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Construction of Modified Carbon Paste Electrodes for Determination of Tramadol in Very Trace Amounts

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Keywords: Tramadol, Nanosilica, Electrodes, Carbon Paste Electrodes.

Article History

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Abstract:

This research includes estimating the drug (Tramadol Hydrochloride, TR) using the potentiometric method by constructing selective electrodes for TR drug with the active ingredient (Ammonium Reinackate, AR) using a plasticizer (Nitro benzene, NB) and adding nanomaterials (Multi wall carbon nanotube (MWCNT), Nanosilica) for carbon paste electrodes to increase selectivity and sensitivity towards the material to be estimated. The results showed that the manufactured electrodes were able to estimate tramadol hydrochloride in the pharmaceutical preparation (tramadol tablets) at very low concentrations (trace amounts**)** up to 5.0×10^{-6} M using the direct and standard methods and proved to have a wide linear range up to 1.0×10^{-8} - 1.0×10^{-2} M. The Nernstatine slope of the prepared TR-AR-NB electrodes is (58.027, 58.251, and 58.694 mV/decade) for Carbon Paste Electrodes (CPE), MCPE (MWCNTs) and MCPE (MWCNTs+ nanosilica), respectively. The lower detection limit (LDL) is 2.39×10^{-7} M for the CPE and 4.98×10^{-8} M for the MCPE (MWCNTs) and 4.7384×10^{-9} M for the electrode MCPE (MWCNTs+ nanosilica) which makes it eligible for the estimation of tramadol hydrochloride in very low concentrations. The study included measuring the selectivity of these electrodes with the presence of interferers where the values of $K_{i,j}^{pot}$ for all studied species were less than 1. The drug was identified in both urine and blood plasma, with a recovery of at least 99.309 for urine and 97.6593 for blood plasma.

Keywords: Carbon Paste Electrodes, Electrodes, Nanosilica, Tramadol.

بناء أقطاب عجينة الكربون المعدلة لتقدير الترامادول بكميات ضئيلة جداً

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الخلاصة:

يتضمن هذا البحث تقدير عقار (هيدروكلوريد الترامادول، TR) باستخدام الطريقة الجهدية من خلال بناء أقطاب انتقائية لعقار TR مع المادة الفعالة (ريناكيت الأمونيوم، AR) باستخدام مادة ملدنة (نيترو بنزين، NB) وإضافة مواد نانوية (أنابيب الكربون النانوية متعددة الجدران (MWCNT)، نانوسيليكا) لأقطاب عجينة الكربون لزيادة الانتقائية والحساسية تجاه المادة المراد تقديرها. أظهرت النتائج أن الأقطاب المصنعة كانت قادرة على تقدير الترامادول المستحضر الصيدلاني (أقراص الترامادول) عند تراكيز منخفضة للغاية (كميات ضئيلة) تصل إلى $5.0 \times 10^{-6} M$ باستخدام الطرق المباشرة والقياسية وأثبتت أن لها نطاق خطي واسع يصل إلى 1.0×10^{-2} M - 1.0×10^{-8}. إن الميل النيرنستي لأقطاب TR-AR-NB المحضرة هو (٥٨,٠٢٧ و ٥٨,٢٥١ و ٥٨,٦٩٤ ملي فولت / عقد) لأقطاب عجينة الكربون (CPE) و MCPE (MWCNTs) و MCPE (MWCNTs + نانوسيليكا) على التوالي. الحد الأدنى للكشف (LDL) هو 2.39×10^{-7} M لـ CPE و $4.98\times10^{-}$ 8M لـ MCPE (MWCNTs) و 4.7384×10^{-9}M للقطب MCPE (MWCNTs + nanosilica) مما يجعله مؤهلاً لتقدير هيدروكلوريد الترامادول في تركيزات منخفضة جدًا. تضمنت الدراسة قياس انتقائية هذه الأقطاب مع وجود متداخلات حيث كانت قيم Ki,jpot لجميع الأنواع المدروسة أقل من ١. باستخدام الطريقة المباشرة وطريقة الإضافة القياسية، تم تقدير العقار في كل من البول وبلازما الدم، مع استرداد لا يقل عن ٩٩,٣٠٩ للبول و ٩٧,٦٥٩٣ لبلازما الدم.

الكلمات المفتاحية: ترامادول، النانو سيليكا، ألاقطاب، قطب عجينة الكربون.

