Journal of Analytical Techniques
Journal of Analytical Techniques
ISSN: 0976–769X & E-ISSN: 0976–7703, Vol. 2, Issue 1, 2011, PP-09-18
Available online at http://www.bioinfo.in/contents.php?id=94

ELECTROCHEMICAL BEHAVIOR AND SQUARE WAVE STRIPPING VOLTAMMETRIC DETERMINATION OF ANTIBACTERIAL DRUG AT IONIC LIQUID MODIFIED CARBON PASTE ELECTRODE

CHANDRASEKAR ANNAMALAI[1], SELVARAJU RADHAKRISHNAN[1], RAGHU KUNJITHAPATHAM[2], AND SANKARAN KUYAM RATHINAVELU[3]*

[1]Department of Technology Transfer, Shasun Pharmaceuticals Ltd, Cuddalore-607005, India
[2]Department of Chemistry, Krishnasamy Engineering College, Cuddalore-607002, India
[3]Department of Chemistry, Annamalai University, Annamalai Nagar-608002, India
*Corresponding Author: Email- profkrs15@gmail.com, Tel: +91 4144- 238601

Received: October 28, 2011; Accepted: November 30, 2011

Abstract-A novel kind of carbon paste electrode (CPE) was prepared by mixing graphite powder, mineral oil and the room temperature ionic liquid (RTILs) 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_4 mim][NTf_2]). The resulting electrode was used to study the electrochemical properties of Sulphamethoxazole, a sulfonamide bacteriostatic antibiotic, were investigated in pH range 2.0–10.0 by cyclic and square wave voltammetry. Compared to a conventional CPE, the oxidation peak currents are largely increased, and the oxidation peak potentials are negatively shifted. The drug was irreversibly oxidized at the electrode surface in one or two oxidation steps, which are pH-dependent. For analytical purposes, a very resolved diffusion controlled voltammetric peak was obtained in Britton–Robinson buffer at pH 9.0 using cyclic and square-wave stripping modes. The peak current varied linearly over the range from 2.0×10^{-6} to 1×10^{-4} mol L^{-1}. The limits of detection and quantification were 2.7×10^{-6} mol L^{-1} and 2.5×10^{-5} mol L^{-1} respectively. The recovery was found in the range from 99.56% to 100.26%. The relative standard deviation was found in the range from 0.429% to 0.845%. The proposed method possesses high sensitivity, accuracy and rapid response. Finally, this method was successfully used to determine Sulphamethoxazole in tablets was described.
Key words- Carbon ionic liquid electrode, Sulphamethoxazole, Square wave voltammetry, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imides

1. Introduction

The development of Sulphonamaide, or supha drugs, has been one of the most fascinating and important studies in medicinal chemistry. The Chemotherapeutic activity of Supha drugs is associated with their competition with *p*-aminobenzoic acid in the synthesis of folic acid [1], which is essential for the growth of both mammalian cells and bacteria. The former can obtain their supply of folic acid by alimentation, while the latter cannot. Therefore, sulphonamides acts by inhibiting the bacterial growth rather than directly affecting the bacteria. Despite the discovery and wide use of other antibiotics, sulphonamides are among the most widely employed antibacterial agent to human and veterinary medicine [2] due to their low cost and efficiency in the treatment of bacterial diseases.

Sulphamethoxazole (4-Amino-N-(5-Methyl-3-isoxazolyl)benzenesulphonamide) Fig-1 is one of the most effective sulpha drug in the treatment of urinary infections. Due to its slow absorption and excretion by human organism, it has greater crystalluria. A number of pharmaceutical combinations are commercialized with the purpose of reducing crystalluria incidence. A good synergetic association is sulphamethoxazole with trimethoprime Fig-1. This combination increases the bacteriostatic effect of sulpha due to an inhibitor effect in more than one step during obligatory sequence of enzymatic reaction in the bacteria. The antibacterial activity of the combination of sulfamethoazole and trimethoprime results from it action on two steps of the enzymatic pathway for the synthesis of tetrahydrofolic acid [3]. In world wide in many countries the combination marketed as Co-trimoxazole.

Owing to concern over the analytical determination of sulpha drugs in pharmaceuticals and residues in food products, a number of analytical techniques have been reported, including high-perfromance liquid chromatography (HPLC) [4-11], capillary electrophoresis [12], gas chromatography [13 - 15], spectrophotometric method [16 , 17], flow injection spectrophotometric method [18] the Bratton-Marshall method [19, 20], the titrimetric assay method [21], liquid chromatography-mass spectrometry. These methods have proven to be sensitive and suitable for sulfonamide determination. However, few electrochemical techniques have been reported for the determination of sulfonamide

compounds [22 – 31], most probably due to issues related electrode deactivation and fouling. Despite this drawback, electrochemical methods offer certain advantages, such as not requiring sample preparation, not being time-consuming and offering a sensitivity and dynamic range comparable to other methods. Table-1 represents the some of the analytical methods proposed in the literature for the determination of Sulphamethoxaole [9, 11, 20, 32-39].

Carbon ionic liquid electrode (CILE), which is made of graphite powder with room temperature ionic liquids (RTILs), has aroused great interests in recent years due to the advantages including high ionic conductivity, fast electron transfer rate, good anti-fouling properties, and inherent catalytic ability and simplicity of preparation [40]. Maleki et al. indicated that CILE exhibited the superiority to different kinds of carbon electrodes such as glassy carbon electrode (GCE), carbon nanotubes (CNTs) modified electrode and edge plane pyrolytic graphite electrode [42]. So CILE can be used as the basal electrode for investigating the electrochemical behaviors of electro active molecules. Safavi et al. investigated the direct electrochemistry and electrocatalytic behavior of hemoglobin (Hb) on n-octylpyridinium hexafluorophosphate (OPPF) based CILE [41]. Musameh et al. also reported an ionic liquid-carbon composite biosensor for the detection of glucose, which was prepared by mixing OPPF with graphite powder and glucose oxidase together [42]. Li et al. combined ILs with different carbon materials such as single walled CNT, order mesoporous carbon and graphene to fabricate IL modified electrode for the biosensor [43 – 45]. Xi et al. combined 1-butyl-3-methylimidazolium tetrafluoroborate and horseradish peroxidase into three-dimensional chitosan hydrogel by electrode position to construct a H_2O_2 biosensor [46]. Xiao et al. introduced an IL-CNTs gel modified electrode for xanthine determination with high sensitivity and selectivity [47]. Wei sun etal. Prepared the different kind of CILE to study the direct electrochemistry of electroactive substances such as Hb, ssDNA, dsDNA, catechol and so on [48 -52]. Recently Prussian blue [53] and DNA [54] modified CILE were fabricated for the detection of H_2O_2 and rutin, respectively.

