Tetrahydrocannabinol (THC)-Modified Screen-Printed Carbon Electrodes (SPCEs): Insights into Stability

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I. Supplementary Information

S1. Statistical Analysis (ANOVA)

The one-way analysis of variance (ANOVA) was conducted to provide statistical evidence associated with the best storage conditions presented in Figures 5-7 in the manuscript.

a. THCi-modified electrodes (130 ng) at -18°C over the span of 6 months

The associated ANOVA analysis (Table S1a(1)) was performed using a significance level (α) of 0.05. With the resultant p-value < α, the null hypothesis is rejected, implying that the mean AUC values for the modified electrodes are significantly different over time when stored at -18°C.

Table S1a(1)): Overall ANOVA analysis performed for the THCi-modified electrodes stored at -18°C for 6 months.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	0.01121	0.00187	250.95651	4.77321E-17
Error	19	1.41506E-4	7.4477E-6		
Total	25	0.01136			

The corresponding percentage of variation (R^2) of 98% (Table S1a(2)) signifies a highly robust model and indicates a strong relationship between the storage conditions and the stability of the electrodes. A coefficient of variation (CV) of 4.28% suggests a relatively low level of variability in the dataset compared to the mean. Hence, there is a high degree of consistency in the AUC values for the modified electrodes stored at -18°C over time. Analyzing Levene's Test (Table

S1a(3)) for determining the homogeneity of variance, the population variances are significantly different at the 0.05 significance level with the p-value < α.

Table S1a(2): Fit Statistics performed for the THCi-modified electrodes stored at -18°C for 6 months.

	R-Square	Coeff Var	Root MSE	Data Mean
	0.98754	0.04283	0.00273	0.06371

Table S1a(3): Levene's Test performed for the THCi-modified electrodes stored at -18°C for 6 months.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	2.79177E-5	4.65294E-6	5.2807	0.00237
Error	19	1.67413E-5	8.81122E-7		

Lastly, the Tukey-Kramer test (Table S1a(4)) was conducted to compare multiple group means for each of the time periods, where Sig = 1 indicates that the difference of means is significant AND Sig = 0 indicates that the difference of means is insignificant at the 0.05 level. The results obtained demonstrate that pairs M2-D7, and M5-M4 consist of statistically insignificant differences with respect to their means. Since these are crucial time periods which mark the beginning and the end of the shelf-life study, the practical significance of using these storage conditions for the long-term stability of the modified electrodes is validated.

Table S1a(4): The Tukey Test performed for the THCi-modified electrodes stored at -18°C for 6 months.

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
M1 D7	-0.01953	0.00223	12.39726	7.19733E-7	0.05	1	-0.02685	-0.01221
M2 D7	-0.00101	0.00193	0.73896	0.99815	0.05	0	-0.00735	0.00533
M2 M1	0.01853	0.00193	13.57616	2.13855E-7	0.05	1	0.01219	0.02486
M3 D7	-0.01136	0.00223	7.20776	0.00105	0.05	1	-0.01868	-0.00404
M3 M1	0.00818	0.00223	5.1895	0.02267	0.05	1	8.5798E-4	0.0155
M3 M2	-0.01035	0.00193	7.58384	5.94763E-4	0.05	1	-0.01669	-0.00401
M4 D7	-0.04372	0.00223	27.74998	0	0.05	1	-0.05104	-0.0364
M4 M1	-0.02419	0.00223	15.35272	5.99538E-8	0.05	1	-0.03151	-0.01687
M4 M2	-0.04272	0.00193	31.30395	0	0.05	1	-0.04905	-0.03638
M4 M3	-0.03237	0.00223	20.54222	6.65008E-8	0.05	1	-0.03969	-0.02505
M5 D7	-0.04288	0.00208	29.09491	0	0.05	1	-0.04973	-0.03604
M5 M1	-0.02335	0.00208	15.84168	5.05698E-8	0.05	1	-0.03019	-0.0165
M5 M2	-0.04187	0.00176	33.61607	0	0.05	1	-0.04766	-0.03609
M5 M3	-0.03153	0.00208	21.38949	1.27438E-7	0.05	1	-0.03837	-0.02468
M5 M4	8.41667E-4	0.00208	0.57106	0.99957	0.05	0	-0.006	0.00769
M6 D7	-0.05224	0.00208	35.44221	0	0.05	1	-0.05908	-0.04539
M6 M1	-0.0327	0.00208	22.18898	1.30518E-7	0.05	1	-0.03955	-0.02586
M6 M2	-0.05123	0.00176	41.1263	0	0.05	1	-0.05701	-0.04544
M6 M3	-0.04088	0.00208	27.73679	0	0.05	1	-0.04773	-0.03403
M6 M4	-0.00851	0.00208	5.77624	0.00939	0.05	1	-0.01536	-0.00167
M6 M5	-0.00935	0.00193	6.85587	0.0018	0.05	1	-0.01569	-0.00302