1. Introduction:

Tramadol (TR) is chemically (±)-*trans*-2-Dimethylaminomethyl-1-(3-methoxyphenyl) cyclohexanol hydrochloride. has a chemical structure shown in **Figure 1** **[1]**. For the temporary alleviation of acute pain, tramadol is utilized. It should only be utilized when non-opioid painkillers are either ineffective or do not assist the patient in regulating their pain **[2,3]**. This drug's content has been assessed using a variety of analytical techniques, including spectroscopy **[4-6]**, due to its medicinal relevance, HPLC **[7-9]**, voltmetery **[10]**, gas chromatography **[11]**, the colorimetric method **[12]**, LC-MS technique **[13]**, fluorometry **[14]**, A Screen-Printed Electrode **[15]** and Electrocatalytic Platform **[16]**. In the analysis procedures, the selective electrodes for ions approach is preferred over many spectrum methods because it is quick, has a broad linear range, is unaffected by the model's color, and is straightforward to set up and operate **[17]**. The goal of the current work is to create a modified electrode containing MWCNTs (MCPE), nanosilica, and unmodified electrodes (CPE) that can be used to assess TR

in pure, pharmaceutical, and biological forms with accuracy, sensitivity, and selectivity. The fundamental analytical parameters were computed and compared for each sensor. The formula for Tramadol hydrochloride is **[18]**.

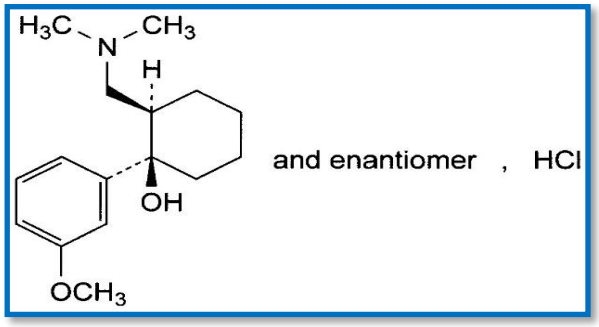


Figure 1: Chemical Structure of Tramadol

Its molecular weight is 299.8 g/mol and its formula is $C_{16}H_{25}NO_2$, HCl. It has the shape of a crystalline powder that is white or almost white. Extremely weakly soluble in acetone, yet freely soluble in water and methyl alcohol **[19].**

2. Material And Methods:

2.1 APPARATUS: Jenway 3310 pH Meter, HANNA Instruments pH Meter 211, calomel electrode Swiss source, JENWAY Hot Plate with Stirrer-Germany, C.H.N Perkin Elmer USA 2400 Series II element analyzer.

2.2 MATERIALS: The chemicals utilized were all very pure and were from Fluka, BDH, and SDI.

2.2.1 Preparation of Tramadol 100 mg/tablet solution: Ten tablets the average weight of one pill 3.941 g, made by the Dimedic Company in the UK, were crushed in an agate mortar to determine the average weight of a tablet. To obtain (1.0×10^{-2}) M, 1.1815 g of the pharmaceutical preparation was obtained and dissolved in 100 ml of deionized distilled water. Additional diluted solutions were prepared for each sample by appropriately diluting with deionized distilled water.

2.2.2 Biological fluid solutions 0.01 M AM: The lowest concentrations of the solutions were made by diluting the samples with deionized distilled water. 4.5 ml of human plasma or urine was obtained, and 0.5 ml of 0.1 M TR was added. The tube was then shaken for one minute.

2.2.3 Preparation of Interfering Solutions: Solutions of $(1.0\times10^{-3}$ M) were created by dissolving the required amounts of these compounds (NaBr, NaCl, $CaCl_2$, KCl, Na_2CO_3, Na_2SO_4, starch, glucose, fructose) in 100 ml of deionized distilled water in volumetric flasks.

2.3 Preparation of the ion-pair: The TR-AR ion-pair was prepared by mixing 10 ml of 0.1 M TR solution with 10 ml of 0.1 M AR solution to create a light red precipitate. The precipitate was then filtered, repeatedly washed with deionized water, and allowed to dry for a day at room temperature. **Table 1** displays the results of the CHN analysis of the TR-AR ion pair.

Table 1: Elemental Analysis for the TR-AR Ion Pair.