In this paper a new kind of CILE was fabricated by using 1-butyl-3-methylimidazolium bis (trifluoromethylsulfonyl)imide ([C_4mim][NTf_2]) as binder and further applied to the investigation on the electrochemical behaviors Sulphamethoxazole. ([C_4mim][NTf_2]) is a hydrophilic imidazolium-type ionic liquid with the electric conductivity as 40μS/cm (25 °C). Due to the specific characteristics of ([C_4mim][NTf_2]), the electrochemical responses of sulphamethoxazole were greatly improved on the CILE with reduced over potentials. The electrochemical behaviors of sulphamethoxazole on the CILE were carefully investigated, and the results indicated that the electro-oxidation reactions appeared on the modified electrode is irreversible diffusion controlled. Based on the cyclic and square wave voltammetric response of sulphamethoxazole, a sensitive electrochemical method for the determination of sulphamethoxazole was established and further applied to the bulk drug and tablets samples detection with satisfactory results.

2. Experimental

2.1. Apparatus

Cyclic voltammetry (CV) and square wave stripping voltammetry (SWSV) were performed using an electrochemical work station (CH Instrument, USA (model 1100A Series and 760C). A three compartment electrochemical cell, incorporating the working electrode (CILE) was used. The reference electrode was Ag/AgCl (3 mol L^{-1} KCl) and a Pt-wire was used s an auxiliary electrode. The operating conditions for SWSV were summarized in table-2.

2.2. Electrode preparation

Carbon paste electrode (CPE) was prepared by hand mixing 1.6 g of graphite powder with 0.5 mL of liquid mineral oil nujal in an agate mortar. A portion of the carbon paste mixture was packed into the end of a glass tube (4.0 mm inner diameter). Electrical contact was made by forcing a copper wire down the glass tube from back side. The preparation process of the ionic liquid modified carbon paste electrode (CILE) was similar to that of the CPE except replacing 0.1 mL of liquid paraffin with the same volume of ([C_4mim][NTf_2]). Prior to use, the CILE surface was smoothed on a weighing paper.

2.3. Reagents

Sulphamethoxazole(99%, Sigma, http://www.sigmaaldrich.com), 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([C_4mim][NTf_2]), (99.0%,Merck, http://www.merck.com and graphite powder & mineral oil (Fluka) were used as received. All the other chemicals were commercially available analytical reagent grade used without further purification. The sulphamethoxazole stock solutions were prepared with dimethyl formamide just before use. Working solution prepared by diluting the appropriate volume of stock solution with B-R buffer pH ranges from 2 -10. 0.2 molL^{-1} Britton-Robinson (B-R) buffer solutions with various pH values were used as the supporting electrolyte, which was composed of a mixture of 0.2 molL^{-1} H_3BO_3, 0.2 molL^{-1} H_3PO_4, and 0.2 molL^{-1} CH_3COOH that was titrated to the desired pH with 0.2 mol L^{-1} NaOH.

2.4. Analytical procedure

2.4.1. Calibration curve preparation

Working solutions ranging between 1×10^{-5} and 1×10^{-4} mol L^{-1} were prepared by diluting the sulphamethoxazole stock solution with B-R buffer of required pH.

2.4.2. Synthetic samples

For recovery studies, excipients such as lactose, magnesium oxide, magnesium stearate and hydroxypropyl cellulose were added to the drug

according to manufacturer's batch formulas for 400 mg of sulphamethoxazole +80mg of trimethoprim per capsule.

2.4.3. Assay

The contents of 10 tablets were mixed and an appropriate amount containing the equivalent of 400mg sulphamethoxazole + 80mg trimethoprime was dissolved in 10mL of dimethylforamide. The solution was sonicated for 15 min and diluted to 25 mL with the same solvent. Further dilution of this solution was performed using B-R buffer to obtain sulphamethoxazole concentration of 2×10^{-5} mol L^{-1}. The final solution was transferred to a voltammetric cell and measurements were recorded at least twice from 0 to 1200 mV. The amount of Sulphamethoxazole, in milligrams, for the sample solution was calculated from the prepared standard calibration curve.

2.4.4. Uniformity of content

No less than 10 commercial tablets of sulphamethoxazole (Septran®, amount declared: 400 mg sulphamethoxazole per tablet) were used. The contents of each individual tablet were suspended in 10mL dimethyl formaide using sonication to ensure complete dissolution of the drug. The solution was diluted to a final volume of 25mL with the same solvent. An aliquot of each solution was diluted to 10mL with B-R buffer to obtain sulphamethoxazole concentration of 2×10^{-5} mol L^{-1}. Each sample solution was transferred to a voltammetric cell and measurements were recorded at least twice from 0 to 1200 mV. The amount of sulphamethoxazole, in milligrams, in the sample solution was calculated from the prepared standard calibration curve.

3. Results and discussions

3.1. Supporting electrolyte selection

The supporting electrolyte plays an important role in the electrochemical response. Its choice can modify the thermodynamics and kinetics of electrochemical processes, as well as mass transfer within the cell. Therefore, 0.04mol L^{-1} Britton-Robinson buffer, 0.05 mol L^{-1} phosphate buffer and 0.1 and 0.5 mol L^{-1} sulfuric acid were tested as supporting electrolytes for sulphamethoxazole oxidation using a CILE electrode. Fig. 2 shows cyclic voltammograms obtained for 1.0×10^{-3} mol L^{-1} sulphamethoxazole in different media. As can be seen, sulphamethoxazole oxidized at 0.976 V, the intensity and resolutions of the voltammetric peaks were better, in Britton- Robinson buffer.

