Voltammetric Determination of Rebamipide in the Presence of its Degradant, in Bulk, Pharmaceutical Preparation, and Biological Fluids

2. MATERIALS & METHODS

2.1. Materials and Reagents

The grade of chemicals used in our work were analytical grade, we implemented a further purification on them. The Egyptian Drug Authority (EDA) supported us with Rebamipide (REBA) standard of purity (99.9%) on an anhydrous basis. Mucosta tablets (REBA tablets) was purchased from local market. We purchased from Sigma-Aldrich the nano-zinc oxide (crystal diameter around 5 nm), the graphite-powder (of 20 µm particle dimension), and multi-walled carbon nanotubes (of purity >95%). Also, we used the same source for Zinc sulphate ZnSO4, ethanol and methanol. Deionized and bi-distilled water utilized was provided by EDA and referred to as "water". We prepared the 0.04 M Britton-Robinson (B-R) buffer by adding 0.04 M boric acid, 0.04 M orthophosphoric acid, 0.04 M glacial acetic acid; then the pH was modified by using 0.2 M NaOH. The holding company of biological blank serum was obtained from VACSERA, Egypt.

2.2. Instrumentation

2.2.1. Apparatus

The Metroham 797 VA Computrace analyzer was used to perform voltametric measurements. The apparatus was equipped with a cell composed of three electrodes: working electrode, Reference electrode: Ag/AgCl (3M KCl), and counter electrode: a platinum wire. We implemented electrical contact with the working electrodes by soldering a copper wire to the contact metallic part of the apparatus. All PH measurements were conducted by a Jenway 3330 Research pH meter. Deionized water and Bi-distilled water were supplied by a Hamilton Aqua-Metric deionized water system. A temperature of 25°C was maintained during the performance of all experiments.

2.2.2. Working Electrodes

Graphite pencil electrode (GPE): It is a lead Rotring HB pencil with 0.5 mm diameter, in addition to a 60 mm length, working as an electrode. To conduct the pencil to the apparatus a copper wire was soldered to the contact part achieving electrical contact and the lead to be fixed inside the pencil. A cloth felt pad (0.05 mm alumina slurry) was used to polish the electrode, 8mm of the electrode was immersed in the solution.

Carbon paste electrode (CPE): CPE is made by blending the pasting liquid with the graphite powder.[32] 250 mg of graphite powder was mixed with 125mg paraffin oil to prepare CPE. The formed paste was loaded into the end of a syringe with internal diameter of 3.0 mm. External electrical contact is set by pushing a copper wire down the syringe.

Multi-wall carbon nano-tubes modified nano-zinc oxide electrode (ZnONP/CNT/ME): the carbon paste was prepared by mixing 425 mg of graphite powder, 25 mg of Zinc oxide nano-particle powder, and 50 mg MWCNT powder several times to get a homogenous mixture in a mortar then a 0.3 mL paraffin oil was added to make homogenous paste. We packed a portion of the resulted paste to the hole of the 3.0 mm syringe that contact the apparatus by a wire of copper.

2.2.3. Stock solution

An appropriate weight of REBA standard was dissolved in 25.0ml methanol and sonicated for 5.0 mins, then moved to a 50.0ml volumetric-flask and completed by water to reach 0.001M. Our stock solution was prepared the same day we conducted the analysis.

Accelerated degradation was implemented through dissolving about 25 mg of pure REBA powder in 5 mL of methanol; then, 25 mL of 0.2M NaOH was provided, and the solution was refluxed for three hours. Then, alkaline degradant was neutralized with 0.1 M HCl. The prepared degradant was evaporated in a 60^0C thermostatic water bath till reaching dryness. The collected degradant was subsequently utilized to elucidate its structure and degradation pathways using IR spectroscopy. (Illustrated below in supplementary data Fig. S1, S2). The same procedure applied to prepare stock standard solutions was used and the residue of degradant was dissolved in water till reaching the final concentration of 100.0 µg/ml. Water was then used to dilute the solution to reach a working degradant solution concentration of 10.0 µg/ml.s.

