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Electrochemical Reduction Behavior Of Mephedrone Drug At A Dropping Mercury Electrode And Its Pharmaceutical Determination In Spiked Human Urine Samples

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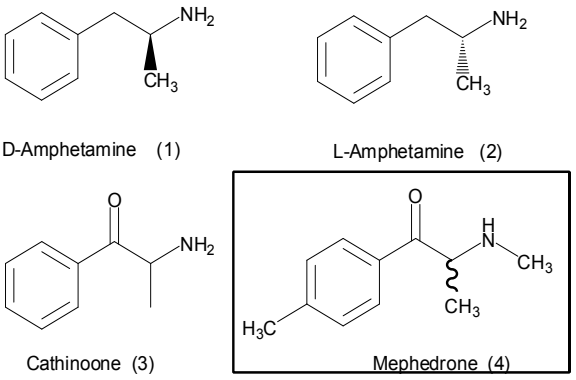
ABSTRACT

The designer drug 1-(4-methylphenyl)-2-methylaminopropan-1-one (4-methylmethcathinone or mephedrone) is reported to possess psychostimulant, entactogenic and hallucinogenic effects. From the structural point of view mephedrone drug has keto (-C=O) functional group. In the present paper, we have reported the reduction behavior of mephedrone was studied on the surface of the dropping mercury electrode (DME) as working electrode in universal buffer of pH range from 2.0 to 12.0 by direct current polarography, differential pulse polarography, cyclic voltammetry and controlled potential electrolysis. Mephedrone showed one reduction peak and the results indicated that the reduction processes of mephedrone is irreversible and was diffusion controlled as evidence from linear plots of id against h1/2. Millicoulometric experiment was performed successfully in estimating the number of electrons and protons involved in the reduction processes and to understand reduction mechanism. Kinetic parameters such as diffusion co-efficient (D) and heterogeneous forward rate constant (Kof, h) are evaluated and reported. Quantitative measurements were successful in the concentration range 1.0 × 10-5 to 1.5 × 10-9 M with lower detection limit 1.25 × 10-8 M.

INTRODUCTION:

The designer stimulant 4-methylmethcathinone (4-MMC) (Mephedrone) is a β-ketoamphetamine (1-(4-methylphenyl)-2-methylaminopropan-1-one) and is among the most popular of the derivatives of naturally occurring psychostimulant cathinone and substituted amphetamines. Mephedrone is one of hundreds of designer drugs that have been reported in recent years, including artificial chemicals such as synthetic cannabis and semi synthetic substances such as methylhexaneamine. These drugs are primarily developed to avoid being controlled by laws against illegal drugs, thus giving them the label of designer drugs [1]. According to the European Monitoring Centre for Drugs and Drugs Addiction, the synthesis of mephedrone was first reported in 1922 by Saem de Bumaga Sanchez in the Bulletin de la Societe Chemique de France, under the name toluyl-alpha-monomethylaminoethylcetone. Abuse of mephedrone has increased dramatically as well as producing the intended stimulant effect, negative side effects occurs when mephedrone used and has become a significant public health problem in the United States and European countries. A drug similar to mephedrone, containing cathinone, was sold legally in Israel from around 2004, under the name hagigat. When this was made illegal, the cathinone was modified and the new products were sold by the Israeli company, Neorganics [2-4]. The products had names such as Neodoves pills, but the range was discontinued in January 2008 after the Israeli government made mephedrone illegal [5-7]. The metabolism of mephedrone has been studied in rats and humans and the metabolites can be detected in urine after usage. Nothing is known about its potential neurotoxicity but scientist have studied and suggested possible dangers associated with its use based on its similarity to other drugs. Several deaths the media attributed to the drug were later determined to have been caused by other factors. Owing largely to its recent emergence, there is no formal pharmacodynamic or pharmacokinetic studies of mephedrone but very little information is available on neurochemical action of mephedrone.

Figure 1. Some biologically active mephedrone like molecules



In recent years the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time when compared to other techniques. Additional application of electroanalytical techniques includes the determination of reaction mechanisms. Redox properties of a drug can give insight into its metabolic fate or its in vivo redox processes or pharmaceutical activity [8-10]. Critical literature survey revealed that no attempt has been made to investigate the electrochemical behavior of mephedrone hydrogen chloride and its pharmaceutical determination in spiked urine samples.

Based on over view, we have developed a simple and prompted electrochemical method for assay of mephedrone hydrogen chloride. The present work includes the reduction behavior of mephedrone hydrogen chloride at dropping mercury electrode and development of a DPP method for its determination in pharmaceutical formulations as well as suggested the mechanism of reduction processes.