b. THCi-modified electrodes (130 ng) with a second modification of PBS (pH 4) at -18°C over the span of 6 months.

The overall ANOVA analysis for the THCi-modified electrodes with PBS(4) at -18°C for 6 months is depicted in Table S1b(1). The p-value < α infers that the mean AUC values for the electrodes are significantly different over time at the 0.05 significance level. The corresponding R^2 value (Table S1b(2)) of 96% implies a solid correlation between the storage conditions and the stability of the electrode at those conditions.

Table S1b(1): Overall ANOVA analysis performed for the THCi-modified electrodes with PBS(4) stored at -18°C for 6 months.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	0.07607	0.01268	118.03968	1.36871E-15
Error	22	0.00236	1.07409E-4		
Total	28	0.07843			

Table S1b(2): Fit Statistics performed for the THCi-modified electrodes with PBS(4) stored at -18°C for 6 months.

	R-Square	Coeff Var	Root MSE	Data Mean
	0.96987	0.05397	0.01036	0.19203

Examining the Levene's Test for the electrodes in Table S1b(3) with the p-value > α, this corroborates that the population variances are not significantly different at the 0.05 level. Hence, the corresponding electrodes demonstrated exceptional stability using the storage conditions described.

Table S1b(3): Levene's Test performed for the THCi-modified electrodes with PBS(4) stored at -18°C for 6 months.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	1.39851E-4	2.33085E-5	0.94961	0.48077
Error	22	5.39998E-4	2.45454E-5		

Analyzing the resultant Tukey Test for the associated electrodes in Table S1b(4), multiple pairs of time periods consisted of insignificant differences between their means at the 0.05 significance level. These pairs comprised of M1-D7, M3-M2, M4-M2, M4-M3, M5-M2, M5-M3, M5-M4, M6-M2, M6-M3,M6-M4, and M6-M5. Therefore, these specific groups do not exhibit statistically significant variation in shelf life, valuable for quality control and the product stability.

Table S1b(4): The Tukey Test performed for the THCi-modified electrodes with PBS(4) stored at -18°C for 6 months.

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
M1 D7	-0.00683	0.00733	1.31708	0.96315	0.05	0	-0.03054	0.01689
M2 D7	-0.11813	0.00733	22.79559	8.56344E-8	0.05	1	-0.14184	-0.09441
M2 M1	-0.1113	0.00733	21.47851	8.39501E-8	0.05	1	-0.13502	-0.08758
M3 D7	-0.11903	0.00695	24.21173	1.39416E-7	0.05	1	-0.14152	-0.09653
M3 M1	-0.1122	0.00695	22.82341	8.5667E-8	0.05	1	-0.1347	-0.0897
M3 M2	-9E-4	0.00695	0.18308	1	0.05	0	-0.0234	0.0216
M4 D7	-0.11154	0.00669	23.57961	1.33739E-7	0.05	1	-0.13319	-0.08989
M4 M1	-0.10472	0.00669	22.13683	8.48275E-8	0.05	1	-0.12637	-0.08307
M4 M2	0.00658	0.00669	1.3917	0.95229	0.05	0	-0.01507	0.02823
M4 M3	0.00748	0.00628	1.68637	0.88964	0.05	0	-0.01283	0.02779
M5 D7	-0.10803	0.00898	17.0211	5.53563E-8	0.05	1	-0.13707	-0.07898
M5 M1	-0.1012	0.00898	15.94571	6.40884E-8	0.05	1	-0.13025	-0.07215
M5 M2	0.0101	0.00898	1.59142	0.91338	0.05	0	-0.01895	0.03915
M5 M3	0.011	0.00867	1.79406	0.85869	0.05	0	-0.01706	0.03906
M5 M4	0.00352	0.00846	0.58772	0.99951	0.05	0	-0.02387	0.0309
M6 D7	-0.12768	0.00733	24.63853	1.43138E-7	0.05	1	-0.15139	-0.10396
M6 M1	-0.12085	0.00733	23.32145	8.62292E-8	0.05	1	-0.14457	-0.09713
M6 M2	-0.00955	0.00733	1.84294	0.84329	0.05	0	-0.03327	0.01417
M6 M3	-0.00865	0.00695	1.75956	0.86906	0.05	0	-0.03115	0.01385
M6 M4	-0.01613	0.00669	3.41054	0.24027	0.05	0	-0.03778	0.00552
M6 M5	-0.01965	0.00898	3.09618	0.34012	0.05	0	-0.0487	0.0094