2.3. Recommended General Procedures

2.3.1. Determination of the pure form of the used Rebamipide

An appropriate volume of stock solution was added to a 10 ml volumetric-flask and completed with suitable electrolyte buffer (B-R) of pH 11.0 then transferred into the voltametric vessel to undergo measurements. Test solutions were purged with nitrogen of five seconds. Working electrode was retained at the favorable accumulation potential for a certain period of time, whereas the solution was stirred at around 2000 rpm for the accumulation period. The stirring was subsequently stopped, and the solution was allowed to rest for 5s, a scan was carried out afterward towards positive potentials over the range 0.5 to +1.2V, and the voltammograms were recorded. Experimental conditions of anodic DPV (A-DPV) were sweep rate, 0.050 V. s-1; voltage step, 0.005 V; pulse amplitude, 0.050 V; voltage step time, 0.1 s; and pulse time, 0.04 s.

2.3.3. Analysis of Pharmaceutical tablets

Ten **Mucosta®** tablets, each labelled to contain 100 mg REBA, were grinded carefully, mixed and weighed. An adequate portion of this powder, equivalent to 10.0 mg of REBA, was transported into a 50 mL calibrated flask and completed the volume with methanol to reach the obtained final concentration. The flask's content was sonicated for 10 min to get a comprehensive dissolution. Centrifugation was implemented afterwards for the solution. Various concentrations for the solution was prepared through moving aliquots of the supernatant to the voltametric 10-ml flask, then completed with the supporting electrolyte. Through standard addition method, the pharmaceutical preparation was spiked with different quantities of the standard drug. The DPVs were recorded according to the mentioned procedure. The %RSD and recoveries were calculated afterwards.

2.3.3. Spiked serum analysis

To a volume of 0.3 mL serum, 0.3 mL 5% ZnSO4 and 5 mL ethanol were added, the mixture was then centrifuged for 15 min at 13,000 rpm, and 1 ml of the clear solution was separated and added to 9 mL B-R buffer solution. The mixture was de-aerated for 5 min and spiked with known amount of REBA. The procedures then follow as mentioned previously[14].