3.2 Cyclic voltammetric behaviour of sulphamethoxazole

The cyclic voltammograms of sulphamethoxazole was recorded using CPE and CILE in acidic, neutral and alkaline conditions at sweep rate 100 mV/s. cyclic voltammogram of sulphamethoxazole exhibited one oxidation peak at 0.966 V. At alkaline condition, peak current peak and peak shape were evident but at neutral medium peak response was low. In acidic medium, the peak was broadened. The background current was recorded for all pH range and subtracted properly in calculating the peak currents and the peak potential. Current of well –defined anodic peaks noticed in the cyclic voltammogram were considered for the study of effect of pH. Fig.3a shows the variation of peak potential with variation of pH, protonation followed by oxidation led to the dependence of the peak potential with pH. As the pH increased protonation decrease and hence higher potential were required for the oxidation of sulphamethoxazole. As the pH increased, the oxidation was facilitated and proceeded at lower potentials and peak current decreased. Sulphamethoxazole exhibited maximum peak current response at pH 9.0 Fig.3b.

Fig. 4a represents the cyclic voltammogram of 300 µg mL^{-1} sulphamethoxazole at pH 9.0. The effect of scan rate showed an increase in peak current with increase in scan rate but non-linearity was observed. Peak current versus square root of scan rate lead to a straight line. Logarithm of peak current were plotted with logarithm of scan rate and straight line was obtained ($\log i_p$ = 0.2231log (v/Vs^{-1}) +0.99, r^2 = 9892 with a slope around 0.2231 Fig. 4b. These facts confirmed diffusion-controlled reaction. A corresponding cathodic peak was not observed in the reverse scan. The $E_{P\ vs}$ logv plot resulted a straight line and a fractional αn value (0.7685) was calculated from the slope. Hence, the electron transfer was irreversible and the overall reaction was diffusion-controlled irreversible oxidation process [55, 56].

The influence of pH on the voltammetric behaviour of sulphamethoxazole has been studied using cyclic voltammetry in the pH range of 2 – 10 and exhibited a single oxidation peak. No cathodic peak was appeared on the reverse scan. Peak current has maximum intensity at pH 9 in B-R buffer.

The plot of the peak potential (E_p) versus pH showed two linear segments with a break at pH 6 Fig.5, which corresponds to pKa value of sulphamethoxazole, and their slope -0.0405 and -0.0385 mV, respectively. The two linear segments can be expressed by the following regression equations:

$$(\text{Sulfamethoxazole})H^+ \rightleftharpoons \text{Sulfamethoxazole} + H^+$$

pH 2 – 6: E (mV) = -0.0405 pH + 1.1705, r = 0.9965; (Sufamethoxazole) H^+ domination

pH 6 – 9: E (mV) = -0.0385 pH + 1.0115, r = 0.9979; Sufamethoxazole domination

This indicates that the protons were involved in the electrode reaction. Hence, a good linear relationship was established between the oxidation peak potential and solution pH. According to the equation [57]: -57.6x/n = -58.5, where x is the hydrogen ion participating the electrode reaction and n is the number of electron transferred. So the loss of electron was accompanied by loss of equal amount of proton and x = n = 2. Hence, there is involvement of two electrons in the oxidation of sulphamethoxazole at electrode surface which attributed to the two electron oxidation of amino group in sulphamethoxazole to the corresponding

iminibenzoquinone according to the currently accepted mechanism Fig-6.

The pKa value of sulphamethoxazole, which is 5.69, is close to our experimental value. Sharpe well defined peak with maximum intensity of the peak obtained at pH-9. The potential shifted less positive value and the peak current decrease with increasing the pH from 2 to 5 and there is increase in peak current with increasing the pH 6 -9 of the solution. This shows, sulphamethoxazole effectively oxidized at higher pH. Also sulphamethoxazole contained both acidic and basic functional groups: the amino functional group with pKa value of 1.65 and amide functional group with a pKa value of 6.5 [10,58]. Only the amino function can be oxidized, which is pH dependent. Hence, in sulphamethoxazole oxidation at electrode surface the conjugate base of amino group is oxidized.

Sulphamethoxazole being the category of sulfonamide compound can be electrochemically oxidized at the $–NH_2$ group, but the reduction of $-SO_2-$ group is very difficult to achieve. The potential at which the reduction occurs is strongly dependent on the characteristic of R (Sulphamethoxazole structure). On the other hand, R has little or no influence on the oxidation potential [59]. Previous investigations [23, 25] have addressed the electrochemical behavior of sulfonamides, and proposed an irreversible two-electron pH dependent reaction for their oxidation in aqueous solution.

3.3. Square wave stripping voltammetric (SWSV) analysis of sulphamethoxazole

Stripping voltammetry involves two-step in which the first step is accumulation of the substrate on the electrode and the second step involves stripping. Cyclic voltammetric results revealed good electroactivity of the substrate on the electrode at pH-9. Square wave mode was employed for stripping voltammetric studies and it is performed well in the determination of sulphamethoxazole. Accumulation potential was varied between -400 to +400 mV, higher peak response was obtained at +300 mV. The accumulation of sulphamethoxazole on the CILE surface under optimized accumulation conditions was understood from the change in electrode surface before and after accumulation. Lesser stripping current revealed lesser accumulation of sulphamethoxazole on the electrode surface and better accumulation of sulphamethoxazole, stripping led to good results and hence stripping parameter were optimized.

For the optimization of instrumental conditions, accumulation potential, deposition time, square wave amplitude, square wave frequency and scan increment were examined. The variable ranges -400 to 400 mV for accumulation potential, 15 – 90s deposition time, 25 – 200 square wave amplitude, 15 -105Hz square wave frequency and 2 – 20 for scan increment. The peak current increases by increasing all of these instrumental parameters. However, the base line current also increases. Optimized experimental parameters are summarized in Table -2.

Deposition time varied between 15 to 90s and maximum peak current was observed at 60s deposition time. The stripping peak current increased with increasing square wave amplitude from 25 – 150 mV. The stripping signal varied to lower responses for higher square wave amplitudes and exhibited good peak response at 50 mV.