TR-AR			
Element	%C	%H	%N
Found	36.74	5.80	17.16
Calculated	36.71	5.85	17.13
Formula	$[C_{16}H_{25}NO_2Cl][C_4H_{10}CrN_7S_4].2H_2O$		

2.4 Preparation of the Electrodes

2.4.1 Construction of carbon and Modified carbon Paste Electrodes (CPE, MCPE): After conducting a number of pilot tests, the selective membrane was created by combining its constituent parts in accordance with weight ratios; the outcomes are displayed in **Table 2** The best electrode for TR-AR-NB CPE is electrode No. 2, the best electrode for CPE (MWCNTs) is electrode No. 5, and the best electrode for CPE (MWCNTs+ nanosilica) is electrode No. 8.

Table 2: Components and Characteristics of CPE and MCPE Electrodes.

CPE no.	Graphite powder (%)	NB (%)	TR-AR (%)	MWCNTs (%)	Nanosilica (%)	Slope $\frac{mV}{decade}$	Linear range $\frac{mol}{L}$	R^2
1	70	20	10	0	0	54.175	$5\times10^{-7}-1\times10^{-2}$	0.9891
2	**65**	**20**	**15**	**0**	**0**	**58.027**	$5\times10^{-7}-1\times10^{-2}$	**0.9999**
3	60	20	20	0	0	56.009	$5\times10^{-7}-1\times10^{-2}$	0.9911
4	62	20	15	3	0	57,208	$1\times10^{-7}-1\times10^{-2}$	0.9940
5	**60**	**20**	**15**	**5**	**0**	**58.251**	$1\times10^{-7}-1\times10^{-2}$	**0.9999**
6	58	20	15	7	0	57.444	$1\times10^{-7}-1\times10^{-2}$	0.9965
7	59	20	15	5	1	57.971	$1\times10^{-8}-1\times10^{-2}$	0.9980
8	**57**	**20**	**15**	**5**	**3**	**58.694**	$1\times10^{-8}-1\times10^{-2}$	**0.9999**
9	55	20	15	5	5	58.089	$1\times10^{-8}-1\times10^{-2}$	0.9975

3. Results and Discussion:

3.1. Effect of pH: It was established what pH range is ideal for maintaining a stable electrode potential. To do this, an electrode potential measurement was conducted in a TR solution (1.0×10-4) M, ranging in pH from 1 to 10. The pH was adjusted using solutions of NaOH and/or HCl. The link between the pH value of the solutions and the electrode potential for each electrode is shown in **Figure 2**. It is clear that the electrodes' pH range was wide (2–7).

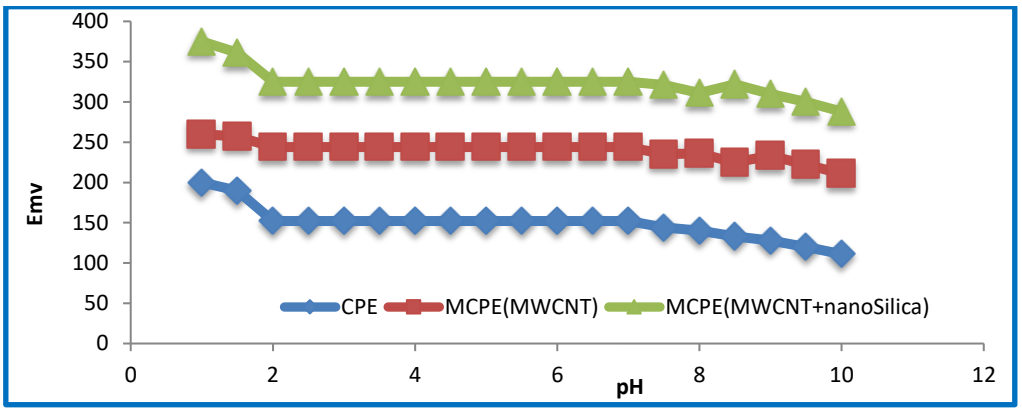


Figure 2: Effect of pH on the potential response of TR-AR-NB Electrodes

3.2. Effect of Temperature: The results in **Figure 3** showed that the ideal temperature for both CPE and MCPE (MWCNTs) electrodes is (10 – 60) °C, whereas the MCPE (MWCNTs+ nanosilica) electrode had a broader temperature range (10 – 70) °C. The results demonstrate all of the development electrodes are extremely thermally steady without noticeably changing their Nernstian slope. The potential change was observed for a concentration of (1×10^{-4}) M) TR solution by modifying the temperature of the solution compared to (5-85) oC.

