Detection of peanut food allergen using a biomimetic labelled electrochemical sensor

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INTRODUCTION

Food allergy can be defined as a medical condition in which exposure to a particular food triggers a harmful immune response. The symptoms can occur from minutes to hours after exposure, and may include difficulty in breathing, low blood pressure, itchy rash, swelling of the tongue, and life-threatening systemic reaction called anaphylaxis.

Peanut allergy is one of the most common food allergies in childhood, with a dramatic increase over the past few decades. It is often lifelong and carries a significant daily burden that adversely affects quality of life.

Ara h1, 2, 3 and 6 are considered to be the major allergens found in peanut which trigger to an immunological response in more than 50% of the allergic population representing the first leading cause of anaphylactic fatalities worldwide.

Therefore, it is essential to develop fast, accurate and easy-to-use analytical methods to determine Ara h1, from different food samples.

APTASENSOR DEVELOPMENT STRATEGY

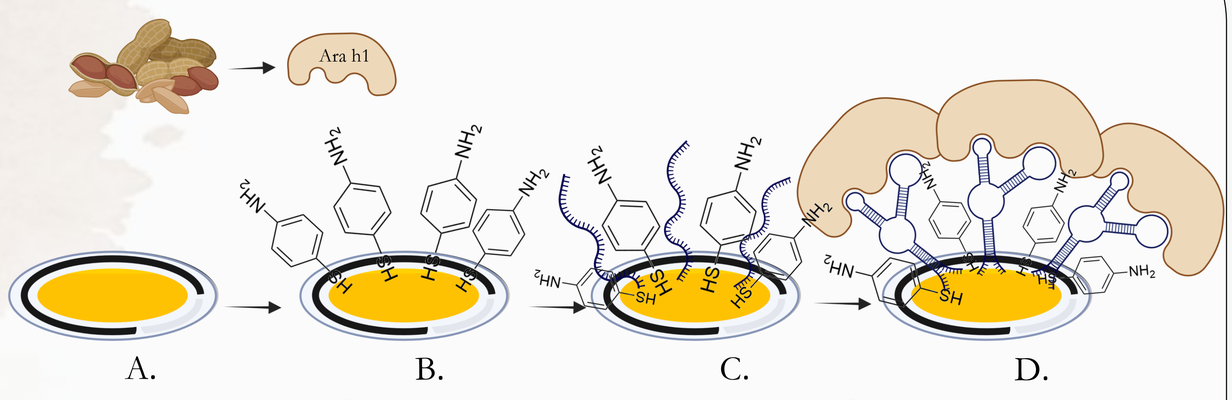


Figure 1. Scheme of aptamer-based sensor development for Ara h1 detection:
A. Activation of the gold surface
B. Para-aminothiophenol (p-ATP) immobilization
C. Thiol group functionalized aptamer (HS-Apt) immobilization
D. Target (Ara h1) incubation and signal evaluation

RESULTS

Electrochemical platform study

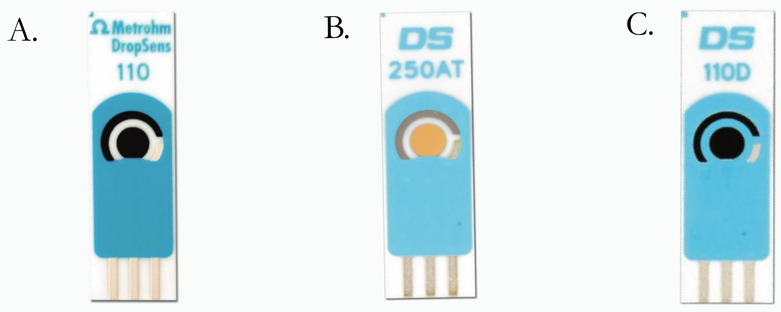


Figure 2. Electrode supports tested for optimal surface construction: A) screen-printed carbon electrode (**SPCE**); B) screen-printed gold electrode (**GSPE**); C) ordered mesoporous carbon modified screen-printed carbon electrode (**SPOMCE**)