c. *THCi-modified electrodes (130 ng) with a second modification of PBS (pH 4) at 4°C over the span of 6 months.*

The overall ANOVA analysis for the affiliated electrodes is presented in Table S1c(1). As per the p-value < α, the AUC means for the modified electrodes are significantly different at the 0.05 level. Approximately 76% of the total variation (Table S1c(2)) associated with the stability of the electrodes can be accounted for by the storage conditions used, specifically, the storage at 4°C in this case. Interpreting the Levene's Test of Absolute Deviations (Table S1c(3)) with the p-

value > α, it can be concluded that the variances in the AUC values of the electrodes are insignificant over time at the 0.05 significance level.

Table S1c(1): Overall ANOVA analysis performed for the THCi-modified electrodes with PBS(4) stored at 4°C for 6 months.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	0.00555	9.25594E-4	15.01965	1.28803E-7
Error	28	0.00173	6.16255E-5		
Total	34	0.00728			

Table S1c(2): Fit Statistics performed for the THCi-modified electrodes with PBS(4) stored at 4°C for 6 months.

	R-Square	Coeff Var	Root MSE	Data Mean
	0.76295	0.09157	0.00785	0.08573

Table S1c(3): Levene's Test (Absolute Deviations) performed for the THCi-modified electrodes with PBS(4) stored at 4°C for 6 months.

	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	6	1.48209E-4	2.47014E-5	1.55813	0.19634
Error	28	4.43892E-4	1.58533E-5		

Examining Tukey's Test for multiple groups means in Table S1c(4), numerous pairs of groups exhibited insignificant differences among the means at the 0.05 significance level. The pairs consist of M2-D7, M3-D7, M3-M2, M4-D7, M4-M2, M4-M3, M5-D7, M5-M2, M5-M4, M6-D7, M6-M3, and M6-M4. Hence, the Tukey Test supports the storage conditions tested for its contribution to extending the stability of the modified electrodes.

Table S1c(4): The Tukey Test performed for the THCi-modified electrodes with PBS(4) stored at 4°C for 6 months.

