*VOLTAMMETRIC DETERMINATION OF SOME PHENOTHIAZINES USING GLASSY CARBON ELECTRODE**

İ.BİRYOL**, Z.ŞENTÜRK , S.A. ÖZKAN , S. DERMİŞ ,B.USLU

Department of Analytical Chemistry,Faculty of Pharmacy,Ankara University,
06100,Ankara, Turkey.

Abstract

In the present study the voltammetric behaviours of some phenothiazines namely Promazine hydrochloride, Promethazine hydrochloride and Fluphenazine hydrochloride were investigated. And it was shown that the electrooxidation of the substances were dependent on the nature of the supporting electrolyte , pH, scan rate. The results of the statistical analysis revealed that the quantitative analysis of these substances could be made using glassy carbon electrode, activated by a simple electrochemical procedure, with good accuracy and precision.

Key Words : Promazine hydrochloride, Promethazine hydrochloride, Fluphenazine hydrochloride, Glassy carbon electrode, Voltammetry.

Introduction

The phenothiazines are among the most widely used drugs in medical practice.Phenothiazine has a three-ring structure in which two benzene rings are linked by a sulfur on a nitrogen atom.

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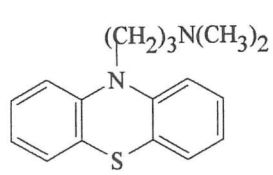
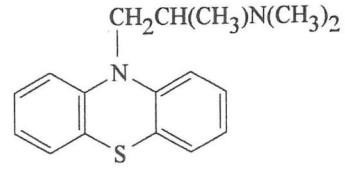
**Correspondence and reprints.

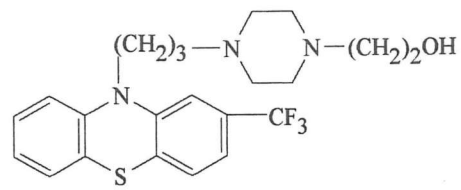
In the present study three members were investigated namely; promazine hydrochloride, promethazine hydrochloride and fluphenazine hydrochloride.The structures of the substances are shown in Scheme 1.



Promazine HCl



Promethazine HCl



Fluphenazine HCl

Scheme 1 . The structures of the phenothiazines used in this study

Promazine hydrochloride and fluphenazine hydrochloride are used as antipsychotics and promethazine hydrochloride is used as an antihistaminic and antiemetic.

Methods for the determination of these drugs include FIA using fluorimetric detector [1,2] , spectrophotometry[3,4], gas chromatography[5], high performance liquid chromatography[6-11] , gas liquid chromatography[12,13], gas liquid chromatography combined with mass spectrometry[14,15] and radioimmuno assays[16,17].

Numerous electroanalytical methods for the determination of phenothiazines are available for example wax impregnated graphite electrode[18] ,fatty acids and lipid modified carbon paste electrode[19,20],nafion coated glassy carbon electrode [21,22] , phospholipid/cholesterol modified glassy carbon electrode [23] ,ruthenium and platinum electrodes [24]

This paper deals with the electrochemical behaviour of these drugs on an electrochemically pretreated glassy carbon electrode.The effects of the factors such as supporting electrolyte and pH on the voltammograms were shown and also the optimum conditions for the determination of the drugs under study were investigated.The statistical

analysis of data revealed that the voltammetric method by the use of glassy carbon electrode was convenient for the quantitative analysis of these substances.

EXPERIMENTAL

Apparatus:

The voltammetric measurements were performed on a PRG-3 polarograph (Tacussel) associated to an EPL-2 recorder (Tacussel).A glassy carbon electrode (Tacussel,Type XM 540,area:1.013 cm^2), a saturated calomel electrode (SCE) and a platinum wire were used as working, reference and counter electrodes respectively.