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EXPERIMENTAL SECTION

MATERIALS AND REAGENTS:

All chemicals and reagents were procured from Sigma-Aldrich, Merck, S. D. Fine. Chem (India, Mumbai) and used without further purification. The stock solutions (1 mM) of the samples were prepared in double distilled ethanol. The supporting electrolyte ranging from pH 2.0-12.0 were prepared using 0.2 M boric acid, 0.05 M citric acid and 0.1 M trisodium orthophosphate [11]. Double distilled ethanol and triple distilled water were used throughout experiment. The desired test solutions were prepared by dissolving required quantity of stock solution with supporting electrolyte to get 10 mL. The solutions were de-aerated by purging with oxygen free nitrogen gas for 20 minutes and then polarogram were recorded. All the experiments were carried out at 298±1 K temperature.

MEPHEDRONE ASSAY:

Mephedrone hydrochloride was obtained from sigma-aldrich and weighted compound in a mortar then crushed into a fine powder. A portion of finely powdered compound equivalent to 1 mM was dissolved in ethanol in 100 mL volumetric flask and sonicated for 20 minutes to effect complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with universal buffer solution in order to obtain a final solution of 1.0 × 10-5 M of mephedrone hydrogen chloride and the voltammograms were subsequently recorded in the optimized conditions. The content of the drug was achieved by the standard addition method.

PREPARATION OF URINE ASSAY:

Aliquot quantity of urine was collected from a health volunteer and added into volumetric cell containing universal buffer of pH – 5.0. The voltammograms are recorded for the blank urine sample. A quantity of mephedrone hydrogen chloride was added to a spiked urine samples of 1 mL was dissolved in ethanol to achieve final stock solution concentration of 1.0 × 10-5 M. It was stirred magnetically for 10 minutes and added 9 mL of universal buffer solution.

INSTRUMENTATION:

D. C. polarography and differential pulse polarography were performed with model 757 VA supplied by Metrohm India Limited, coupled with a three electrode system consisting of a dropping mercury electrode (DME) (Surface area = 0.0026 cm2) the polarographic bottom was fitted with a saturated calomel electrode (SCE) worked as reference electrode and platinum wire worked as a auxiliary electrode. Metrohm unit E 506 polar record coupled with E 612 VA-scanner, E 648 VA-controller and digital electronics x-y/t recorder are used for cyclic voltammetry. pH values were measured with pH meter. A magnetic stirrer (REMI) and stirring bar provided the convective transport during pre-concentration.

RESULTS AND DISCUSSIONS

Figure. 1 shows that different biologically active mephedrone like molecules, in which compound (4) indicates the structure of the mephedrone. The polarographic and voltammetric techniques were employed to study the electrochemical behavior of mephedrone. Electrochemical reduction behavior of mephedrone hydrogen chloride has been examined over the pH range of 2.0 to 12.0. As buffer medium is essential for reduction process of organic compound because most of the reductions of organic compounds are dependent on the pH of the solutions [12], Therefore in present study supporting electrolytes used as buffers. The electrochemical reduction of mephedrone hydrogen chloride was giving a single well defined cathodic peak at a dropping mercury electrode in acidic and neutral solutions (pH 2.0-6.0). There was no peak obtained in the basic medium (pH 8.0 to 12.0) due to the precipitation of the electro active species (absence of protons). The peak was developed in acidic medium. The concentration 2.0 × 10-5 M solution of mephedrone hydrogen chloride at pH 4.0 and drop time 2 second was showed D.C. polarogram (Fig.2). When the concentration of mephedrone hydrogen chloride increases the half wave potential values are found to change to more negative values. Figure. 3 show the cyclic voltammogram of mephedrone hydrogen chloride at pH 2.0 with a scan rate of 40 mVs-1 and concentration 2.0 × 10-5 M. A peculiar behavior was observed that absence of anodic peak in reverse scan and cathodic peak was obtained which may be due to the reduction of carbonyl group to saturated hydroxyl derivative which is reversible in nature. Reduction process of most carbonyl groups is irreversible [13]. The peak height decreases with increases in pH and gave a characteristic Ep in all the buffer systems because of the decreased available of protons. As result, the Ep shifts to more positive potential values with increasing pH (thus, decreasing the concentration of protons in the electrolyte solution). The electrode process was found to be diffusion controlled in all the buffer systems studied, as shown by the linear plots of id vs h1/2 and im vs t2/3 that pass through the origin indicating the absence of adsorption complications. When the concentration of the mephedrone hydrogen chloride increases resulted the slight variation of E1/2, Ep and Em shifted towards more negative values indicating the electrode process to be irreversible. The marginal variation of peak potential (Em) with concentration, nonlinearity in the plot of im vs (1-σ/1+σ) in differential pulse polarography and disobedience of Tomes" criterion also confirm the irreversible nature of the electrode process. For the irreversible, the value of αna was calculated from the equation below and the values are illustrated in Table 2.