	MeanDiff	SEM	q Value	Prob	Alpha	Sig	LCL	UCL
M1 D7	0.03081	0.00496	8.77657	2.00334E-5	0.05	1	0.01506	0.04656
M2 D7	0.0078	0.00496	2.22234	0.70059	0.05	0	-0.00795	0.02355
M2 M1	-0.02301	0.00496	6.55423	0.00131	0.05	1	-0.03876	-0.00726
M3 D7	-0.00624	0.00496	1.77628	0.8656	0.05	0	-0.02199	0.00951
M3 M1	-0.03705	0.00496	10.55284	8.09407E-7	0.05	1	-0.0528	-0.0213
M3 M2	-0.01404	0.00496	3.99862	0.10491	0.05	0	-0.02979	0.00171
M4 D7	-0.00165	0.00496	0.46885	0.99987	0.05	0	-0.0174	0.0141
M4 M1	-0.03246	0.00496	9.24542	8.38147E-6	0.05	1	-0.04821	-0.01671
M4 M2	-0.00945	0.00496	2.69119	0.49498	0.05	0	-0.0252	0.0063
M4 M3	0.00459	0.00496	1.30743	0.96531	0.05	0	-0.01116	0.02034
M5 D7	0.0122	0.00496	3.47394	0.21361	0.05	0	-0.00355	0.02795
M5 M1	-0.01862	0.00496	5.30263	0.01275	0.05	1	-0.03437	-0.00287
M5 M2	0.00439	0.00496	1.2516	0.97193	0.05	0	-0.01136	0.02014
M5 M3	0.01843	0.00496	5.25022	0.01397	0.05	1	0.00268	0.03418
M5 M4	0.01384	0.00496	3.94279	0.11368	0.05	0	-0.00191	0.02959
M6 D7	-0.00877	0.00496	2.49693	0.58047	0.05	0	-0.02452	0.00698
M6 M1	-0.03958	0.00496	11.2735	2.49058E-7	0.05	1	-0.05533	-0.02383
M6 M2	-0.01657	0.00496	4.71927	0.03431	0.05	1	-0.03232	-8.18687E-4
M6 M3	-0.00253	0.00496	0.72065	0.9985	0.05	0	-0.01828	0.01322
M6 M4	-0.00712	0.00496	2.02808	0.77914	0.05	0	-0.02287	0.00863
M6 M5	-0.02096	0.00496	5.97087	0.00386	0.05	1	-0.03671	-0.00521

S2. XPS Characterization

Table Table S2a: XPS survey data (atomic percentage) for the most concentrated elements in the materials.

Samples	**Elements (At. %)**					
	C 1s	**O 1s**	**N 1s**	**Cl 2p**	**Si 2p**	**Fe 2p**
P-Z*	82.2	8.7	0.4	7.9	0.9	0.3
Control	77.4	9.1	2.2	10.1	1.0	0.2
RT	81.1	9.5	1.4	7.4	0.4	0.2
4CT	82.2	10.2	1.5	5.9	-	0.2

-18CT	73.6	21.4	0.7	-	2.8	1.4

*P-Z: Pristine Zensor electrode

Table S2b: The peak-fitting results of C1s high-resolution signal of materials.

Samples	Assignment	E_B (eV)	FWHM (eV)	At. %
P-Z	**C1s** C=C aromatic	284.4	0.6	16.2
	C1s C-C, C-H	285.0	1.3	57.6
	C1s C-OH, C-O-C, C-Cl	286.5	1.3	22.0
	C1s O-C=O	289.0	1.3	4.2
Control	**C1s** C=C aromatic	284.4	0.7	18.2
	C1s C-C, C-H	285.0	1.5	51.1
	C1s C-OH, C-O-C, C-Cl	286.4	1.5	26.3
	C1s O-C=O	288.8	1.6	4.4
RT	**C1s** C=C aromatic	284.2	0.9	20.4
	C1s C-C, C-H	285.0	1.4	49.4
	C1s C-OH, C-O-C, C-Cl	286.3	1.5	26.0
	C1s O-C=O	288.7	1.7	4.2
4CT	**C1s** C=C aromatic	284.3	0.8	17.7
	C1s C-C, C-H	285.0	1.5	55.9
	C1s C-OH, C-O-C, C-Cl	286.4	1.5	23.8
	C1s O-C=O	288.7	1.6	2.7
-18CT	**C1s** C=C aromatic	284.4	1.0	24.8
	C1s C-C, C-H	285.0	1.4	55.5
	C1s C-OH, C-O-C, C-Cl	286.5	1.4	9.9

	C1s O-C=O	288.4	1.7	9.8

Table S2c: The peak-fitting results of O1s high-resolution signal of materials.

