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Research Article

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Spectral, electrochemical behaviour of Alprazolam and Voltammetric assaying of pharmaceutical formulation

V. Krishna*[a], B. Umamaheswara Rao[b] and N. Raju[c]

[a]*Department of Chemistry, Malla Reddy Engineering College (Autonomous), Hyderabad, Telangana*
[b]*Department of Chemistry, Gokaraju Rangaraju Institute of Engineering and Technology(Autonomous), Bachupally, Hyderabad, Telangana*
[c]*Department of Chemistry, National Institute of Technology Warangal, Telangana*

ABSTRACT

*The spectral and voltammetirc behavior of Alprazolam(ALP) in aqueous buffers of varied pH is presented. Spectrophotometry and cyclic voltammetry were utilized to study its proton and electron transfer characteristics respectively. Relevant thermodynamic and electrochemical data such as deprotonation constant (pK_a), charge transfer co-efficient (α_{na}), forward rate constant ($k^0_{f,h}$), etc, have been evaluated. An excellent electroanalytical assaying of **ALP** has been developed in differential pulse polarography at pH = 8. Molecular modeling on various acid-base conjugates of **$1H_2$** and their several conformers has been carried out to arrive at the thermodynamic and conformational issues to correlate to the spectral and electrochemical observations.*

Key words: Alprazolam, cyclic voltammetry, isosbestic points

INTRODUCTION

Alprazolam is widely prescribed sedative and antidepressant benzodiazepine (BDZ) drug. Chemically known as {8-chloro-1-methyl-6-phenyl-4H-s-triazolo(4,3-a) (1,4)benzodiazepine} and it belongs to the class of anxiolytic, sedative and hypnotic anticonvulsant [1-3]. It is believed that alprazolam (ALP) is more fairly safe and it rapidly reduces the symptoms of anxiety through control of the central nervous system (CNS) excitability by a selective and potent enhancement of inhibitory gammaamino butyric acid (GABA) mediated neurotransmission [4].

The photostability of alprazolam was evaluated at pH 2.0, 3.6 and 5.0. The drug was exposed to UVA–UVB radiations, the photodegradation of alprazolam was followed by high-performance liquid chromatographic (HPLC) and the developed spectrofluorimetric assay allowed determination of the photodegradation products at very low concentrations ($>10^{-5}$ M). The photoinstability was found to increase with the pH value decreasing; consequently acidic media should be avoided during the drug-development process [5].

Hydrolysis of the diazepinone ring is one the most frequently observed degradation routes for benzodiazepinones [6-8]; nevertheless, in the case of alprazolam, we have determined that hydrolysis under several different conditions, is not a major degradation source [9-10].

On the other hand, to the best of our knowledge, there are no reports on electrochemical studies carried out with alprazolam, previous to our work. We have observed that the UV-visible spectrophotometric method [11], and the

high-performance liquid chromatographic (HPLC) method [12], usually used for the alprazolam assays. More sophisticated methods such as HPLC–tandem mass spectrometry (electrospray ionization MS–MS), recently developed for the quantitation of alprazolam in human fluids [13-15] and hair [16].

EXPERIMENTAL SECTION

2.1 Materials

Ten tablets of the alprazolam labeled as contain 25-mg per tablet were crushed dissolved in methanol by mechanical shaking for 10 min and lastly centrifuged for 5min filtered the solution and stored at 5^oC. A portion of stock is diluted with the supporting electrolyte to achieve desired concentration. Then, electronic spectral and voltammetric studies were carried at different pHs. All of the reagents employed were of analytical grade. Solutions for pH measurements were buffered using Britton–Robinson buffer adjusted with NaOH to a desired pH.

2.2 Instruments

The voltammetric measurements were performed with metrohm 663 VA electrochemical instrument. Three-electrode system was employed: Static Mercury Drop Electrode (SMDE), Ag/AgCl reference electrode and glassy carbon counter electrode. The UV spectrum was recorded using Analytik jena specord-205 spectrophotometer.

2.3 Procedure for CV-pH investigations

In electrochemical cell 14 ml of BR buffer of different pHs was transferred, degassed for 10 min with high purity nitrogen and 1 ml of ALP stock solution was added to make its final concentration of 6 x 10^{-5} M. The solution was purged for another 3 min and cycle voltammograms were recorded.

RESULTS AND DISCUSSION

3.1 Electronic Spectra and Kinetic Stability of Alprazolam:

The electronic spectral profile of Alprazolam in methanol shows two characteristic peaks at ~ 290 and ~ 480 nm. Azomthine group is vulnerable for hydrolysis [17]. To know the stability we recorded electronic of ALP in high acidic and basic pHs for every 5min over the period of 1 hr. The unchanged absorbance and spectral profile indicating that ALP stable in the pH range 2-12.

The effect of pH on the electronic spectra of ALP were recorded in the Britton-Robinson buffers of pH range, 2-11 and shown Fig. 1.