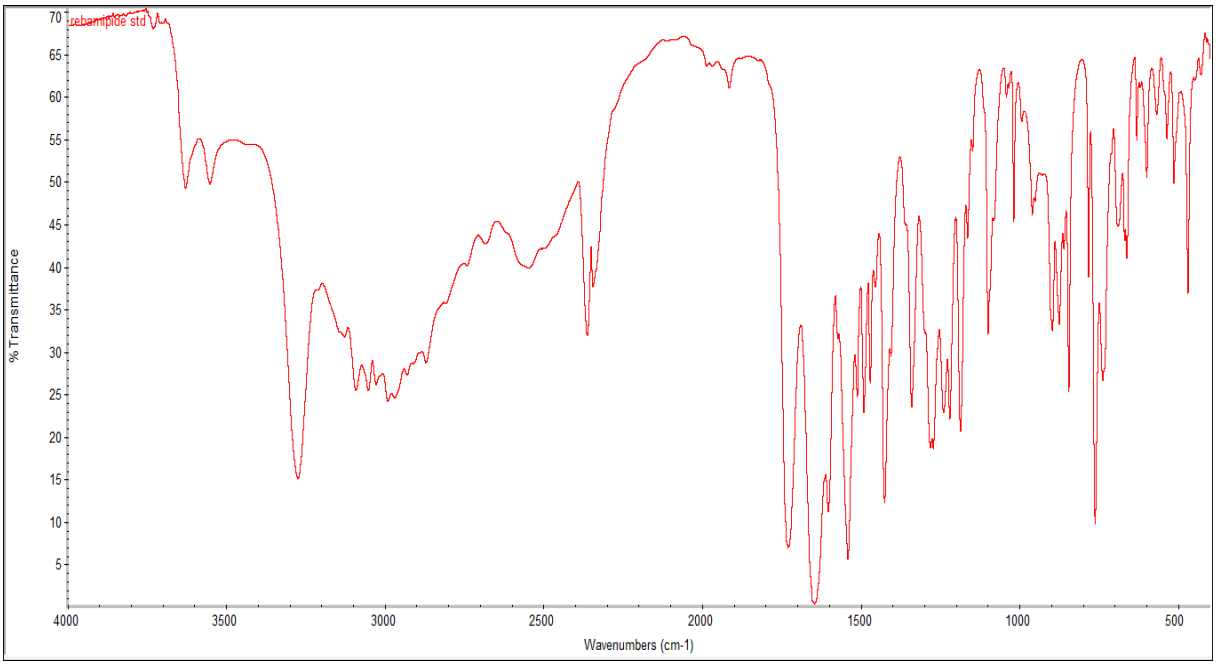

Figure S1(a). IR spectra of intact REBA.

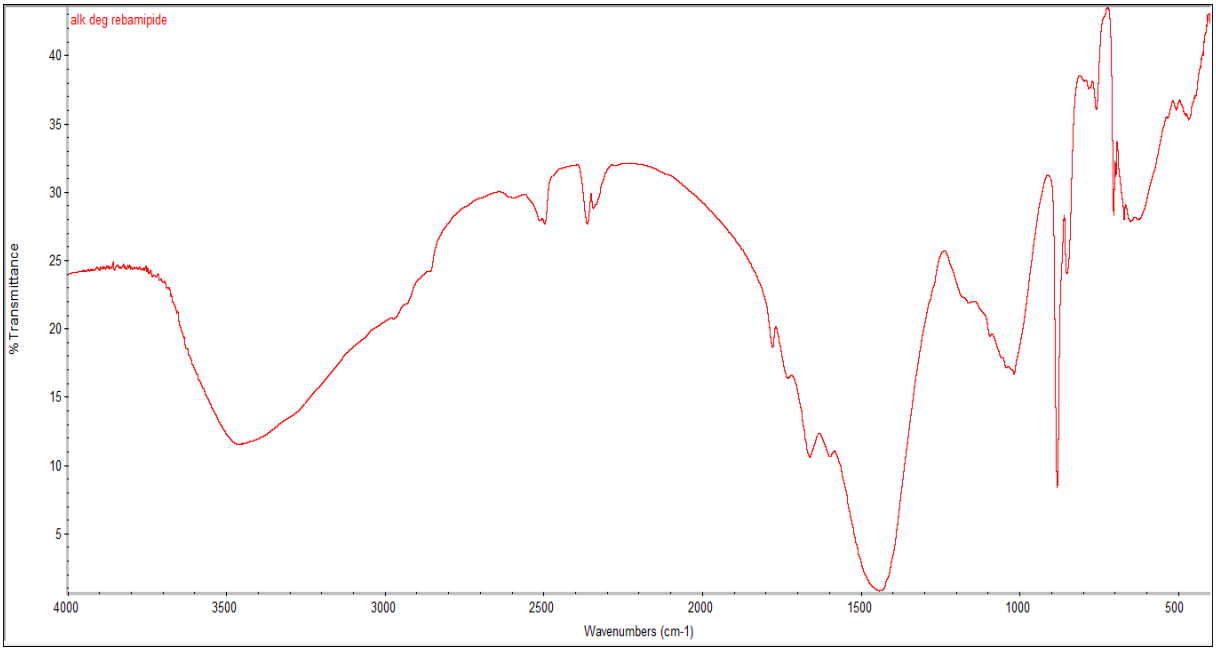

Figure S1(b). IR spectra of Alkaline degradant of REBA.