Samples	Assignment	E_B (eV)	FWHM (eV)	At. %
P-Z	**O1s** C=O	532.5	1.5	68.3
	O1s O*-(C=O)-C, C-O aromatic	533.5	1.6	31.7
Control	**O1s** C=O	532.2	1.6	51.5
	O1s O*-(C=O)-C, C-O aromatic	533.5	1.7	48.5
RT	**O1s** C=O	532.1	1.6	43.4
	O1s O*-(C=O)-C, C-O (aromatic)	533.4	1.8	56.5
4CT	**O1s** C=O	532.2	1.6	45.4
	O1s O*-(C=O)-C, C-O (aromatic)	533.4	1.8	54.6
-18CT	**O1s** C=O	532.4	1.6	60.6
	O1s O*-(C=O)-C, C-O (aromatic)	533.6	1.8	39.4

S3. Additional modifiers

Organic compounds, including Ascorbic Acid (AA) and Citric Acid (CA) were used to perform a second modification on the THCi-modified electrodes to perform their role as antioxidants and radical scavengers in controlling the oxidation of THC. Both, AA-modified and CA-modified electrodes were vacuum sealed with O_2 absorbers and stored at 4°C for a period of one week. Although the corresponding results (Figure S3a) depict similar electrochemical behaviour to THCi-modified electrodes with a second modification of PBS (pH 4), the signals only remained stable for up to day three, after which the intensity of the signal was observed to decrease on day

7. Additionally, electrodes with a second modification of these organic compounds also exhibited significantly low reproducibility.

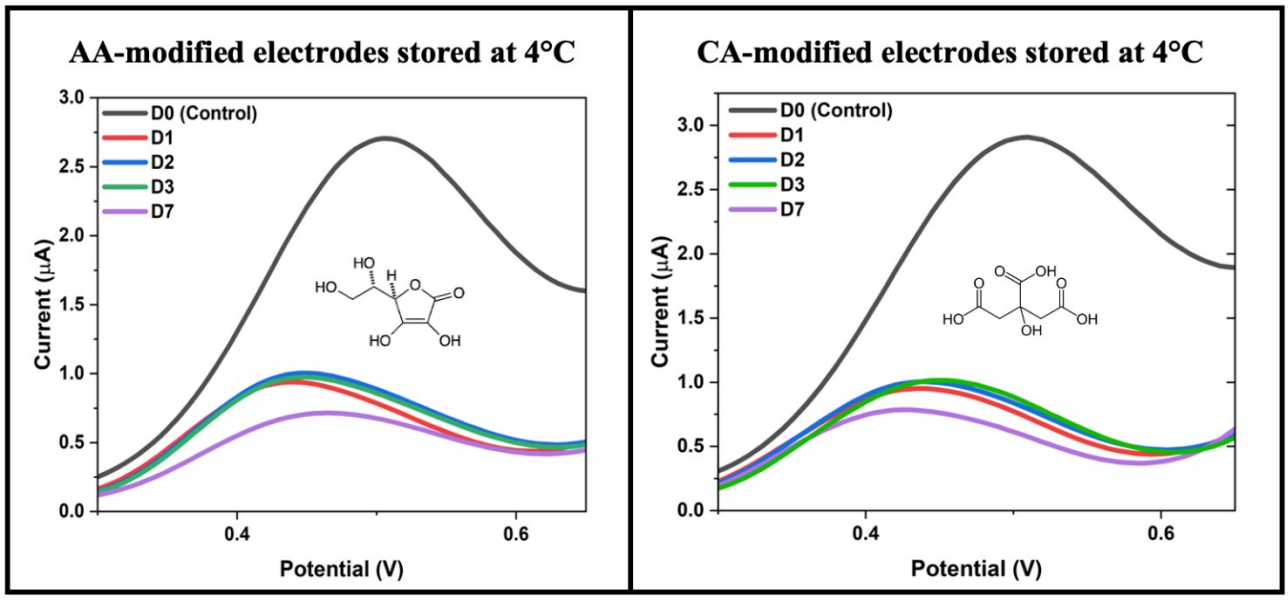

Figure S3a: AA-modified electrodes (13 ng) and CA-modified electrodes (13 ng) stored at 4°C for a period of one week.

BHT is yet another organic chemical which functions by blocking the propagation of free radicals, and hence, it was used for its antioxidant properties in this study. The THCi-modified electrodes were modified with a second modification of BHT in conjunction with Transcutol as was previously reported in the literature for THC [S1], vacuum sealed with O_2 absorbers and stored on the lab bench at RT for a period of one month. The results showcase (Figure S3b) that the modified electrodes only remained stable for a period of two days at RT, after which flat signals were observed for the subsequent days.