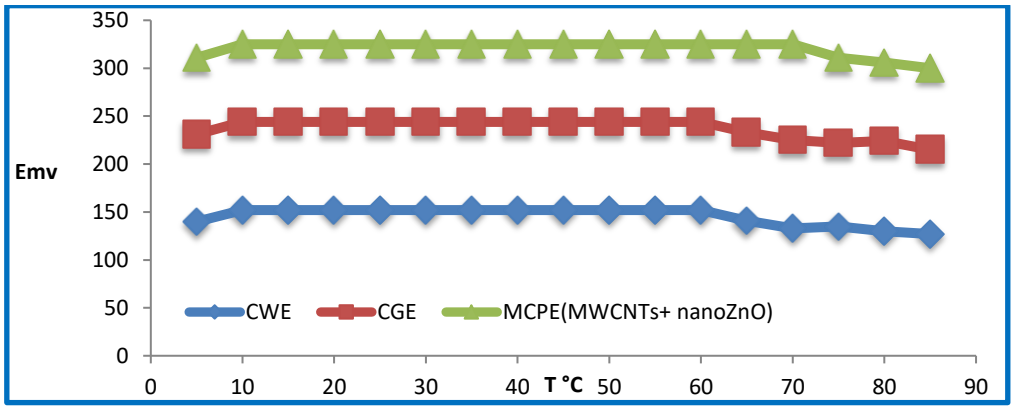


Figure 3: Effect of Temperature on the potential response of TR-AR-NB Electrodes

3.3. Calibration Curve and Detection Limit: Figure 4 displays the calibration curve that was created after several tests were conducted to determine the ideal pH and temperature. **Table 3** lists the electrode parameters. When it is evident that the electrode made with MCPE (MWCNTs+ nanosilica) is superior to the other electrodes in terms of benefits.

Table 3.: Analytical Characteristics of the Electrodes that Were Manufactured

Electrode type	Liner range M	Slope (mV decade^{-1})	Upper detection limit M (UDL)	Lower detection limit M (LDL)
MCPE (MWCNTs+ nanosilica)	1.0×10^{-8} - 1.0×10^{-2}	58.694	0.0201	4.7384×10^{-9}
MCPE (MWCNTs)	1.0×10^{-7} - 1.0×10^{-2}	58.251	0.0205	4.9827×10^{-8}
CPE	5.0×10^{-7} - 1.0×10^{-2}	58.027	0.0203	2.3920×10^{-7}

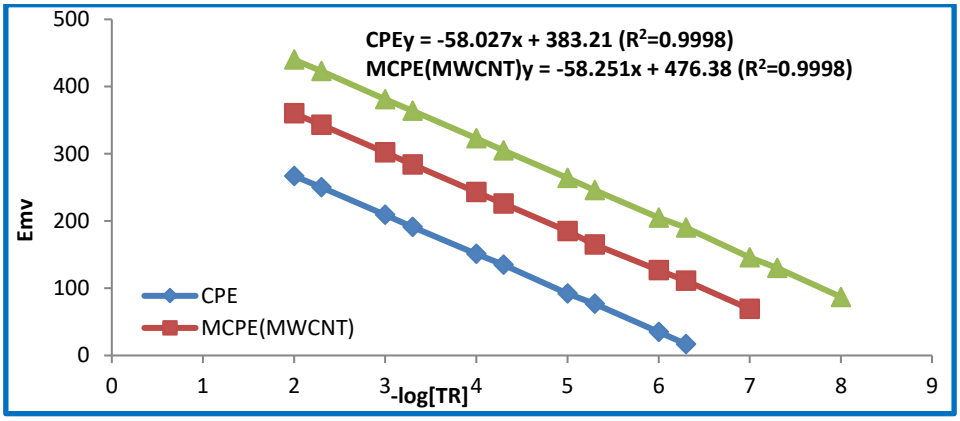


Figure 4: Calibration Curve of TR-AR-NB Electrodes.

3.4. Precision and accuracy: By measuring the potential of various drug concentrations within the linear range of the calibration curve for seven consecutive readings under ideal conditions, precision and accuracy were investigated. The results are displayed in **Table 4** and suggest that the set electrodes might be employed for determining the TR drug with precision as well as accuracy.

Table 4: Precision and accuracy of findings for TR-AR-NB electrodes.