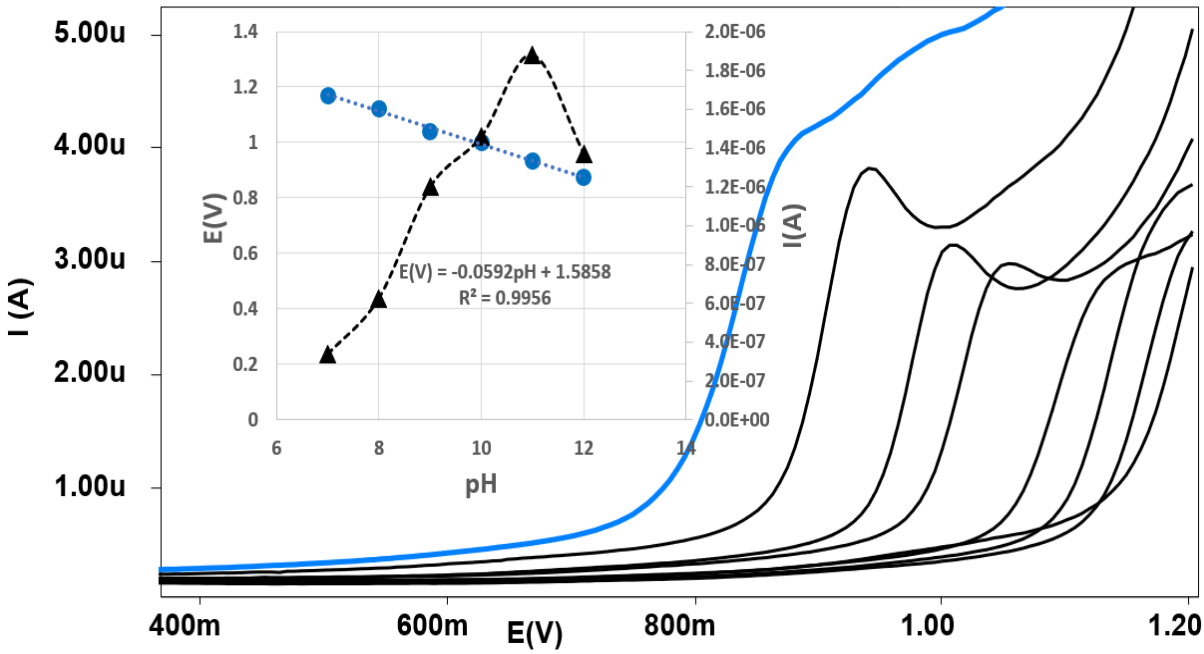

Figure S2. Effect of pH on the peak current (I) and potential (E) of 20µM of REBA at ZnONP/CNT/ME.