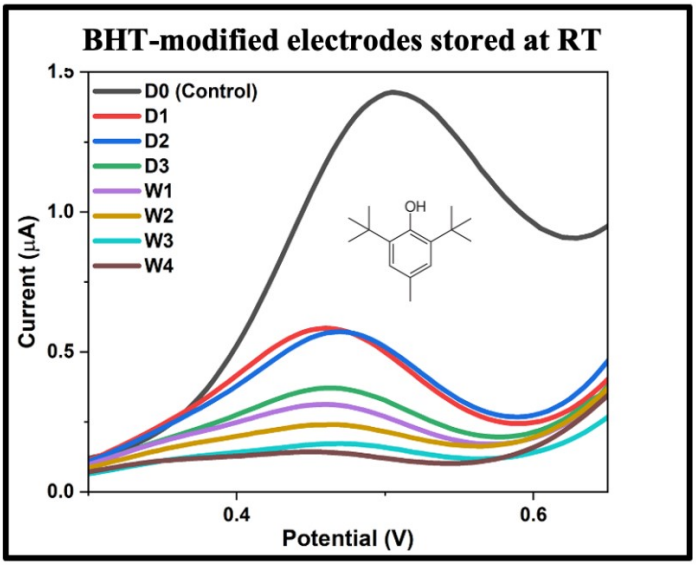

Figure S3b: BHT-modified electrodes (5.2 ng) stored at RT for one month.

S4. Alternative modifiers

THCA is the inactive and the more stable precursor of THC due to the presence of an extra carboxylic group. The TE-100 Zensor electrodes were manually modified with 130 ng of THCA, vacuum sealed with O_2 absorbers, placed in a mylar bag and stored on the lab bench at RT and in the refrigerator at 4°C for a period of one week (Figure S4a). The electrodes were tested using PBS (pH 7.4) via SWV, during which the electrochemical signals displayed an additional second peak during detection (SCAN 2). The presence of the second peak denotes the occurrence of the partial decomposition of THCA into its decarboxylated product, THC.

Comparing the different temperatures for storing the THCA-modified electrodes, storage at both RT and 4°C provided stable electrochemical signals for the entire span of one week. With reproducibility and high intensity values being prime factors in engendering stability, in addition to the second peak observed, the resultant intensities obtained in the graph are too low to be used for the purposes of THC detection.

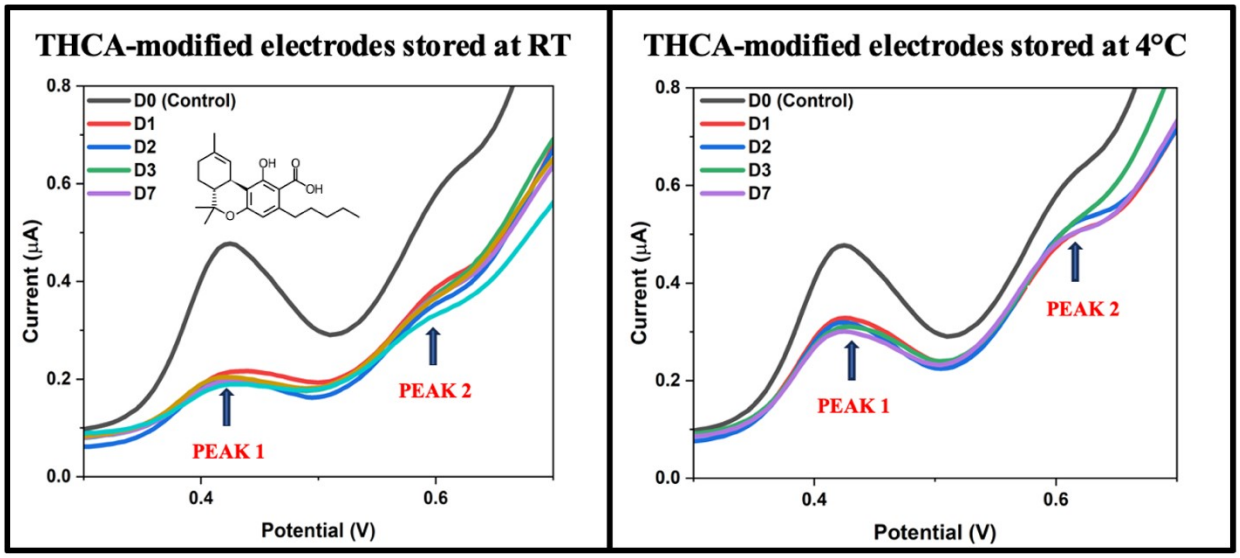

Figure S4a: THCA-modified electrodes (130 ng) stored on the lab bench at RT and at 4°C for a period of one week.