The dependency of the peak intensity on the frequency was studied between 15 Hz to 105 Hz. At a constant step potential of 2 mV, the maximum peak current was at 75 Hz. As the frequency increased above 75 Hz, the peak current decreased and the peak was broadened. When the step potential increased to higher value 2 – 20 mV, a decrease in peak current was noticed. Hence, a frequency of 75 Hz and step potential of 8 mV were used. The use of higher frequency and step potentials led to distorted peaks and poor resolution. The square wave stripping voltammogram obtained under optimum experimental conditions (Table-2) for sulphamethoxazole at various concentration are illustrated in Fig.7a.

3.4. Analytical curve and validation parameters of the method proposed for sulphamethoxazole determination

3.4.1 Linearity

The experimental results showed that the peak current increased with the increase in concentration of the sulphamethoxazole Fig.7b. The peak potential did not shift the following the addition of sulphamethoxazole. The applicability of the proposed SWSV procedures as analytical methods for the determination of sulphamethoxazole was examined by measuring the stripping peak current as a function of concentration of the bulk drug at least three times under the optimized operational parameters. The calibration plot of the peak current versus the concentration was found to be linear over the range $2x\ 10^{-5}$ to $1x10^{-7}$mol $L^{-1,}$ and the linear regression equation is expressed as i_p = 0.0998C+1.1276; r^2=0.9978 in SWSV, where i_p is the stripping peak current and C is the concentration of sulphamethoxazole. Limit of detection (LOD) and limit of quantification LOQ) were obtained for SWSV is 1.05 μg mL^{-1} and 2.15 μg mL^{-1} respectively. The data shown in (Table -3) show some of the validation parameters for the proposed method for sulphamethoxazole. LOD and LOQ were calculated using the following equation [60 – 62]:

LOD = 3s/m LOQ = 10s/m

Where s is the standard deviation of peak current and m is the slope of the calibration curve.

The regression plots showed that there is a linear dependence of the current intensity on the concentration in the proposed SWSV. The good linearity of the calibration graphs and the negligible scatter of the experimental points are clearly evident by the values of the correlation coefficient and SD. The specificity of the method was investigated by observing any interference encountered from the excipients of the tablets mass. It is

shown that, in the proposed method, co administered drugs did not interfere.

3.4.2. Specificity

Specificity is the ability of the method to measure the analyte response in the presence of all the potential impurities. The specificity of the optimized procedure for estimation of sulphamethoxazole was examined in the presence of excipients such as lactose, magnesium oxide, magnesium stearate and hydroxypropyl cellulose, which were added to dosage form. Samples containing 0.1 µg mL^{-1} bulk sulphamethoxazole and different concentrations of the excipient under evaluation were analyzed by means of the proposed procedure. The obtained mean percentage recoveries (%R) and the relative standard deviations (%RSD) based on the average of seven replicate measurements is (99.0+ 0.8 to 100.5 +.2 showed no significant interference from excipients. Thus the proposed procedure can be considered specific.

3.4.3. Repeatability

The repeatability was examined by performing seven replicate measurements for 0.1 µg mL^{-1} bulk drug followed preconcentration for 200 s under the same operational conditions. Percentage recoveries (%R) of 99.7, 99.8, 99.2 99.8, 98.9, 100.2 and 100.5 were achieved with a mean value of 99.86 and (%RSD) of 0.6, which indicates repeatability and high precision of the proposed procedure Table-4.

3.4.4. Robustness

The robustness was examined by evaluating the influence of small variation of some of the most important procedure variables including preconcentration potential (Eacc) and preconcentration time (tacc). The obtained result provided an indication of the reliability of the proposed procedure for the assay of sulphamethoxazole, and hence it can be considered robust. The obtained mean percentage recoveries based on the average of seven replicate measurements were not significantly affected within the studied range of variations of some operational parameters, and consequently the proposed procedure can be considered robust.

3.4.5. Accuracy

Accuracy and precision of the proposed method were determined by replicate analyses of five different concentration of sulphamethoxazole, the results were shown in Table -5. The recovery was found in the range from 99.65% to 100.85% and relative standard (RSD) was in the range of 0.51% to 2.89%.

3.4.6. Precision and Stability

The intraday and inter-day precision of the proposed procedure was estimated by analyzing 0.1 µg bulk sulphamethoxazole solutions four times in 4 successive days using proposed SWSV method. The percentage recoveries based on the average of four separate determinations are given in Table 5. The results confirmed both the good precision of the proposed procedure and the stability of the drug's solution.

3.4.7. Ruggedness

The ruggedness test of the analytical assay method is defined as the degree of reproducibility of assay results obtained by the successful applications of the assay over time and multiple laboratories and analysts. Two analysts analyzed the same standard with the proposed method using the same instrument. The methods were found to be rugged with the results of variation coefficients 1.0 and 1.5% for first and second analysts, respectively. The results show no statistical differences between different analysts.

3.5. Application: Determination of sulphamethoxazole in commercial pharmaceutical products

The proposed voltammetric method was successfully applied to determine sulphamethoxazole in dosage form (Septronm bactrim and cotrimoxazole tablets) indicating that there is no interference from some common excipients used in pharmaceutical preparation such as lactose, magnesium oxide, magnesium stearate and hydroxypropyl cellulose. The linearity range was from $4.0x10^{-7}$ to $5.2x10^{-6}$ mol L^{-1} with mean recovery of 99.98% and mean relative standard deviation of 0.77%. The results were compared with those obtained with approved reference method [9] Table-6. The results obtained were compared statistically with those from published method [9] by using student's t-test for accuracy and variance ratio F-test (for precision). The results show that the t and F values were smaller than the critical values, indicating that the student t-test and variance ratio F-test excluded any significance difference between the proposed stripping voltammetric method and the published method with respect to accuracy and precision.