Reagents:

Promazine hydrochloride (Sandoz Drug Industries Inc. Istanbul,TURKEY), Promethazine hydrochloride (Günsa Drug Industries Inc., Adana, TURKEY) , Fluphenazine hydrochloride (Fako Drug Industries Inc.,Istanbul,TURKEY) were used without further purification.All other reagents were analytical grade. Doublly distilled water was used to prepare the solutions. Various electrolytes e.g., sulphuric acid (0.5M), phosporic acid (0.2M , pH 2), acetate buffer (pH 3.5 - 6.2), phosphate buffer (pH 4.7 - 8.5)were evaluated a suitable media for the determination of these phenothiazines.

Acetate buffers of pH 3.5 , 4.7 , 6.2 were prepared by the addition of proper volumes of 5M sodium hydroxyde to 0.2 M acetic acid solution ; and phosphate buffers of pH 4.7 , 6.2 , 7.0 , 8.5 were prepared by the addition of proper volumes of 5M sodium hydroxyde to 0.2 M sodium dihydrogen phosphate solution.

Stock solutions were prepared daily by dissolving these phenothiazines in selected supporting electrolytes.

Pre-treatment of the working electrode:

Because the apparent rates of electron transfer are slower at glassy carbon electrodes than at metallic electrodes a lot of investigations have been reported in the literature in order to activate these electrodes. Among these activation procedures metallographically polishing and electrochemical pre-treatments were also took place[25-29] .Studies on glassy carbon indicate the existence of various surface oxide forms, such as hydroxyl, carbonyl, carboxy and quinoidal structures. When a potential is applied to a glassy carbon electrode these surface structures come into redox equilibrium with the applied potential slowly because the rates of solid-state surface reactions are low [27] .

There is no agreement on a standard electrochemical pre-treatment for glassy carbon electrode, because the activation method also depends on the material being analysed.

For our purpose activation of the glassy carbon electrode was obtained by polishing the electrode with alumina for every 10-15 measurements and by applying a potential of +1.5 V for 5 min and -1.0 V for 2-3 s in 0.1 M potássium nitrate solution before each experiment. By this pre-treatment it was observed that the reproducibility of the curves were satisfactory. When the tests were performed using a glassy carbon electrode activated by only polishing with alumina reproducibility was poor.

RESULTS and DISCUSSION

It has been proposed that phenothiazine derivatives undergo two one-electron oxidation steps in 6 M sulphuric acid solution.The first step is the oxidation of $R^{\cdot}$ to R^{+} and the second is the oxidation of R^{+} to S , where $R^{\cdot}$ represents the initial form of the compound, R^{+} represents the free radical obtained by 1- electron loss and S represents the corresponding sulfoxide[30]. However in a weakly acidic solution the cation radical is unstable and undergoes chemical reaction e.g. hydrolysis.In recent studies on phenothiazine cation radical hydrolysis in aqueous buffer solutions it has been reported that the cation radical is attacked by a weak nucleophile to form an adduct which is oxidized by another molecule of the cation radical to produce $R^{\cdot}$ and S[31] .

Voltammograms were obtained in sulphuric acid solutions,acetate buffer of pH 3.5 ; 4.7 ; 6.2 and phosphate buffer of pH 4.7 ; 6.2 ; 7.0 ; 8.5.

Fig. 1 shows the behaviours of the drugs in 0.5 M sulphuric acid solution.Generally three oxidation steps are seen on the curves and the peak potentials of promazine HCl seem to be lower than the others.At the scan rates below 100 mVs^{-1} the shape of the peaks changes to limiting currents.At the cathodic branches promazine HCl and promethazine HCl show three steps at about 600 , 300 and 100 mV but fluphenazine seems to be more irreversible and no peak was observed.Some examples about the curves obtained in acetate and phosphate buffers are seen in Fig.2. It was observed that the peak potentials shifted to less positive values with the increase in pH as can be seen on the curves.The first oxidation peak of promethazine which was observed in Fig.1 disappeared and the peak current of the second peak increased. The third peak can be seen only on the curve of fluphenazine.