Dopamine is a neurotransmitter with a similar electrochemical behaviour to that of THC i.e., with an irreversible oxidation potential at approximately 0.45 V. An amount of 100 ng of DA was manually deposited onto the WE of the pristine electrodes after incubating the deposition sample for 0 minutes and 30 minutes. Irrespective of the sample incubation time tested, the DA-modified electrodes exhibited a broad electrochemical signal comprised of two different oxidation potentials and hence, were unable to detect the oxidation of THC (Figure S4b). In this case, DA displayed a reversible oxidation peak at 0.25 V, implying electro-oxidation of the molecule. After conducting the first scan, the subsequent four scans for the molecule presented a flat signal via SWV.

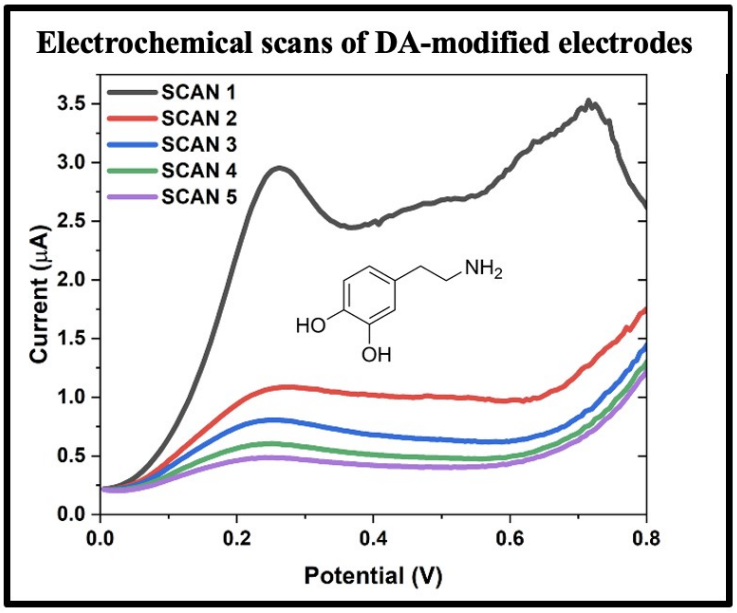

Figure S4b: The electrochemical behaviour of DA-modified electrodes (100 ng).

Subsequently, the manual deposition of 130 ng of CBD was performed onto the WE of the TE-100 Zensor electrodes after incubating the deposition sample for 40 minutes. The CBD-modified electrodes were vacuum sealed with O_2 absorbers and stored on the lab bench at RT, and at 4°C for a period of one week. CBD is known to undergo the course of oxidation at the same oxidation potential value as THC (0.48 V) due to the analogous redox active moieties present in both molecules. The resultant graphs (Figure S4c) obtained in PBS (pH 7.4) via SWV display the same electrochemical behaviour as that of THC at both temperature conditions. The peak current values demonstrate stability at both RT and 4°C until day 3, after which the signal is observed to decrease.