4. Conclusion

In conclusion, we were able to examine the voltammetric behavior of sulphamethoxazole in CILE using Britton Robinson buffer as supporting electrolyte. The electrochemical oxidation of sulphamethoxazole under the condition described in this work is an irreversible diffusion controlled process. The validated SWSV procedure could be used successfully to determine sulphamethoxazole in bulk and pharmaceutical formulation. In the proposed method, the high percentage of recovery shows that the compounds are almost completely extracted from tablet formulations, and the results indicate that the developed method can be used to quantify sulphamethoxazole without interference from other ingredients. The oxidation peak potential and current value were a function of the pH of the electrolyte. The developed method with the detection limit of $1x10^{-6}$ mol L^{-1}, is more sensitive than the already reported spectroscopic method and HPLC method for the determination of pharmaceutical dosage form. The usage of ionic liquid in the preparation CILE may

represent the attractive alternative for the analysis of the drug with direct analytical procedure in aqueous, pharmaceutical formulations and biological sample. In addition, no sophisticated instrument is required. Consequently, the proposed methods have the potential of good analytical alternative for the determination of sulphamethoxazole in the pharmaceutical formulation.

References

[1] Wormser G.P. and Keusch G.T. (1979) *Ann. Intern. Med.* 9, 420 – 429.

[2] Wang S., Zang H.Y., Wang L., Duan Z.J. and Kewnnady I. (2006) *Food Adult. Contam.* 23, 362-384.

[3] Hitching G.H. (1961) *Ann. N.Y. Acad. Sci.* 23, 700-705.

[4] Porter S. (1994) *Analyst* 119, 2753 – 2756

[5] Klimes J. and Mokry M. (1997) *Parmazie* 52, 448 – 450.

[6] Furusawa N. and Kishida K. (2001) *J.Anal. Chem.* 371, 1031 – 1033.

[7] Koseukwiwat U., Jayanta S. and Leepipatpiboon N. (2007) *J. Chromatogr. A* 1140, 147 – 156.

[8] Carla G. B., los A. Mallmann, Daniel R. Arsand, Francieli M. Mayer, Ayrton F. Martins (2011) *Clean – Soil, Air,Water* , 39, 28–34.

[9] Amini H. and Ahmadiani A. (2007) *J.Pharm. Biom. Anal.* 43, 1146 – 1150.

[10] Rao T.N., Sarada B.V, Tryk D.A. and Fujishma A. (2002) *J.Electroanal. Chem.* 491, 175-181.

[11] Worapun A.P, Watanakul C., Einaga Y., Grudpan K., Motomizu S. and Chailapakul O. (2006) *Talanta* 68, 1726-1731.

[12] You T.Y, Yang X.R. and Wang E.K. (1998) *Analyst* 123, 2357-2360.

[13] Reeves V.B. (1999) *J.Chromatogr.* B 723, 127-137.

[14] Chiavarino B., Crestonol M.E., Marzion A. and Fornarini S. (1998) *J.Chromatogr.* B 706, 269 – 277.

[15] Assassi N., Tazerouti A. and JCanselier J.P. (2005) *J.Chromatogr.* A 107, 71 -80

[16] Issa M.Y and Amin S.A. *Anal. Lett*. 27, 1147 - 1158.

[17] Nagaraja P., Naik S.D., Shreshta A.K. and Shivakumar A. (2007) *Acta Pharm*. 57, 333-42.

[18] Fan J., Chen Y., Feng S., Ye C. and Wang J. (2003) *Anal. Sci.,* 19, 419.

[19] Whelpton R., Watkins G. and Curry S.H (1981) *Clin. Chem.* 27, 1911.

[20] United States Pharmacopoeia, XXIth revision, National formulary XVIth edn (1985) *US Pharmacopeial Convention: Rockville.*

[21] Kumar K.G. and Indrasenan P. (1998) *Analyst,* 113, 1369.

[22] Voorhies J.D. and Adams R.N. (1958) *Anal. Chem,* 30, 346-350.

[23] Astrid M.V, Maria E., Carrera B., Dietrich V.B. and Carls B. (1984) *Anal. Chem.Acta* 159, 119-127.

[24] Msagati T.A.M and Ngila J.C. (2002) *Talanta* 58, 605–610.

[25] Kotoucek M., Skopalova J., and Michalkova D. (1997*) Anal. Chim. Acta* 353, 61–69.

[26] Abdullin I.F., Chernysheva N.N. and Budnikov G.K. (2002) *J. Anal. Chem.* 57, 629–631.

[27] Ali A.M.M. (1993) *Anal. Lett.* 26, 1635–1647.

[28] Bishop E. and Hussein W. (1984) *Analyst* 109, 913–921.

[29] Diaz T.G., Cabanillas A.G., Valenzuela M.I.A. and Sallinas F. (1996) *Analyst* 121, 547–552.

[30] Sabry S.M. (2007) *Anal. Lett.* 40, 233–256.

[31] Carrazon J.M.P., Recio A.D. and Diez L.M.P. (1992) *Talanta* 39, 631–635.

[32] Granero G., Garnero C. and Longhi M. (2002) *J. Phar. and Bio Analysis,* 29, 51–59

[33] Hassouna M.E.H. (1997) *Analytical Letters,* 30, 13 , 2341 – 2352.

[34] Bedor D.C.G., Goncalves T.M., Ferreira M.L.L., D'Sousa C.E.M., Menezes A.L., Oliveira E.J. and De Santana D.P. (2008) *J. of Chrom B,* 863, 46–54.

[35] Issac S. and Kumar K.G. (2009) *Drug Test Anal.* 1, 2009, 350 – 4.

[36] Andrade L.S., Rocha-Filho R.C., Cass Q.B. and Fatibello-Filho O. (2010) *Anal. Methods,* 2, 402–407

[37] Ozkorucuklu S.P., Sahin Y. and Alsancak G. (2008) *Sensors, 8*, 8463-8478

[38] Cristine D.S., Otoniel C.B., Iolanda C.V. and Almir S. (2008) *Sensors and Actuators* B 135, 66–73.

[39] Wei D. and Ivaska A. (2008) *Anal Chim Acta* 607 126 – 135.

[40] Maleki N., Safavi A. and Tajabadi F. (2006) *Anal Chem* 78, 2006, 3820 – 3826.

