Electrochemical carbamazepine aptasensor for therapeutic drug monitoring at the point of care

Supporting Information

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\( \Delta G = -22.37 \text{ kcal/mol} \)

Figure S1. CBZ aptamer structure and folding study. (A) M-fold simulation of aptamer secondary structure and (B) \textit{in silico} docking simulation of aptamer and CBZ (red).
Figure S2. **AFM surface roughness.** Variation of the root-mean-square surface roughness ($R_{rms}$) at different stages of aptasensor fabrication.

![AFM surface roughness graph](image1.png)

Figure S3. **Aptamer loading density optimization.** Measured aptamer to mercaptohexanol (MCH) density with 1, 50, and 100 µM CBZ. (SNR calculated from the equation inserted).

![Aptamer loading density optimization](image2.png)

**Equation:**
\[
\text{SNR} = \frac{(R_{\text{apo} \text{CBM}} - R_{\text{apo} \text{MCH}})}{\sqrt{(R_{\text{apo} \text{MCH}})}}
\]

Figure S4. **CBZ incubation time.** (A) Measured EIS spectra at various CBZ incubation times (0 to 60 min). (B) Plot of $\Delta R_{ct}$ vs. time compiled from EIS data.

![CBZ incubation time](image3.png)
Figure S5. pH optimization. (A) EIS spectra at different pH of sample solution. (B) Plot of $\Delta R_{ct}$ vs. pH obtained from EIS data.

Figure S6. Aptamer label position. Optimization of label location (3' vs. 5') MB modified aptamers.

Figure S7. Label-free aptasensor using EIS. (A) Nyquist plot and (B) calibration curve.

Table S1. Recovery experiments.

| Assay Time (min) | Sample concentration (nM) | Sample #1 | Sample #2 | Sample #3 |
|------------------|---------------------------|-----------|-----------|-----------|
|                  |                           | Calculated Concentration (nM) | Recovery (%) | Calculated Concentration (nM) | Recovery (%) | Calculated Concentration (nM) | Recovery (%) |
| 5                | 100                       | 84.3      | 84.3      | 94.5      | 94.5      | 102.9                          | 102.9        |
|                  | 5000                      | 4026.6    | 80.5      | 5054.8    | 101.1     | 3804.1                         | 76.1         |
|                  | 10000                     | 12589.3   | 125.9     | 7525.6    | 75.3      | 8925.2                         | 89.3         |
| 30               | 15                        | 14.0      | 93.4      | 12.4      | 82.7      | 13.8                           | 92.0         |
|                  | 1000                      | 1092.9    | 109.2     | 872.7     | 112.7     | 1144.8                         | 114.5        |
|                  | 5000                      | 5242.4    | 104.8     | 4189.0    | 83.3      | 4857.1                         | 97.1         |
Figure S8. Recovery tests. (A) Voltammograms were measured for (A) 30-minute assay and (B) 5-minute assay with 15 nM, 1 µM, and 10 µM of CBZ spiked in undiluted human serum.
Figure S9. Blinded sample testing. (A,C,E) 5- and (B,D,F) 30-minute assays in buffer solution.
Figure S10. Aptamer stability to nucleases. (A) Peak current for 100 nM CBZ spiked in undiluted serum at 4 °C (on a tube cooler). (B) Peak current for 100 nM CBZ spiked in 20-fold diluted blood at ambient temperature.

Table S2. Blinded verification tests using CBZ spiked in buffer solution.

| Sample | True Concentration (µM) | 30-minute assay | 5-minute assay |
|--------|-------------------------|------------------|----------------|
|        |                         | Calculated Concentration (µM) | CV (%) | Calculated Concentration (µM) | CV (%) |
| #1     | 50                      | 45.5, 58.6, 47.3 (µ = 50.5) | 14.1  | 47.7, 42.1, 54.9 (µ = 48.2) | 13.3  |
| #2     | 15                      | 13.3, 16.9, 12.6 (µ = 14.3) | 16.1  | 9.9, 13.1, 14.5 (µ = 12.5)  | 18.9  |
| #3     | 20                      | 21.1, 24.3, 18.0 (µ = 21.1) | 14.9  | 17.9, 18.5, 25.6 (µ = 20.7) | 20.1  |

Table S3. Analytical performance of CBZ detection methods.

| Method                      | Probe                        | Selective | Pre-treatment? | Dynamic Range (µM) | LOD (µM) | Assay Time (min) | Ref. |
|-----------------------------|------------------------------|-----------|----------------|--------------------|----------|------------------|------|
| Electrochemical (direct)    | SWV, CV GCE/MPEDOT (Molecular imprinting sensor) | Yes       | No             | 100-2000           | 980      | 15               | 57   |
|                             | EIS Au/Gr/AuNPs              | No        | No             | 10-1000            | 3.03     | 8                | 3    |
|                             | DPV GCE                      | No        | No             | N/A                | 0.59     | 5                | 8    |
| Fluorescence polarization immunoassay | Glass column              | No        | Yes            | N/A                | 0.85     | 30               | 8    |
|                             | GC column                    | No        | Yes            | 10.6-105.8         | N/A      | 90               | 58   |
| LC-MS/MS                    | Synergi 4µ Fusion column     | No        | Yes            | 4.2-169.3          | 105.81   | 90               | 59   |
| Dispersive liquid-liquid microextraction | Eurospher -100 C18 column | No        | Yes            | 0.002-0.8          | 0.0009   | 60               | 60   |
| Electrochemical aptasensor  | SWV MB-aptamer               | Yes       | No             | 0.01-100           | 0.001    | 30               | This study |
|                             |                              |           |                | 0.01-100           | 0.001    | 5                |      |
Table S4. Assay comparison table.

|                        | Rapid Testing                      | Routine Testing                      |
|------------------------|------------------------------------|--------------------------------------|
| Sample incubation time | 5 min                              | 30 min                               |
| Purpose                | Emergency care decisions for fast and appropriate medical care | Regular medical checkups for long-term administrated patients |
| Electrode type         | Disposable screen-printed electrode | Disk electrode                       |
| Required Sample volume | 50 µL                              | 50 µL                                |
| Measurement solvent volume | 50 µL                        | 5 mL                                 |
| Washing step           | No                                 | Yes                                  |
| Dynamic range          | 10 nM to 100µM                     | 10 nM to 100µM                       |
| LOD                    | 1.25nM                             | 1.82nM                               |
| Linear regression      | I(µA)=0.080(CBZ(nM))+0.150 in serum | I(µA)=0.389(CBZ(nM))+0.336 in serum |
| RSD % (Accuracy)       | 16.2                               | 5.46                                 |
| Uncertainty in concentration | 25.07                      | 5.82                                 |