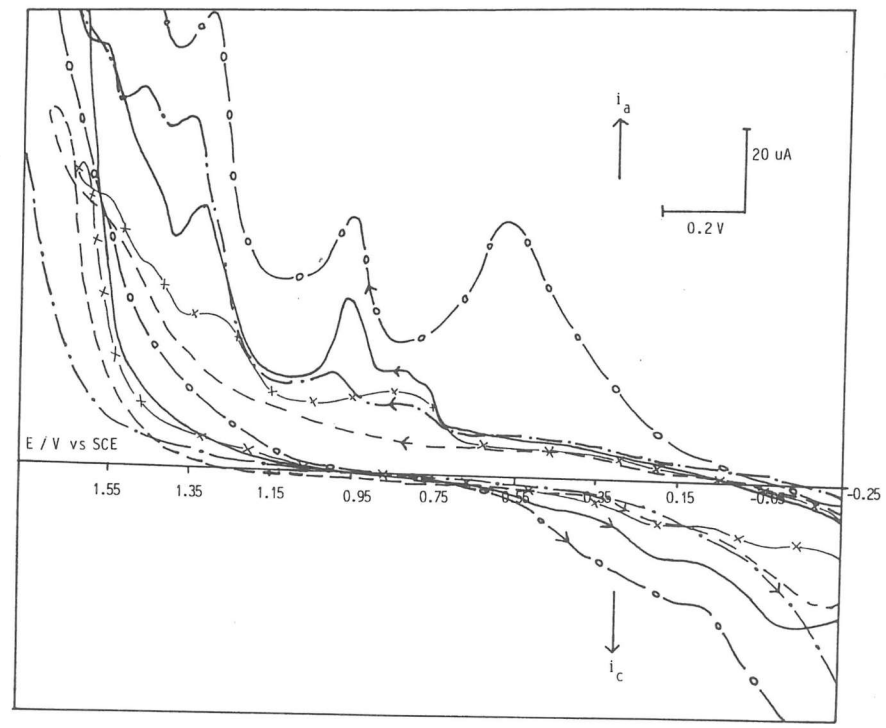


Fig.1 : Cyclic voltammograms of 4.10^{-4} M drugs in 0.5 M H_2SO_4 solution.
(-----), Supporting electrolyte, (-·-·-),Fluphenazine HCl,
(——),Promethazine HCl, (-o-o), Promazine HCl, scan rate,100 mV/s.
(-x-x-) Promethazine HCl , scan rate, 50 mV/s.

Takamura et al [32] observed three oxidation steps for chloropromazine (CPZ) in the phosphate buffer of pH 3.9 at 650 ; 950 and 1100 mV vs SCE.At the end of the analysis of the products at these potentials they reported that at 650 mV CPZ cation radical formed ($CPZ^{+\cdot}$)and finally CPZO was obtained.At the peak potential of 950 mV they reported that the final product was also CPZO which formed by the subsequent oxidation of $CPZ^{+\cdot}$. The third peak was found as the result of the further oxidation of CPZO.