[41] Safavi A., Maleki N., Moradlou O. and Sorouri M. (2008) *Electrochem Commun* 10, 420 – 423.

[42] Musameh M.M., Kachoosangi R.T., Xiao L., Russell A. and Compton R.G. (2008) *Biosens Bioelectron* 24, 87 – 92.

[43] Li C.M., Zang J.F., Zhan D.P., Chen W., Sun C.Q., Teo A.L., Chua Y.T., Lee V.S. and Moochhala S.M. (2006) *Electroanalysis* 1, 2006, 8713.

[44] Sun W., Guo C.X., Zhu Z.H. and Li C.M. (2009) *Electrochem Commun* 11, 2105 – 2108.

[45] Guo C.X., Lu Z.S., Lei Y. and Li C.M. (2010) *Electrochem Commun.* 12, 2010, 1237 – 1240.

[46] Xi F.N., Liu L.J., Wu Q. and Lin X.F. (2008) *Biosens Bioelectron* 24,29 – 34.

[47] Xiao F., Ruan C.P., Li J.W., Liu L.H., Zhao F.Q. and Zeng B.Z. (2008) *Electroanalysis* 20, 361 – 366.

[48] Sun W., Gao R.F., Jiao K. (2007) *J Phys Chem* B 111, 4560 – 4567.
[49] Sun W., Li Y.Z., Yang M.X., Liu S.F. and Jiao K. (2008) *Electrochem Commun* 10, 298 – 301.
[50] Sun W., Li Y.Z., Gao H.W. and Jiao K. (2009) *Microchim Acta* 165, 2009, 313 – 317.
[51] Sun W., Li Y.Z., Yang M.X. and Jiao K. (2008) *Sens Actuators B Chem* 133, 387 – 392.
[52] Sun W., Jiang Q., Xi M.Y. and Jiao K. (2009) *Microchim Acta* 166, 343 – 348.
[53] Li Y.H., Liu X.Y., Zeng X.D., Liu Y., Liu X.S., Wei W.Z. and Luo S.L. (2009) *Microchim Acta* 165, 393 – 398.
[54] Wang Y., Xiong H.Y., Zhang X.H. and Wang S.F. (2010) *Microchim Acta* 170, 27.
[55] Raghu K., Chandrasekar A. and Sankaran K.R. (2010) *Inter. J. Chem. Research,* 2, 5 – 16.
[56] Chandrasekar A., Raghu K. and Sankaran K.R. (2011) *J. Bio sci. Res* ., 21, 142 – 159.
[57] Nicholson R.S. (1965) *Anal.Chem*. 37, 1351.
[58] Sabry S.M. (2007) *Anal. Lett*. 40, 233 – 256.
[59] Msagati T.A.M. and Ngila J.C. (2002) *Talanta* 58, 605 – 610.
[60] ICH Guideline (1994) *Validation of Analytical Procedures: Current Step 4 Version*, *Parent guideline* 27th-October.
[61] Miller J.C., Miller J.N. (1993) *Statistics for analytical chemistry Ellis Horwood Series, PTR Prentice Hall, New York, London,* 119–121
[62] Miller J.N. (1991) *Analyst,* 116, 3 – 14.

Table 1- Analytical techniques developed for the Analysis of Sulfamthoxazole in Bulk Drugs and /or commercial products

Method	Detector	Linearity Range μmol L^{-1} or M or μg/mL or ppm)	LOD (mol L^{-1})	LOQ (mol L^{-1})	Reference
UV	Second order derivative	1.60 – 16.5 μg/mL	-	-	20
Spectrophotometric	UV	4.0 – 20.0 μg/mL	-	-	32
HPLC	FIA - AMP	0.050 – 100 ppm	0.12 ppm	0.040 ppm	11
HPLC	UV	0.39 – 50 μg/mL	-	0.39 μg/mL	9
SPE-LC-MS/MS	MS	0.5 – 60 μg/mL	-	1250 pg	33
SPE-LC-UV	UV	0.5 – 60 μg/mL	-	7500 pg	33
CE	EC	0.13 – 100 μM	0.1 μM	-	34
Voltammetric	MWCNT/GCE	1.0×10^{-2} – 5.0×10^{-5} M	1.0×10^{-5}		35
DPV	HT-BDD	1.0 - 8.0 mg L^{-1}	6.51×10^{-8} M	21.0×10^{-8} M	36
DPV	PGE	0.75×10^{-3} – 2.5×10^{-7} M & 2.0×10^{-3} – 0.75×10^{-3} M	3.59×10^{-7} M	1.20×10^{-6} M	37
DPV	CMCPE	1.0×10^{-2} – 1.0×10^{-8} M	1.5×10^{-9} M	-	38
SWV	BDD	6.10×10^{-6} – 6.01×10^{-5} M	1.15×10^{-6} M	-6.5×10^{-8} M	39
SWSV	ILCE				Present

HPLC – High performance liquid chromatography, CE - Capillary electrophoresis, FIA - AMP- Flow-injection analysis - amperometric, UV-Ultraviolet spectrophotometry, SWSV - Square wave stripping voltammetry, SWV - Square wave voltammetry, DPV – Differential pulse voltammetry, SPE-LC-MS – Solid phase extraction-liquid chromatography-mass spectrometry, SPE-LC-UV - Solid phase extraction-liquid chromatography-ultraviolet spectrometry, EC – End-Column, HT-BDD – Hydrogen terminated boron doped diamond electrode, BDD- Boron doped diamond electrode, PGE – Pencil graphite electrode, CMCPE – Chemically modified carbon paste electrode, MWCNT/GCE – Multi wall carbon nano tube / Glassy carbon electrode, ILCE – Ionic liquid carbon paste electrode, MS – Mass spectrometry

Table 2 Optimum experimental conditions in SWSV

S.No	Variable	Optimized condition
1	pH	9
2	Buffer (mL)	10
3	Temperature	24±2°C
4	Purge time (s)	300
5	Accumulation Potential (mV)	300
6	Accumulation Time (s)	60
7	Square wave amplitude (mV)	50
8	Square wave frequency (Hz)	75
9	Scan Increment (mV)	12