Sample	Taken [TR] M	Found [TR] M	%Recovery	%RE
MCPE(MWCNT +nanosilica)	1×10^{-2}	9.9210×10^{-3}	**99.21**	-0.79
	1×10^{-3}	9.8026×10^{-4}	**98.02**	-1.97
	1×10^{-4}	1.0073×10^{-4}	**100.73**	-0.73
	1×10^{-5}	9.9530×10^{-6}	**99.53**	-0.46
	1×10^{-6}	9.8342×10^{-7}	**98.34**	-1.65
	1×10^{-7}	9.7169×10^{-8}	**97.16**	-2.83
	1×10^{-8}	9.6000×10^{-9}	**96.00**	-3.99
%Mean ± SD	98.4316 ± 1.5673			
N	7			
Variance	2.4564			
%RE	-1.5683			
%RSD	1.5922			
Sample	Taken [TR] M	Found [TR] M	%Recovery	%RE
MCPE (MWCNT)	1×10^{-2}	1.0048×10^{-2}	100.48	0.48
	1×10^{-3}	1.0148×10^{-3}	101.48	1.48
	1×10^{-4}	9.8524×10^{-5}	98.52	-1.47
	1×10^{-5}	9.9507×10^{-6}	99.50	-0.49
	1×10^{-6}	1.0049×10^{-6}	100.49	0.49
	1×10^{-7}	1.0150×10^{-7}	101.50	1.50
%Mean ± SD	**100.3335 ± 1.1571**			
n	**6**			
Variance	**1.3390**			
%RE	**0.3335**			
%RSD	**1.1533**			
Sample	Taken [TR] M	Found [TR] M	%Recovery	%RE
CPE	1×10^{-2}	9.9382×10^{-3}	99.38	-0.61
	1×10^{-3}	9.9489×10^{-4}	99.48	-0.51
	1×10^{-4}	9.9596×10^{-5}	99.59	-0.40
	1×10^{-5}	9.5824×10^{-6}	95.82	-4.17
	1×10^{-6}	9.9809×10^{-7}	99.80	-0.19
	5×10^{-7}	4.8862×10^{-7}	97.49	-2.50
%Mean ± SD	**98.5991 ± 1.5994**			
n	**6**			
Variance	**2.5583**			
%RE	**-1.4008**			
%RSD	**1.6222**			

3.5. Response Time: By submerging the electrode in ($1\times10^{(-6)}$-$1\times10^{(-2)}$M) drug solutions, monitoring the potential for each solution, and timing the electrode's reaction, the response time of the electrodes was examined. The response times of the created TR-AR-NB electrodes vary between 15–49 seconds, 20–53 seconds, and 23–55 seconds for MCPE(MWCNT+nanosilica), MCPE(MWCNT), and CPE electrodes, correspondingly, as can be seen from the data displayed in **Figure 5**.