Where i was the cathodic current in μA, id was the cathodic diffusion current in μA.

In polarography, the theoretical equation for the maximum diffusion current obtained with a dropping mercury electrode, which was first derived by Ilkovic [14-17] was given by

id = 708 nCD1/2m2/3t1/6

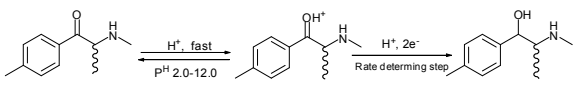
The diffusion coefficients values were obtained in a good agreement indicating the diffusion controlled and adsorption free nature of the electrode process. The variation of diffusion current with the pH of the supporting electrolyte influences the diffusion coefficient values also to vary in the same manner. The reason for slight variation in diffusion coefficient values with increases in pH may be attributed to the decrease in the availability of protons with increases in pH of the supporting electrolyte.

Millicoulometry employed at pH 2.0 to find out the number of electrons involved in the electrode process. The results showed that the number of Reduced electrons to be two for mephedrone hydrogen chloride. From the slope of E1/2 vs pH plot, the number of protons involved in the rate determining step of the electrode process are found to be two. Controlled potential electrolysis experiments are carried out at -0.27V vs saturated calomel electrode at pH 4.0. Ether was added to the content and extracted the product into the ether layer. The ether was evaporated on water both and the resulted product was employed to IR spectrometer. The isolated product was identified as hydroxyl product and was confirmed by IR spectral data, in which the absorption band at 1678 cm-1 assigned for –C=O functionality was disappeared and the absorption band was appeared at 3357 cm-1 assigned for presence of the –OH group.

The number of protons (Z) involved in the rate determining step of the electrode reaction is given by

Δ E1/2 / Δ pH = -0.059P/αna

The number of protons was determined to be 1.54 i. e. two protons were probably consumed in the rate determining step of the reaction. The heterogeneous forward rate constant values (K0fh) are found to decreases with increasing pH indicating that the electrode process reaction is more and more irreversible with increasing pH of the solution. Based on the results obtained, the proposed electrochemical reduction mechanism of mephedrone hydrogen chloride can be represented in scheme. 1.



Scheme 1 the reduction electrode mechanism of carbonyl functional group in mephedrone

The differential pulse polarography was used for the determination of mephedrone hydrogen chloride. The polarographic

peaks obtained in the pH range 2.0 to 6.0 are well resolved and reproducible. Calibration plots are linear for mephedrone in the concentration over the range from 5.0 × 10-8 M to 1.0 × 10-5 M, the height of the peak was a linear function of the concentration at any pH value. The lower detection limit was calculated as 4.2 × 10-8 M using the expression dl = 3 × Sd/m, where Sd was the standard deviation and m was the slope of the calibration plot.

Recommended analytical procedure

Mephedrone hydrogen chloride was analyzed by the standard addition method. The standard solution (1.0 × 10-5 M) was prepared by dissolving the appropriate amount of the electroactive species in ethanol. One mL of the standard solution is transferred to the polarographic cell and made up to 10 ml with the supporting electrolyte to get the required concentration and then deoxygenated by bubbling nitrogen gas for 10 min. After recording the polarogram, small increments of the standard solution (0.5 mL) were added and then polarogram were recorded for each addition under similar conditions. The optimum conditions for determination of mephedrone hydrogen chloride at pH 4.o were found to be a drop time of 2 sec, a pulse amplitude of 50 mV and an applied potential of -0.27 V vs saturated calomel electrode. The relative standard deviation and correlation coefficient values were found to be 1.64% and 0.94423 respectively for 10 replicates.

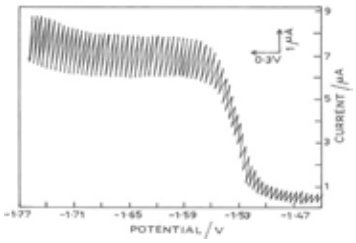


Figure 2 Typical D. C. Polarograme of mephedrone hydrogen chloride at pH 4.0; concentration: 2.0 × 10-5M; Drop time: 2 sec

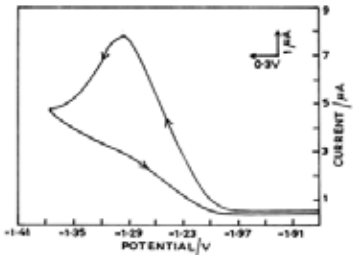


Figure 3 Typical cyclic voltammogram of mephedrone HCl at pH 2.0; concentration: 2.0 × 10-5M; scan rate: 40 mVs-1.