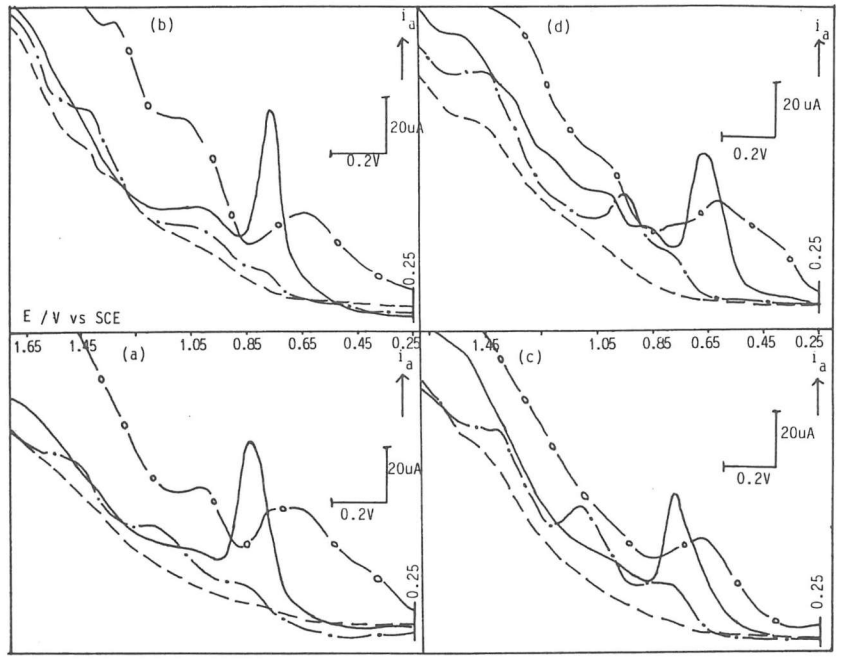


Fig. 2 : Voltammograms of 4.10^{-4} M drugs in different buffer solutions and different pHs. ***(a)*** in acetate buffer,pH 3.5 ; ***(b)*** in acetate buffer, pH 4.7; ***(c)*** in phosphate buffer,pH 4.7 ***(d)*** in phosphate buffer , pH 6.2; (-----), Supporting electrolyte, (----),Fluphenazine HCl, (——),Promethazine HCl, (-o-o-), Promazine HCl.

At the present study the potentials of the two peaks seem to be in accordance with the observations of Takamura et al for promazine HCl but for Promethazine HCl and Fluphenazine HCl the potentials are more positive (Fig. 2a) indicating that the reactions are energetically less favourable.When the peak currents of the first peaks were plotted against pH in phosphate and acetate buffer (Fig. 3) in phosphate buffers an increase with pH was observed which supports the catalytic mechanism at higher pHs in accordance with the literature [32] .

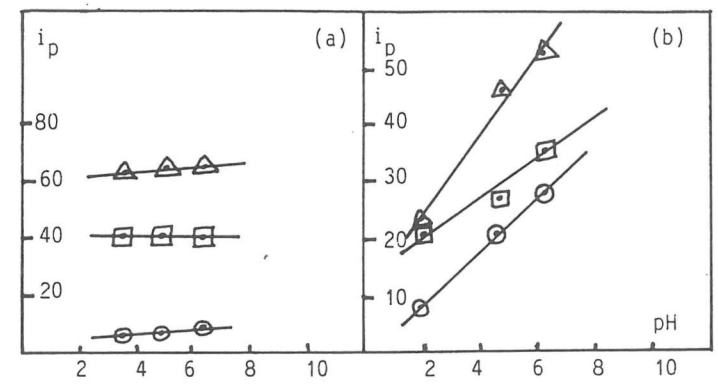


Fig.3 : Effects of the pH on the Fluphenazine HCl, Promethazine HCl and Promazine HCl peak currents.Drug concentration 4.10^{-4} M. Scan rate,100 mV/s.
(a) Acetate buffer , pH 3.5 ;4.7 ; 6.2 (b) Phosphate buffer, pH 2 ; 4.7 ; 6.2
(o) Fluphenazine HCl ,(Δ) Promethazine HCl , (□)Promazine HCl