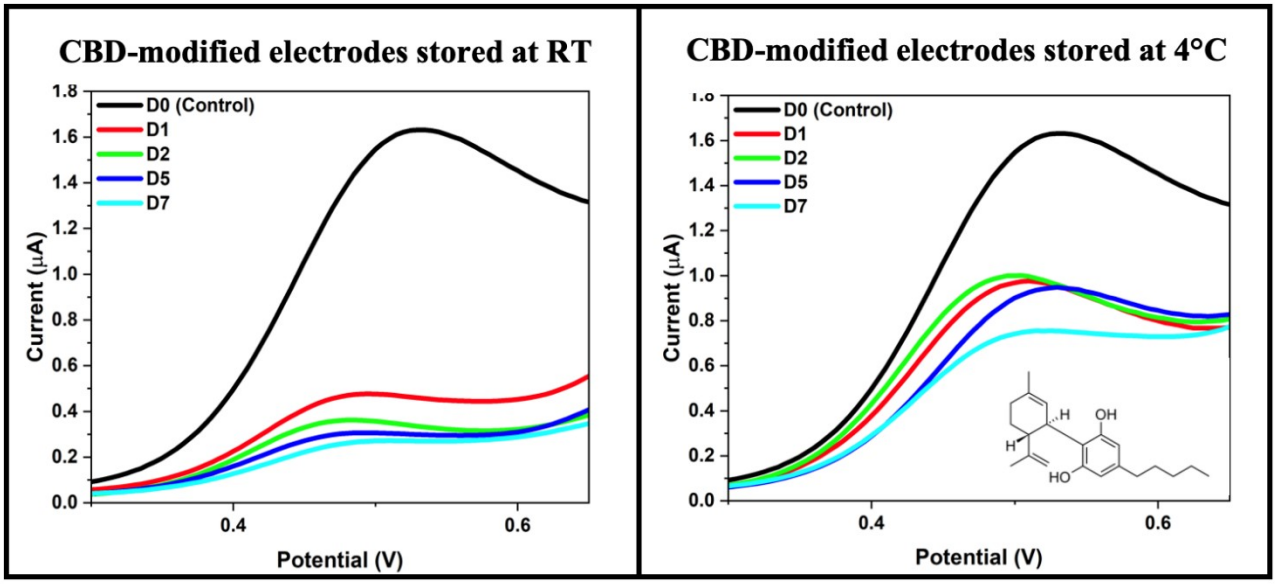

Figure S4c: CBD-modified electrodes (130 ng) stored on the lab bench at RT and at 4°C for a period of one week.

Lastly, pristine electrodes were manually modified with THC-OAc (130 ng) and subjected to SCAN 2 using SWV at RT in PBS (pH 10) and PBS (pH 12). The resultant graph (Figure S4d) demonstrates the lack of signals due to THC oxidation despite a high basic pH, invalidating this modification for sensing purposes. Hence, the flat signals obtained were unfavourable for the purposes of extending stability for our research.

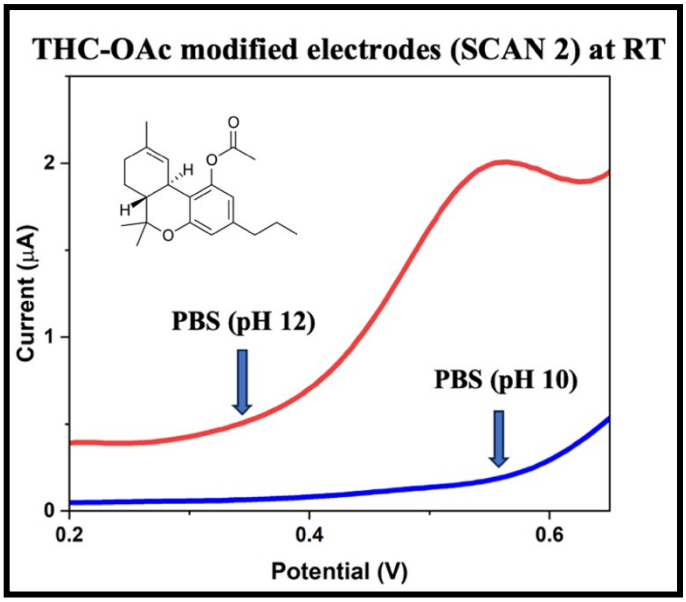

Figure S4d: THC-OAc-modified electrodes (130 ng) SCAN 2 performed in PBS(10) and PBS(12) at RT.

References

S1. G. Gonzalez-Cuevas et al., “Unique treatment potential of cannabidiol for the prevention of relapse to drug use: Preclinical proof of principle,” *Neuropsychopharmacology*, vol. 43, pp. 2036–2045, 2018, doi: 10.1038/s41386-018-0050-8.