Gold deposition – multipulse-assisted procedure

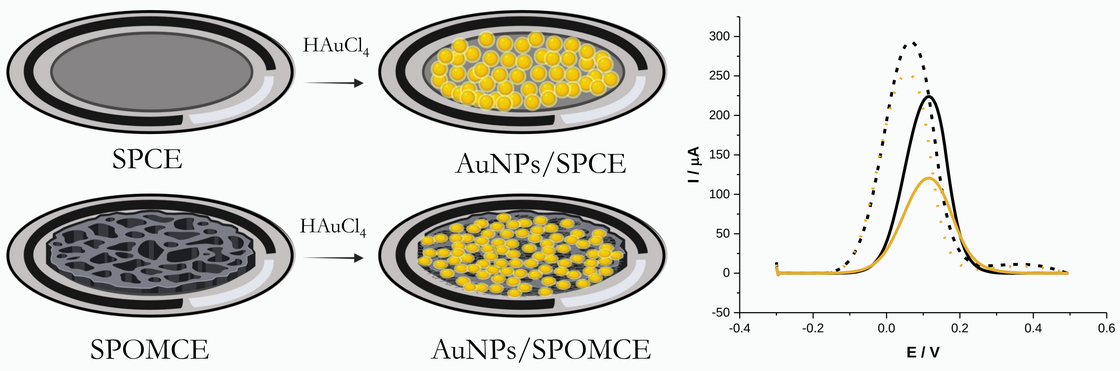
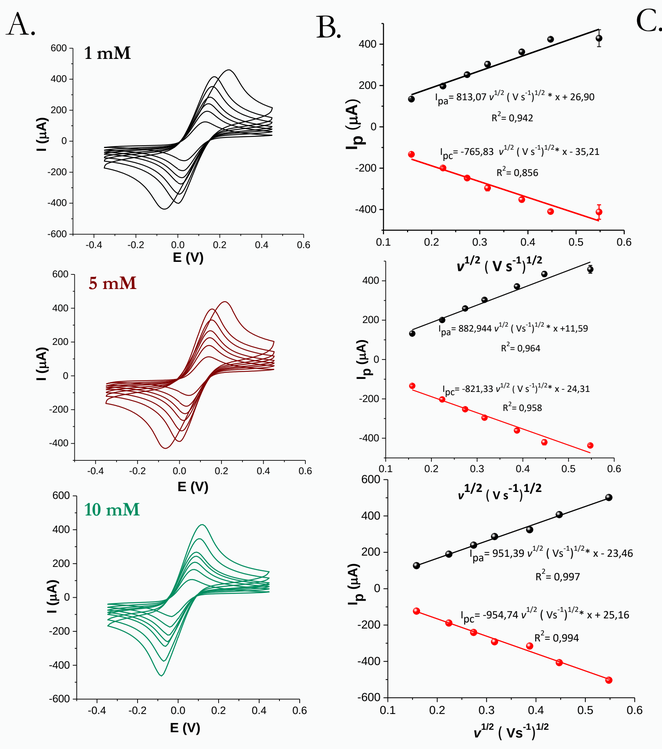


Figure 3. Characterization by DPV of SPCE (yellow) and SPOMCE (black) before (straight line) and after (dot line) gold nanoparticle deposition from a 10 mM $HAuCl_4$ solution in 0.5 M H_2SO_4 *via* multipulse amperometry

$HAuCl_4$ solution concentration optimization



C.