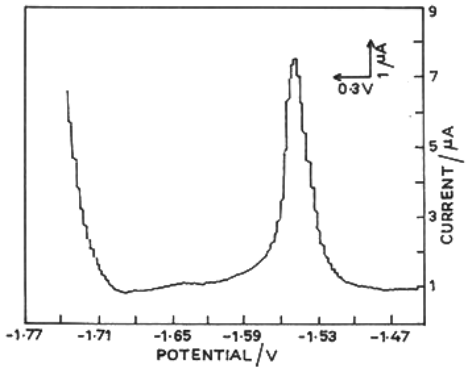


Figure 4 Typical differential pulse polarogram of mephedrone hydrogen chloride at pH 4.0; concentration: 2.0 × 10-5M; drop time: 2 sec; pulse amplitude: 50 mV.

For analysis of mephedrone hydrogen chloride in urine samples, different amounts of mephedrone hydrogen chloride were added to a fixed volume of urine.

Transfer 1.0 mL of human urine samples into a centrifugation tube adds aliquots of mephedrone stock solution and mixed well. Transfer the content of the centrifugation tube quantitatively into a 25 mL beaker and added 9.0 mL of universal buffer solution then transfer the whole content into a polarographic cell. Then, pass the nitrogen gas for 15 minutes for inert environment.

The content of drug in urine samples was subjected to polarography and estimated the drug with the help of the calibration graph. The intension of the experiment with urine samples was to study the various possibilities to overcome a bio-matrix effect and to find the best solution but to explore the way for direct, simple, reliable and fast determination mephedrone hydrogen chloride in urine by its dilution by employing differential pulse polarography.

The recovery was found to be 99.6% with relative standard deviation of 0.2% at the lowest concentration level and 99.36% with relative standard deviation of 0.33% at the concentration of μg/mL and higher. The determination of mephedrone hydrogen chloride in pharmaceutical formulations and urine samples are presented in Table 3.

Table 1 Typical kinetic data of mephedrone hydrogen chloride, concentration 0.5 mM, drop time: 2 sec

pH of the supporting electrolyte	D. C. Polarography Drop time: 3 sec			Cyclic Voltammetry Scan rate: 40 mv/s			D. P. Polarography Drop time: 2 sec		
	-E1/2 volts	D × 105 cm2/s	K0fh cm/s	-E1/2 volts	D × 105 cm2/s	K0fh cm/s	-E1/2 volts	D × 105 cm2/s	K0fh cm/s
2.0	1.30	2.84	8.56 x 10-4	1.32	2.79	7.27 × 10-4	1.34	2.89	7.87 × 10-4
3.0	1.43	2.63	6.28 x 10-4	1.47	2.67	4.84 × 10-4	1.47	2.64	2.49 × 10-4
4.0	1.56	2.49	4.27 x 10-5	1.58	2.53	5.53× 10--5	1.56	2.39	6.86 ×10-5
5.0	1.64	2.26	1.64 x 10-5	1.67	2.12	3.12 × 10-5	1.67	2.18	2.28 × 10-5
6.0	1.69	1.98	5.48 x 10-6	1.66	2.07	6.27 × 10-6	1.69	2.05	4.15 × 10-6

Table 2 Effect of pH on the polarographic behavior of mephedrone hydrogen chloride

pH of the supporting electrolyte	-E1/2 volts	id (μA)				αna
2.0	1.30	8.40	1	0.15	0.15	0.82
3.0	1.43	7.95	1	0.08	0.08	0.77
4.0	1.56	7.30	1	0.07	0.07	0.72
5.0	1.64	6.70	1	0.05	0.05	0.67
6.0	1.69	6.10				0.58

Table 3 Polarographic assay of mephedrone hydrogen chloride by DPP in pharmaceutical formulations and in spiked human urine samples.

S. No	Sample	Type	Labeled amount mg/L	Average amount found mg/L	Recovery %	Standard deviation	% Relative standard deviation
1	Mephedrone	Pharmaceutical formulation	200	199.10	99.50	0.50	0.200
2	Mephedrone	Urine	200	198.50	99.20	0.80	0.326

Conclusion:

The electrochemical behavior of mephedrone hydrogen chloride at dropping mercury electrode was examined in universal buffer over a pH range of 2.0 to 12.0. The DPP procedure provides a convenient and efficient method for assay of mephedrone hydrogen chloride. The diffusion coefficient, transfer coefficient and heterogeneous forward rate constant values were noticed to be in good agreement in all the techniques. Proton involvement seems to make the reduction easier. But in basic media, the reduction process is not easily facilitated owing to the non-availability of protons. DPP techniques have been applied for the quantitative estimation of the drug in pharmaceutical formulations and biological media. The proposed method is one of the best analytical tools for their estimation, simple, accurate, sensitive, selective, reliable and low cost of analysis in less time consuming. Further, it was used for color and excipient solutions without prior separation before the analysis.

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