Table-3 Validation experimental paramaters

No.	Parameter	Range studied	Optimum range
1	pH	9	9
2	Accumulation potential (mV)	-400- 400	300
3	Accumulation time (sec)	15 - 90	60
4	Square wave amplititude (mV)	25 - 200	50
5	Frequency (Hz)	15 - 105	75
6	Scan increment (mV)	2 - 20	12
7	Scan rate (mV/sec)	10 - 80	40
8	Stirring rate (RPM)	50 - 300	300
9	Rest period (sec)	2 - 10	5

Table 4- Recovery test of Sulfamethoxazole

Added in (M)	Found in (M)	Recovery (%)
2.00×10^{-6}	1.95×10^{-6}	97.50
4.00×10^{-6}	4.04×10^{-6}	101.00
6.00×10^{-6}	5.94×10^{-6}	99.00
8.00×10^{-6}	7.96×10^{-6}	101.30
9.00×10^{-6}	8.92×10^{-6}	99.10
1.00×10^{-5}	0.98×10^{-5}	98.00
2.00×10^{-5}	2.06×10^{-5}	101.50
3.00×10^{-5}	2.92×10^{-5}	98.00
4.00×10^{-5}	3.97×10^{-5}	99.25
6.00×10^{-5}	5.94×10^{-5}	99.00
8.00×10^{-5}	7.96×10^{-5}	99.50
1.00×10^{-4}	0.98×10^{-5}	98.00

Table 5- Analytical precision and accuracy of Sulfamethoxazole determination by the proposed SWSV method

Added / µg / ml	Intra-day			Inter-day		
	Found / µg / ml	Precision[a]	Accuracy[b] (% of Relative error)	Found / µg / ml	Precision[a]	Accuracy[b] (% of Relative error)
0.43	0.42	X=0.42±0.005 µg / ml s = 0.011 µg / ml RSD 2.89%	-2.33	0.42	X=0.42±0.004 µg / ml s = 0.008 µg / ml RSD1.97%	-2.33
3.40	3.39	X=3.39±0.022 µg / ml s = 0.042 µg / ml RSD 1.24%	-0.29	3.30	X=3.30±0.023 µg / ml s = 0.047 µg / ml RSD 1.42%	-2.94
5.08	5.03	X=5.03±0.013 µg / ml s = 0.028 µg / ml RSD 0.55%	-0.98	5.10	X=5.10±0.024 µg / ml s = 0.041 µg / ml RSD 0.81%	0.39
9.13	9.11	X=9.11±0.023 µg / ml s = 0.047 µg / ml RSD 0.51%	-0.22	9.09	X=9.09±0.032 µg / ml s = 0.0.77 µg / ml RSD 0.84%	-0.44

a X = Mean ± standard error, s – Standard deviation, RSD – Relative standard deviation
b Accuracy = [(found-added)/added] x 100

Table 6- Determination of sulfamthoxazole in Pharmaceutical Formulation by the proposed and reference methods

Pharmaceutical product	Labeled amount mg / Tablet	SWSV			Reference method HPLC		
		Found (mg / tablet)	Recovery (%)	RSD (%)	Found (mg / tablet)	Recovery (%)	RSD (%)
Brand – A	400	414.51 ± 2.788	103.63	0.673	404.14 ± 1.708	101.03	0.423
Brand – B	400	413.78 ± 1.666	103.45	0.403	404.97 ± 2.654	101.24	0.655
Brand – C	400	394.65 ± 1.550	98.66	0.393	392.39 ± 2.331	98.29	0.594
Brand – D	800	827.73 ± 1.973	103.47	0.238	791.03 ± 2.661	98.88	0.336
Brand – E	800	809.64 ± 2.437	101.2	0.301	809.97 ± 2.177	101.25	0.269

mean ± standard deviation (n = 6)
RSD - relative standard deviation

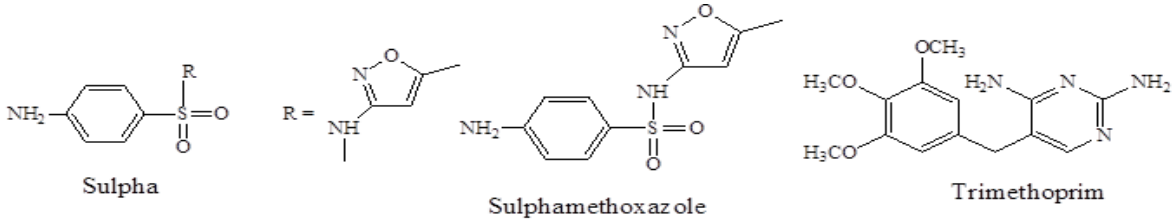

Fig- 1 Chemical structure of Sulphamethoxazole and Trimethoprim

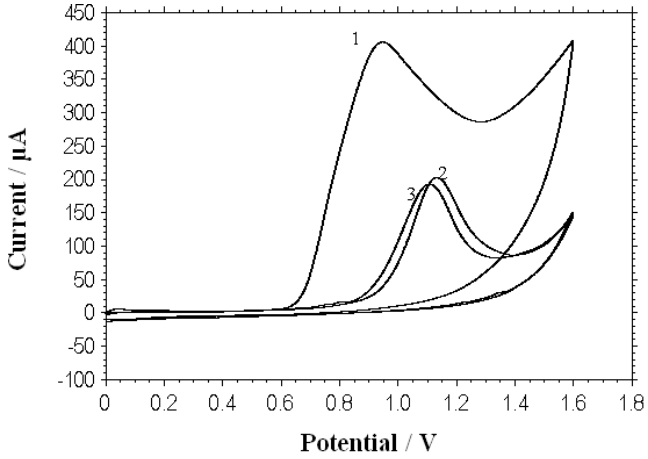

Fig- 2: Cyclic voltammogram of 1x10^{-3} mol L^{-1} Sulphamethoxazole in 1). 0.04mol L^{-1} Britton-Robinson buffer, 2) 0.05 mol L^{-1} phosphate buffer and 3) 0.1 and 0.5 mol L^{-1} sulfuric acid