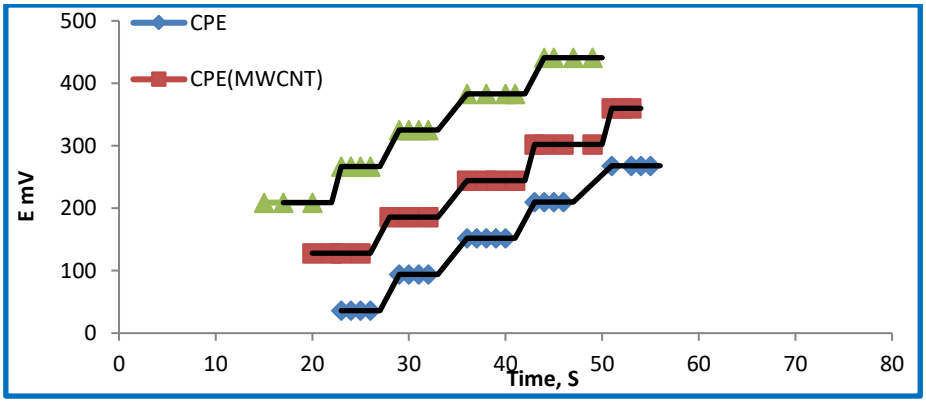


Figure 5: Response Time of TR-AR-NB Electrodes

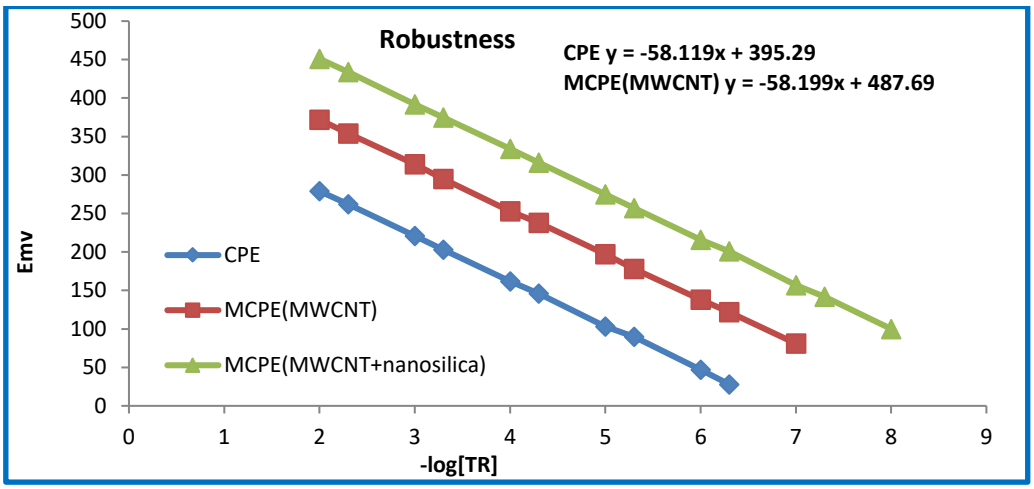
3.6. Electrode Life Observation: By establishing the calibration curve for every single set electrode two or three times over the course of a week, the lifetime of the TR-AR-NB electrodes was estimated. For the MCPE(MWCNT+nanosilica), MCPE(MWCNT), and CPE electrodes, respectively, no aberration in the Nernstain slope was observed for 52 days, 65 days, and 70 days. The prepared electrode is kept dry between readings.

3.7. Selectivity: The method of separate solutions was used to evaluate the selectivity **[20]**. First, the drug solution's potential was checked at a concentration of 1×10^{-3} M with no interfering ion (Ei), and then the interfering ion solution's potential was observed at a concentration of 1×10^{-3} M alone (Ej). The chemicals and interfering ions used for this investigation did not appear to have any effect on the electrodes' excellent drug selectivity. According to **Table 5**, every selectivity coefficient value is lower than one.

Table 5.- Selectivity coefficient values.

the interfering ion M1 × 10^{-3}	Selectivity coefficient values $K_{i,j}^{pot}$		
	TR-AR-NB		
	CPE	MCPE(MWCNT)	MCPE(MWCNT+nanosilica)
Na^{1+}	7.15×10^{-1}	6.00×10^{-1}	5.39×10^{-1}
K^{1+}	6.11×10^{-1}	5.34×10^{-1}	4.00×10^{-1}
Ca^{2+}	8.70×10^{-2}	6.65×10^{-2}	3.79×10^{-2}
Cl^{1-}	8.59×10^{-2}	8.32×10^{-2}	7.81×10^{-2}
Br^{1-}	1.26×10^{-1}	9.75×10^{-2}	7.17×10^{-2}
CO_3^{1-}	5.98×10^{-2}	5.20×10^{-2}	5.00×10^{-2}
SO_4^{2-}	9.00×10^{-3}	3.90×10^{-2}	2.81×10^{-2}
Glucose	3.23×10^{-3}	2.22×10^{-3}	1.34×10^{-3}
Fructose	4.94×10^{-2}	4.11×10^{-2}	3.87×10^{-2}
Starch	2.90×10^{-2}	2.28×10^{-2}	1.99×10^{-2}

3.8. Ruggedness and Robustness: Using ethanol as a solvent for the making of drug solutions, the robustness of this method employing the TR-AR-NB electrodes for each kind was investigated. The reliability of such electrodes for the measurement of TR drugs can be seen in **Figure 6**. The Ruggedness of this study was conducted using another potentiometer (HANNA Instruments 211 pH Meter).



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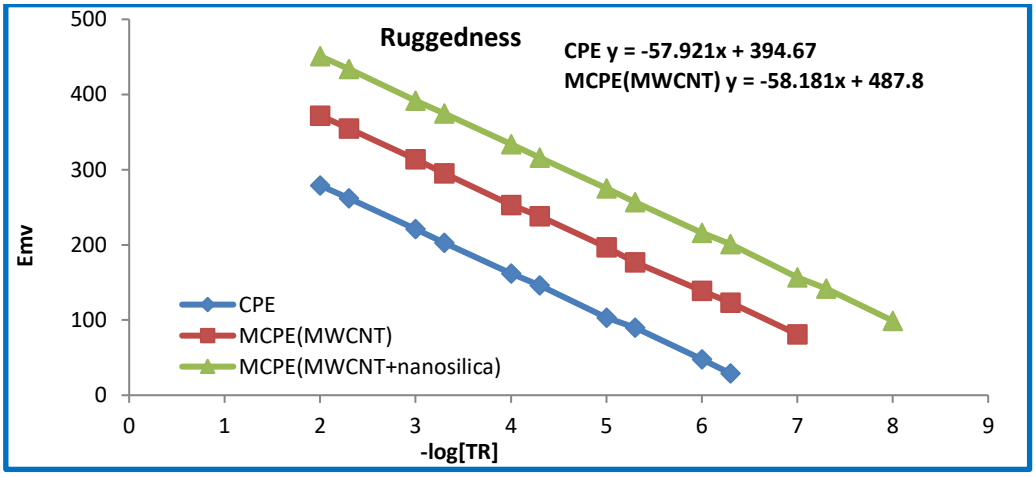