From the voltammograms recorded for various concentrations for the three drugs the homogenous reaction orders were calculated using $\left(\frac{\partial \log i}{\partial \log c}\right)_E$ equation [33]. In 0.5 M sulphuric acid solution for promazine HCl at the potential of 700 mV in the concentration range of 4.10^{-5}-1.10^{-4} M it was found as about 1 and in the concentration range of 4.10^{-4}-8.10^{-4} M this value decreased and found as about 0.4 indicating that the oxidation mechanism of this substance depends on the concentration.For promethazine in the concentration range of 8.10^{-5}-4.10^{-4} M , for fluphenazine between 2.10^{-5}-8.10^{-4} M ,the concentration ranges for analytical evaluation, the degree of the reactions were found as about 1 and no concentration dependence were observed in the range under study.In acetate and phosphate buffers the degree of the reactions were found different indicating that the anions take part in the mechanism. Fig.3 shows that i_p is nearly independent of pH in acetate buffers and a linear dependence exists between i_p and pH in phosphate buffers for all the three substances in accordance with the results in literature [31,32] . In

phosphate buffers the reaction is accelerated at higher pH. An addition reaction occurs between the supporting electrolyte and phenothiazine cation radical. With phosphate nucleophile a proton may be produced, this makes the rates of the cation radical decay highly pH dependent while nucleophiles, derived from monoprotic acids do not show such a dependence.

The curves obtained in various buffer solutions were analytically evaluated and it was statistically shown that the quantitative determination of promazine HCl, promethazine HCl and fluphenazine HCl could be made using glassy carbon electrode.Table I shows the optimum conditions and the results of the linear regression analysis. From analytical view point best results were obtained in different solution for all the three substances as can be seen in Table I. The statistical analysis showed that the most reproducible results could be obtained in 0.5 M sulphuric acid for Fluphenazine , in acetate buffer of pH 4.7 for Promethazine and in acetate buffer of pH 6.0 for Promazine.

Although peak currents for Fluphenazine are higher in phosphate buffers than in the other solutions the reproducibility is poor in these media this may be because of catalytic mechanism is dominant in phosphate buffers.

CONCLUSION

This study revealed that the electrooxidation mechanism of phenothiazines changes depending on pH and the nature of the supporting electrolyte solutions. From analytical view point it was shown that the analysis of Promazine, Promethazine and Fluphenazine could be made with enough accuracy and precision using a glassy carbon electrode which was simply pretreated by the application of an electrochemical procedure.

Table I : Linear regression results of Fluphenazine, Promethazine and Promazine hydrochloride.
Peak currents were taken at 1.35 V for Fluphenazine ; 0.80 V for Promethazine ; 0.70 V for Promazine.

Sample	*Medium*	*Linearity Range / M*	*Equation*	*Correlation Coefficient*	*St.Error of Slope*	*St.Error of Intercept*
Fluphenazine	0.5 M sulphuric acid	2.10^{-5}-8.10^{-4} (n=9)	$y=1,6.10^{5}\ x +5.26$	0.999	$2,58\ .10^{3}$	0.95
Promethazine	Acetate buffer (pH 4.7)	6.10^{-5}-8.10^{-4} (n=7)	$y=6,5.10^{4}\ x -1.23$	0.999	$1,4\ .10^{3}$	0.58
Promazine	Acetate buffer (pH 6.2)	6.10^{-5}-8.10^{-4} (n=7)	$y=1,11.10^{5}\ x -1.05$	0.999	$1,43\ .10^{3}$	0.59