The poor spectral variation with pH indicates that the protonation-deprotonation of the heterocyclic nitrogen hardly affect the molecular orbital energies. The slight absorbance gain at low pH range at ~500 nm might be due to an auxochromic phenomenon rather than a chromophoric effect.

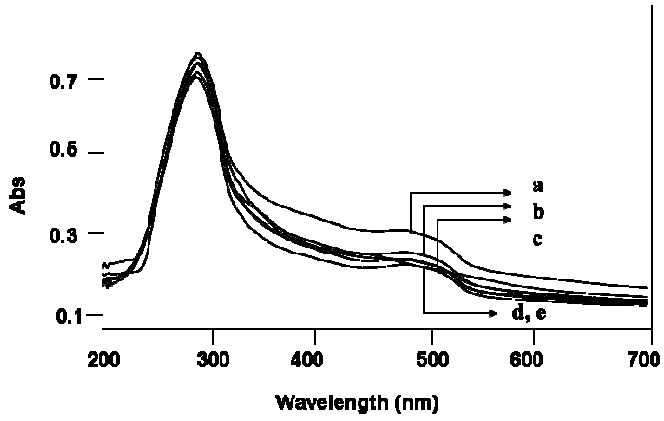


Fig 1: Electronic spectra of ALP (8 x 10^{-5} M) at pH a) 1.5, b) 2.5, c) 3.5 d) 6.00 and e) 11.00

3.2 Voltammeter Studies:

The cyclic voltammograms of ALP at a few selected pH values are shown in Fig. 2. Alprazolam exhibits well-defined cathodic response in the entire pH range. The peak potentials, E_p, shift cathodically with pH. The cyclic voltammetric response is completely irreversible. A displacement of peak potential to more negative values with an increase in pH indicates the involvement of protons in the electrochemical process.

Further, absence of anodic peak means that the electron transfer and electrochemical reduction is irreversible. The E_p value shifts cathodically, by ~30 mV for a 10-fold increase in scan rate. This also supports the irreversible nature of the electrochemical reaction [18].

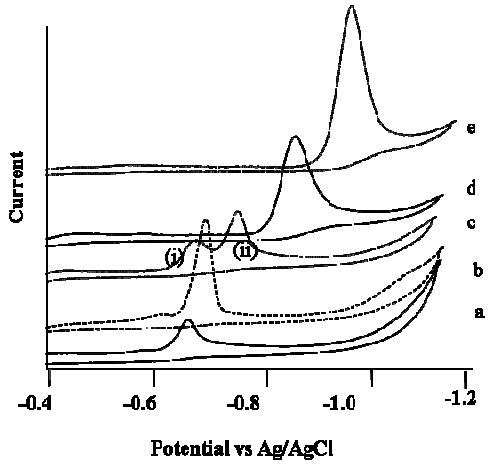


Fig 2: Cyclic voltammograms of the ALP (6 x 10^{-5}M) at pH a)1.5, b) 2.0, c)3.0, d)7.3, and e)11.0

In the pH range 1-4, two reduction peaks are observed a small peak at ~ -0.65 V and another at ~ -0.75 V. As the pH increase one obtain only single reduction peak ~-0.9 V. To understand whether the two peaks appearing at low pH range are interdependent, we followed the ratio of the peak current as a function of scan rate. The peak current ratio (i_{pi}/i_{pii}) is near independent at scan rate above 100 mV/sec whereas it is increases with decreasing scan rate. The plot of peak current ratio vs scan rate is shown in Fig 3.

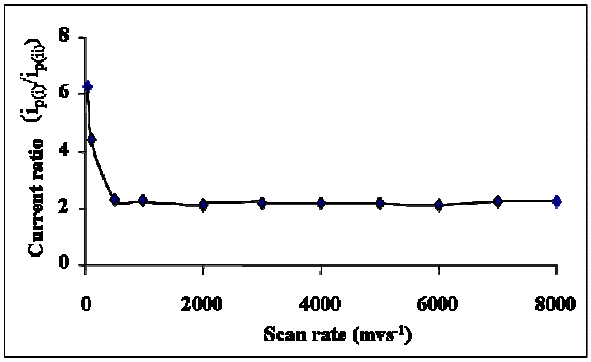


Fig 3: Peak current ratio vs scan rate of ALP at pH 3.00

The irreversible electron transfer of ALP at mercury electrode is confirmed from the plot of current function ($i/\upsilon^{1/2}$) vs scan rate. The current function of the second peak is near constant at high scan rates supports the irreversible electron transfer property [19-20]. Alprazolam has two electroreducable sites; one is the azomethine site in cycloheptane ring and the other is the carbon-halogen (-C-X) bond. The electrochemical reductive cleavage of C-X bond appears at sufficiently higher negative potential than the reduction of the azomethine group [21-24]. The possibility of the reduction of C-Cl bond in ALP is ruled out because there was no fall in pH when ALP was electrolysed at a potential cathodic to the E_p of the CV profile.

Hence, the cathodic response in the potential ranges -0.7-1.2V is tentatively attributed to the electrochemical reduction of azomethine electrophore in 7-membered heterocycle. Enhanced peak current observed at pH 9.00, used for further analytical studies.