Scan rate (V/s)	Average I_{pa} (µA)	RSD I_{pa} (%)	Average I_{pc} (µA)	RSD I_{pc} (%)
0.025	134.33	2.27	−133,00	−1.99
0.050	197.33	2.97	−199,00	−2.80
0.075	252.67	3.59	−247,67	−3.64
0.100	303.00	4.54	−296,00	−4.55
0.150	362.67	3.05	−352,00	−3.16
0.200	423.67	1.83	−409,67	−1.80
0.300	428.67	9.55	−412,00	−8.50

A = 0.181 cm^2

Scan rate (V/s)	Average I_{pa} (µA)	RSD I_{pa} (%)	Average I_{pc} (µA)	RSD I_{pc} (%)
0.025	131.67	0.44	−134.00	−4.74
0.050	200.67	1.52	−203.00	−4.55
0.075	259.67	3.21	−252.67	−2.74
0.100	303.33	2.56	−295.67	−2.15
0.150	371.67	2.90	−360.67	−2.40
0.200	434.33	2.92	−420.67	−3.16
0.300	458.33	4.24	−437.00	−1.32

A = 0.198 cm^2

Scan rate (V/s)	Average I_{pa} (µA)	RSD I_{pa} (%)	Average I_{pc} (µA)	RSD I_{pc} (%)
0.025	126.67	2.41	−123.67	−2.60
0.050	189.67	2.49	−189.00	−3.22
0.075	239.33	2.78	−240.67	−3.38
0.100	286.67	4.21	−292.00	−1.78
0.150	324.33	12.12	−315.67	−16.70
0.200	407.33	5.65	−407.00	−6.55
0.300	501.33	7.46	−503.33	−8.19

A = 0.284 cm^2

Figure 4. Electroactive area calculation for different AuNPs/SPOMCE platforms. A) Cyclic voltammograms at different scan rates after gold deposition using different concentration $HAuCl_4$ solutions (1, 5 and 10 mM); B) Graphical representation of the anodic and cathodic currents vs. the square root of the scan rate; C) I_{pa} (µA) and I_{pc} (µA) values and the corresponding RSD obtained after CV measurements

Optimization of the Ara h1 specific aptasensor

- **p-ATP immobilization**

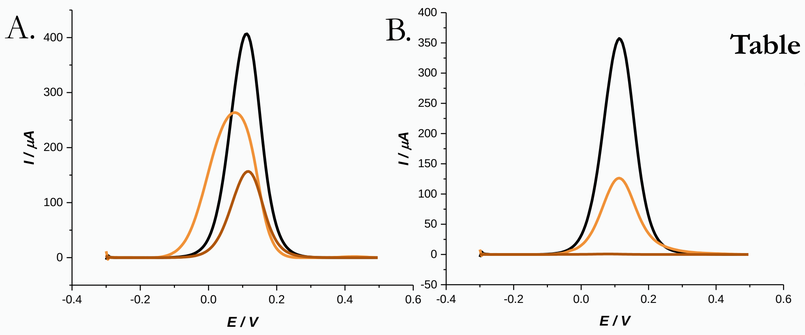


Table I. Deposition time optimization of multipulse-assisted technique

Deposition procedure	Deposition time (min)	Signal change (%)
Multipulse-assisted technique	1	-43.11
	2	-68.25
	3	-88.00

Figure 5. DPV signal obtained after immobilization of different p-ATP concentrations (0 mM – black; 5 mM – orange; 10 mM – brown) on GSPE *via* A) multipulse amperometry (MPA) and B) overnight incubation