REFERENCES

1. Calatayud ,J.M.,Sancho,T.G., ***Pharmazie,***47, (1992),557.
2. Romero,A.M.,Benito,C.G.,Calatayud,J.M.,***Anal.Lett.***,25, (1992),1289.
3. Liu,B.,Chang,Y.,***Zhongguo Yiyao Gongye Zazhi***,24, (1993),468.
4. Bhongade,S.L.,Kasture,A.V.,***Indian J.Pharm.Sci.***,55, (1993),101.
5. Sane,R.T.,Surve,S.R.,Gangrade,M.G.,Bapat,V.V.,Chonkar,N.L.,***Indian Drugs***,30, (1993), 66.
6. Balikova,M.,***J. Chromatogr.***,581, (1992),75.
7. Mathew,M.,Das Gupta,V.,Bethea,G.,***Drug Dev. Ind.Pharm.***,20, (1994),1693.
8. Tracqui,A.,Kintz,P.,Kreissig,P.,Mangin,P.,***J.Liq.Chromatogr.***,15(8),1381-1396,1992.
9. Davis,C.M.,Fenimore,D.C.,***J.Chromatogr.***,272, (1983),157.
10. Hoffman,D.V.,Edkins,R.D.,Shillcutt,S.D.,Salama,A.,***J.Chromatogr.***,414, (1987),504.
11. Cooper,J.K.,Hawes,E.M.,Hubbard,J.W.,Midha,K.K.,***Ther.Drug Monit.***,11,(1989),354.
12. Franklin,M.,Wiles,D.H.,Harvey,D.J.,***Clin.Chim.***,24, (1978),41.
13. Jawaid,J.I.,Dekirmenjian,H.,Liskevych,V.,Lin,R-L.,Davis,J.M., ***J.Chromatogr. Sci*** .,19, (1981), 439.
14. Mckay,G.,Hall,K.,Edom,R.,Hawes,E.M.,Midha,K.K. ,***Biomed.Mass Spectrom*** .,10, (1983), 550.
15. Jernal,M.,Ivashkiv,E.,Both,D.,Koski,R.,Cohen,A.,***Biomed,Environ .Mass Spectrom.***,14, (1987),699.
16. Hawes,E.M.,Shetty,H.V.,Cooper,J.K.,Rauw,G.,Mckay,G.,Midha,K.K.,***J.Pharm.Sci.***,73, (1984),247.
17. Lo,E.E.S.,Fein,M.,Hunter,C.,Suckow,R.F.,Cooper,T.B.,***J.Pharm. Sci.***,77, (1988), 255.
18. Jarbawi,T.B.,Heineman,W.R.,***Anal.Chim.Acta***,186, (1986),11.
19. Kauffmann,J-M. ,Chastel,O. , Quarin, G. ,Patriarche, G.J. ,Khodari ,M. ,***Bioelectrochem. Bioenerg.***,23, (1990),167.
20. Hu,X.Y.,Leng,Z.Z.,***Yaoxue Xuebao***,27, (1992), 283.
21. Zhou,J.,Wang,E.,***Anal.Chim.Acta***,249, (1991), 489.
22. Khodari,M.,Kauffmann,J-M.,Patriarche,G.J.,Ghandour,M.A.,***J. Pharm.Biomed.Anal.***, 7, (1989),1491.
23. Wang,J.,Lu,Z.,***Anal.Chem.***,62, (1990), 826.
24. Dermiş,S.,Biryol,İ.,***J.Pharm.Biomed.Anal.***,8, (1990), 999.

25. Laser,D.,Ariel,M., ***Electroanal.Chem. and Interfac.Electrochem.***, 52, (1974),291.
26. Aoki,K.,Ishida,M.,Tokuda,K., ***J.Electroanal.Chem.***,251, (1988),63.
27. Blaedel,W.J.,Jenkins,R.A., ***Anal.Chem.***, 46, (1974), 1952.
28. Nagaoka,T.,Sakai,T.,Ogura,K.,Yoshino,T., ***Anal.Chem.***, 58, (1986), 1953.
29. Özkan,S.,Biryol,İ.,Şentürk,Z., ***Tr.J. Chem.***, 18, (1994), 34.
30. Merkle,F.H.,Discher,C.A.,***Anal.Chem.***,36, (1964), 1639.
31. Sackett,P.H.,Mc Creery,R.L.,***J.Med.Chem.***,22, (1979),1447.
32. Takamura,K.,Inoue,S.,Kusu,F.,Otagiri,M.,Vekama,K.,***Chem.Pharm.Bull.*** , 31, (1983), 1821.
33. Vetter,K.J.,***Electrochemical Kinetics***, Academic Press,New York,London ,pp.440,1967.

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