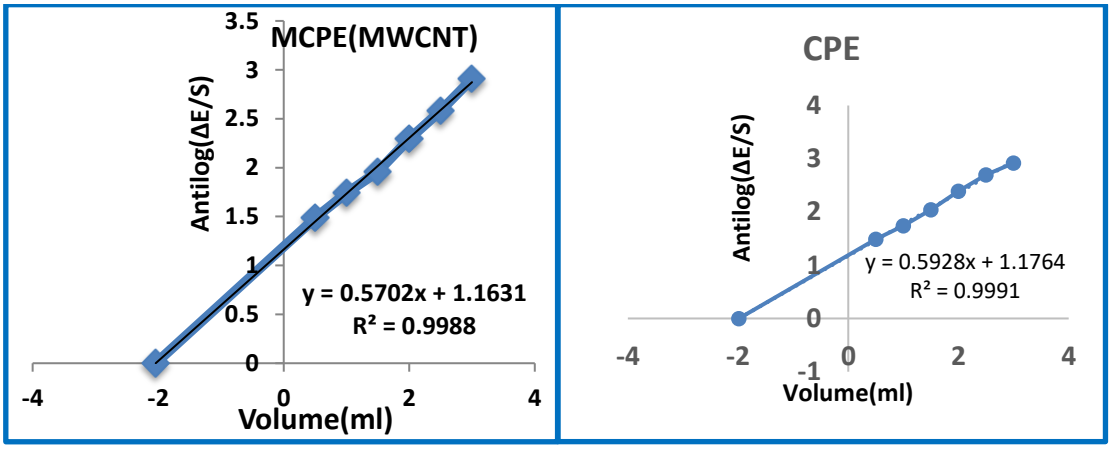
(b)

Figure 6: Robustness And Ruggedness of The TR-AR-NB Electrode-Based Analytical Technique.

3.9. Analytical Applications: Using the prepared TR-AR-NB electrodes, the TR drug was identified in the pharmaceutical composition, Tramadol tablets, and biological fluid by both direct and standard addition approaches. The results, which are displayed in **Table 6** and **Figure 7**, verify the high accuracy and precision of the assessment of AM drugs using the electrodes mentioned above.

Table 6: The direct and standard addition methods for assessing the drug.

Electrode	Taken [TR] M	Found [TR] M	%Recovery	%RSD	%RE
Direct method					
MCPE(MWCNT +nanosilica)	5×10^{-5} 5×10^{-6}	4.9715×10^{-5} 5.0095×10^{-6}	99.4314 100.1910	0.1293 0.1119	-0.56 0.19
MCPE(MWCNT)	5×10^{-5} 5×10^{-6}	5.0315×10^{-5} 5.0817×10^{-6}	100.632 101.635	0.4012 0.4979	0.63 1.63
CPE	5×10^{-5} 5×10^{-6}	5.0731×10^{-5} 4.8810×10^{-6}	101.462 97.619	0.7876 0.9933	1.462 -2.38
Standard addition method					
MCPE(MWCNT+ nanosilica)	2×10^{-4}	2.0253×10^{-4}	101.2660	1.9282	1.26
MCPE(MWCNT)	2×10^{-4}	2.0398×10^{-4}	101.9905	2.4325	1.99
CPE	2×10^{-4}	1.9843×10^{-4}	99.21558	3.8527	-0.78
Urine					
MCPE(MWCNT +nanosilica)	2×10^{-3}	1.9861×10^{-3}	99.3093	0.1371	**-0.69**
MCPE(MWCNT)	2×10^{-3}	2.0268×10^{-3}	101.3438	0.2969	1.34
CPE	2×10^{-3}	2.0322×10^{-3}	101.6125	0.3940	1.61
Plasma					
MCPE(MWCNT +nanosilica)	2×10^{-3}	2.0255×10^{-3}	101.2766	0.2094	1.27
MCPE(MWCNT)	2×10^{-3}	2.0109×10^{-3}	100.5458	0.0767	0.54
CPE	2×10^{-3}	1.9531×10^{-3}	97.6593	0.2798	-2.34