- **Aptamer immobilization**

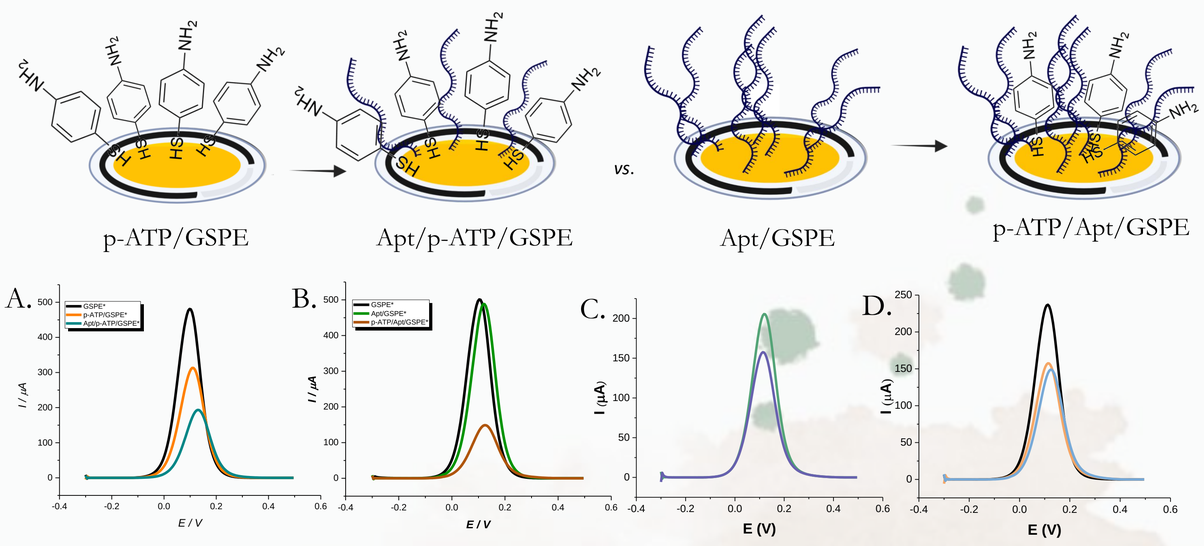


Figure 6. Platform characterization by DPV of A) Apt/p-ATP/GSPE and B)p-ATP/Apt/GSPE; C) Optimization of the Apt (2.5 µM) immobilization method by overnight incubation (green) *vs.* MPA (purple); D) Optimization of Apt concentration immobilized by MPA (0 µM – black; 2.5 µM – orange; 5 µM – blue).

- **Ara h1 determination**

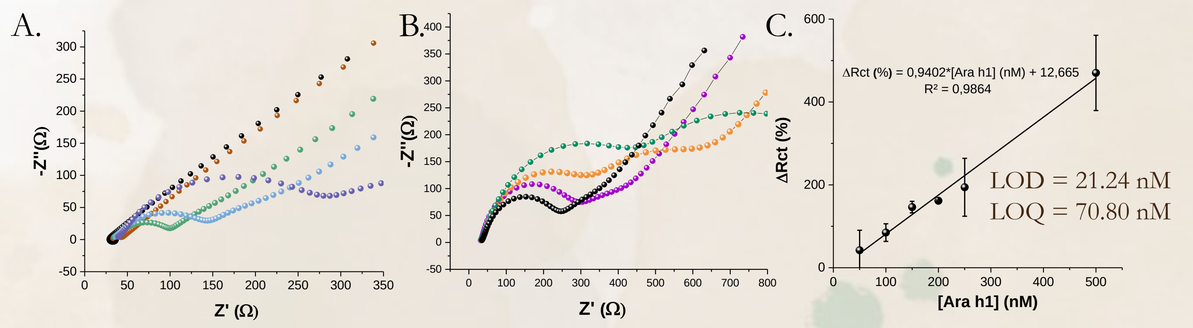


Figure 7. A) Platform characterization by EIS after each step of modification: GSPE - black; GSPE(activated) - brown; p-ATP/GSPE - green; Apt/p-ATP/GSPE - blue; Ara h1/Apt/p-ATP/GSPE - purple; B) EIS signals obtained after incubation of the sensing platform with different Ara h1 concentrations (0 nM – black; 100 nM – purple; 150 nM – orange; 200 nM – green); B) Calibration curve obtained from the EIS measurements.

The measurements were conducted in 5 mM $[Fe^{+2}(CN)_6]^{4-}/[Fe^{+3}(CN)_6]^{3-}$ solution in 0.1 M KCl
Electrochemical techniques used: CV – cyclic voltammetry; DPV – differential pulse voltammetry; EIS – Electrochemical impedance spectroscopy

CONCLUSIONS

- Work in progress: optimization of the sensing platform to lower the detection limit, interference studies and real sample analysis.
- This work could be a starting point for aptamer based electrochemical detection platforms which target multiple allergens from food samples.

References:
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