 and 41 mV/dec for sandwiching and crosslinking methods respectively. The response time was 3 min.

• These results suggest that proposed biosensor could be used for the diagnosis of galactosemia.
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