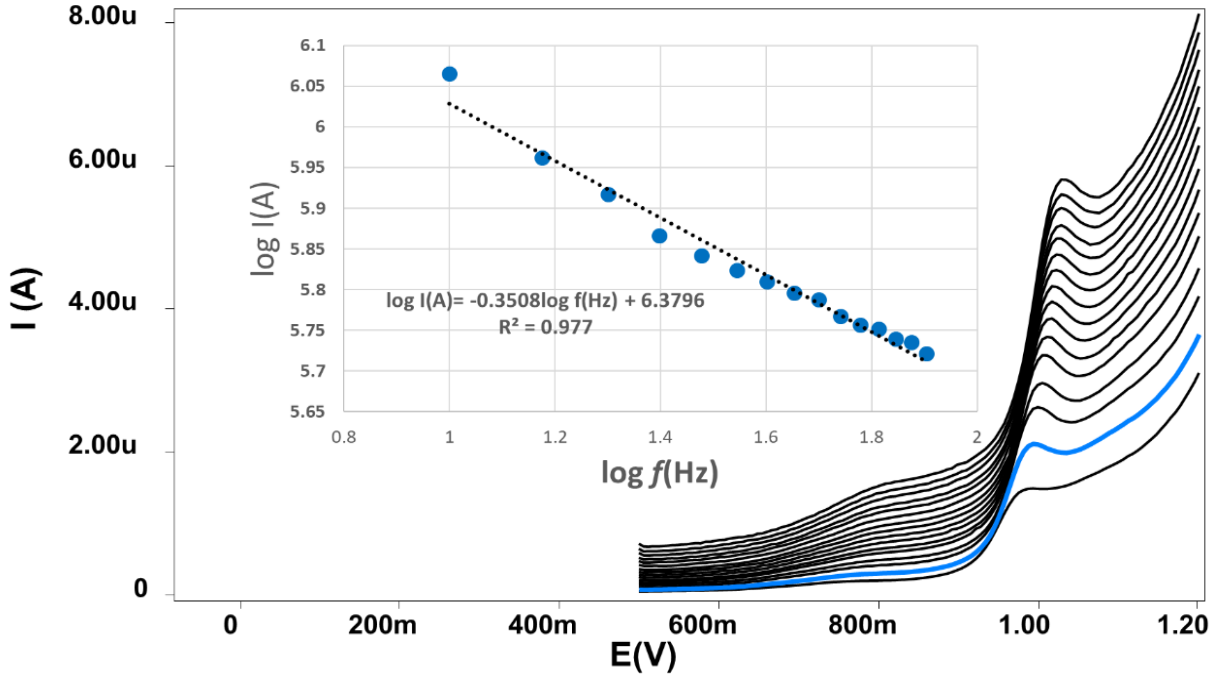

Figure S3. Plot of log Frequency (Hz) vs peak current (I_P) of 20µM of REBA at ZnONP/CNT/ME.

Table 1S. Molecular orbital calculation of REBA with different theories.

Atom	Atom Type (MM2)	Charge (MM2)	Charge (MMFF94)	Charge (Huckel)	Mulliken Charges (Mopac Interface)
N (1)	N Amide	0	-0.547	0.408103	-0.72339
C (2)	C Carbonyl	0	0.6156	0.355757	0.649614
C (3)	C Alkene	0	-0.1356	-0.15208	-0.40649
C (4)	C Alkene	0	-0.1666	0.101421	0.127648
C (5)	C Alkene	0	0.0284	-0.01497	-0.18517
C (6)	C Alkene	0	-0.15	-0.01988	-0.10544
C (7)	C Alkene	0	-0.15	-0.0816	-0.28432
C (8)	C Alkene	0	-0.15	-0.02087	-0.09681
C (9)	C Alkene	0	-0.15	-0.08432	-0.3287
C (10)	C Alkene	0	0.117	0.165278	0.292234
C (11)	C Carbonyl	0	0.659	0.599041	0.621175
C (12)	C Alkane	0	0.3611	0.048199	0.047219
C (13)	C Alkane	0	0.1382	-0.05116	-0.42758
O (14)	O Carbonyl	0	-0.57	-0.64326	-0.48932
O (15)	O Carboxyl	0	-0.65	-0.16995	-0.61057
O (16)	O Carbonyl	0	-0.57	-0.86031	-0.50447
C (17)	C Carbonyl	0	0.5438	0.388182	0.661165
C (18)	C Alkene	0	0.0862	-0.03633	-0.15154
C (19)	C Alkene	0	-0.15	-0.01601	-0.1247
C 20)	C Alkene	0	-0.15	-0.10189	-0.24344
C (21)	C Alkene	0	0.177	0.179325	0.036543
C (22)	C Alkene	0	-0.15	-0.10159	-0.22607
C (23)	C Alkene	0	-0.15	-0.01181	-0.14926
O (24)	O Carbonyl	0	-0.57	-0.85785	-0.50573
N (25)	N Amide	0	-0.7301	0.229736	-0.79902
Cl (26)	Cl	0	-0.177	0.050646	-0.07215