The linear dependence of peak current on the scan rate (R^2=0.9989) instead of the square root of scan rate (R^2=0.9789) suggests adsorption controlled electrochemical process for ALP. Some of the plots of i_p vs scan rate are shown in Fig 4.

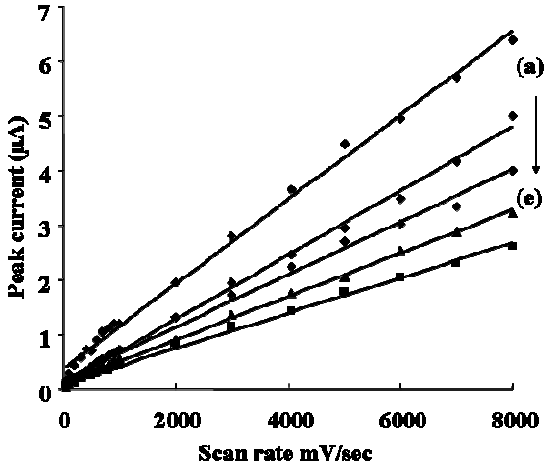


Fig 4: Variation of peak current with scan rate of ALP at pH a) 7.3 b) 1.65 c) 6.3 d) 9.3 and e) 11.00

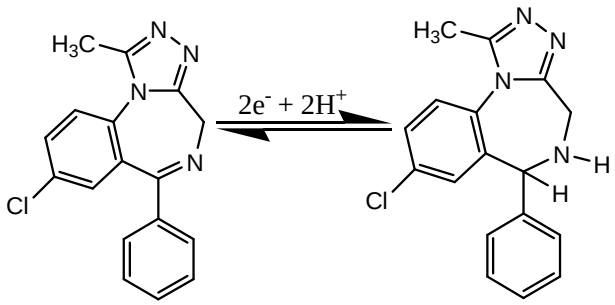
The adsorptive electrochemical behaviour of ALP is also confirmed from the plots of log i_p vs log υ; straight lines were obtained with slope value of 0.7-0.9 mV, which were very close to the theoretical value of 1.0 mV [19]. The Ep–log *v* plots at different pH values were linear with slope values of 31-60 mV (slope = 59/*α*n*a*, mV). Values of αna (0.98-1.9) and α (0.49-0.95) were obtained. Relevant voltammetric data was colleted in Table 1.

The number of electrons involved in electrochemical reduction was calculated using controlled potential coulometry whereas the number of protons as mentioned above from the slope of E_{pc} vs pH as 2 both values turned out to be 2 each at pH >5.

Table 1: Cyclic voltammetric data of alprazolam

pH	E_p	i_p	Slope of E_p vs log υ mV	αn_a	α	D_o x 10^6 (cm^2sec^{-1})	$k_{f,h}$ x10^{-10} (cm^2sec^{-1})
1.65	0.68	0.117	31	1.90	0.95	1.63	5.66
3.00	0.775	0.092	32	1.67	0.83	1.24	0.50
4.70	0.806	0.088	34	1.82	0.91	1.05	4.36
6.30	0.894	0.102	35	1.62	0.81	1.57	5.53
7.30	0.983	0.137	41	1.42	0.71	3.20	8.57
9.80	1.08	0.299	43	1.36	0.68	1.62	0.10
11.00	1.16	0.118	60	0.98	0.49	3.40	0.10

Based on the above observations a 2electron 2proton electrochemical process of reduction is attributed to reduction of alprazolam as presented in Scheme 1.



pH>5

Scheme 1

3.3 Electrochemical assaying of alprazolam

The calibration curve is presented for alprazolam in the buffer of pH = 9.00 under the optimally chosen conditions Figure 5. The regression equation associated with calibration the calibration plots exhibits good linearity (r=0.9968). The linear curve fit to an equation i_p = 1.1017X+0.0209. The relative standard deviation (RSD) of the analytical signals was calculated by using the calibration data, and found to be 5%. The proposed analytical method is examined in absence and presence of common excipients. It is found to be freedom from interference by the excipients.

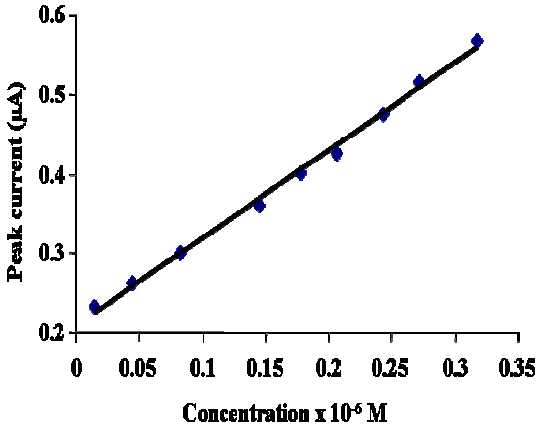


Fig 5: Differential pulse polarographic calibration curve of alprazolam in buffer of pH 9.00

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