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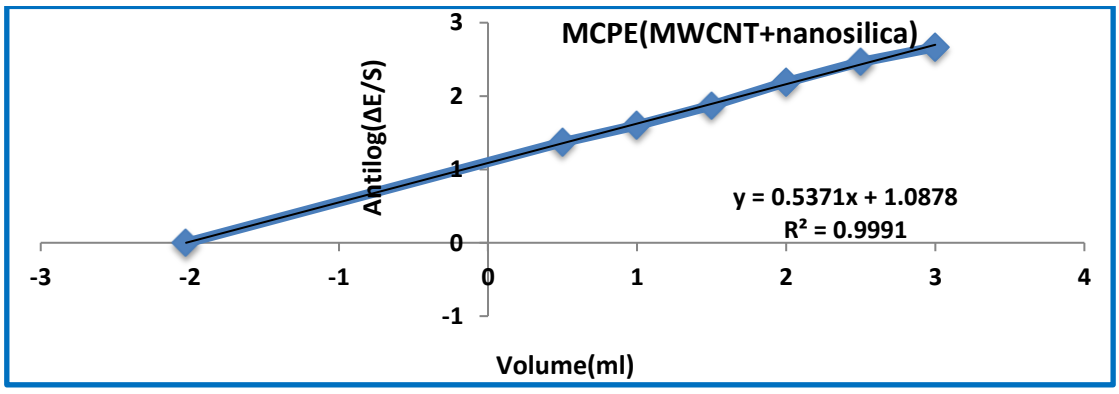

(b)

Figure 7: Standard addition curves of TR-AR-NB Electrodes.

3.10 Assessment of the net results: Using the t-test and F-test to assess the validity of employing the created electrodes to assess pharmaceutical preparations, the outcomes shown in (**Table 7** and **Table 8**) demonstrate that the developed electrodes were favorable and that there was absolutely no distinction between the recommended approach and the standard method.

Table 7: The F- And T-tests are Used to Evaluate the Results.

Electrode	%Mean ±SD	n	Calculated t-test	tabulated t-test for %95	Calculated F-test	tabulated F-test for %95
MCPE (MWCNT +nanosilica)	98.4317 ± 1.5673	7	1.7535	2.45	0.3818	4.53
MCPE(MWCNT)	100.3335 ± 1.1571	6	-1.0016	2.57	0.7005	5.19
CPE	98.5991 ±1.5994	6	1.1497	2.57	0.3666	5.19
Standard method* (HPLC)	100.85± 0.9685	5	0.00	0.00	0.00	0.00

* The HPLC technique for Wadi Al-Rafidain Pharmaceutical Industries according to the Constitution (USP41).

Table 8. Differentiation of the suggested tramadol electrodes with published electrodes.

Ref.	Slope $\frac{mV}{\text{decade}}$	Liner range M	LDL (M)
[21]	57.8	9.2×10^{-6} - 1.0×10^{-1}	6.2×10^{-6}
[22]	56.5	5.5×10^{-6} - 1.0×10^{-1}	1.8×10^{-6}
[22]	58.1	1.0×10^{-5} - 1.0×10^{-1}	1.0×10^{-5}
Present work			
CPE	58.027	5.0×10^{-7} - 1.0×10^{-2}	2.39×10^{-7}
MCPE(MWCNTs)	58.251	1.0×10^{-7} - 1.0×10^{-2}	4.98×10^{-8}
MCPE(MWCNTs+ nanosilica)	58.694	1.0×10^{-8} - 1.0×10^{-2}	4.7384×10^{-9}

4. Conclusions

When comparing the electrodes developed in this research with other previous electrodes, the modified carbon paste electrode parameters containing MWCNTs and nanosilica were superior and can be used to evaluate drugs such as tramadol in the field of forensic evidence because they have a very low detection limit of up to 4.7384×10^{-9} M. The integrated sensors have a very excellent slope, good recovery, wide pH range (2-7), very low detection limit, voltage balance, and high sensitivity. Assessing TR using the developed electrodes (CPE and MCPE) in biological fluids and pharmaceutical preparations at very low concentrations of up to 5×10^{-6} M. All the developed electrodes are suitable for application in the field of pharmacology, based on statistical analysis.

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