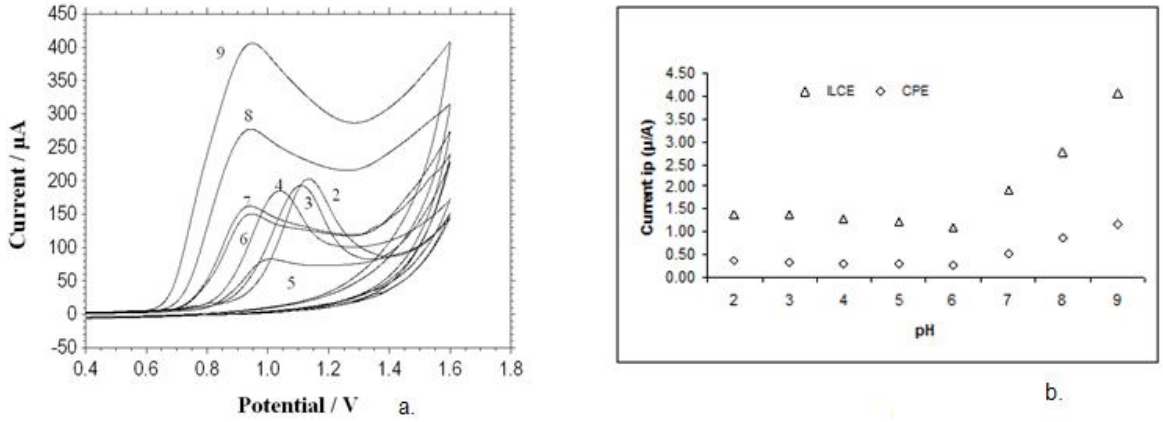

Fig- 3

a. Cyclic voltammograms of 1 x 10^{-3} molL^{-1} sulphamethoxazole in BR buffer at various pH ranges from 2 – 9 at the electrode surface of ILCE.
b. Plot of Current Vs pH for 1x10^{-3} mol L^{-1} sulphamethoxazole solutions in 0.04mol L^{-1} Britton-Robinson buffer at the surface of ILCE and CPE

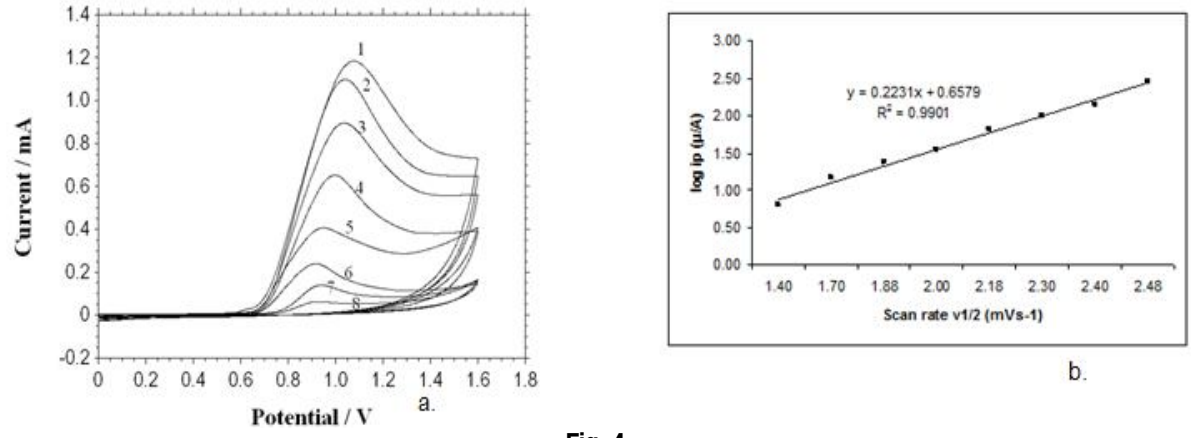

Fig- 4

a. Cyclic voltammograms of 300 µg mL^{-1} sulphamethoxazole in BR buffer at 9 at the electrode surface of ILCE with the scan rate of 1) 300 2) 250 3) 200 4) 150 5) 100 6) 75 7) 50 & 8) 25 meV/s
b. Plot of logarithm of peak current vs. logarithm of scan rate for 300 µg mL^{-1} sulphamethoxazole solutions in 0.04mol L^{-1} Britton-Robinson buffer at pH-9 & the electrode surface of ILCE

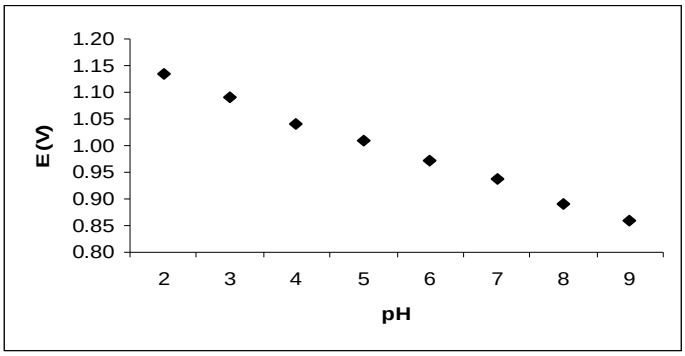

Fig- 5 Plot of peak potential vs. pH with the scan rate of 100 meV for 300 µg mL^{-1} sulphamethoxazole solutions in 0.04mol L^{-1} Britton-Robinson buffer at pH 2 - 9 & the electrode surface of ILCE

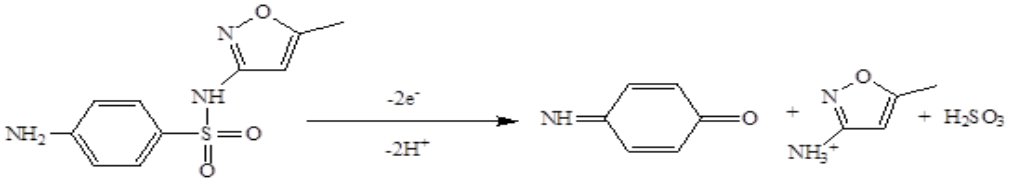

Figure -6: Proposed reaction mechanism of sulphamethoxazole at electrode surface

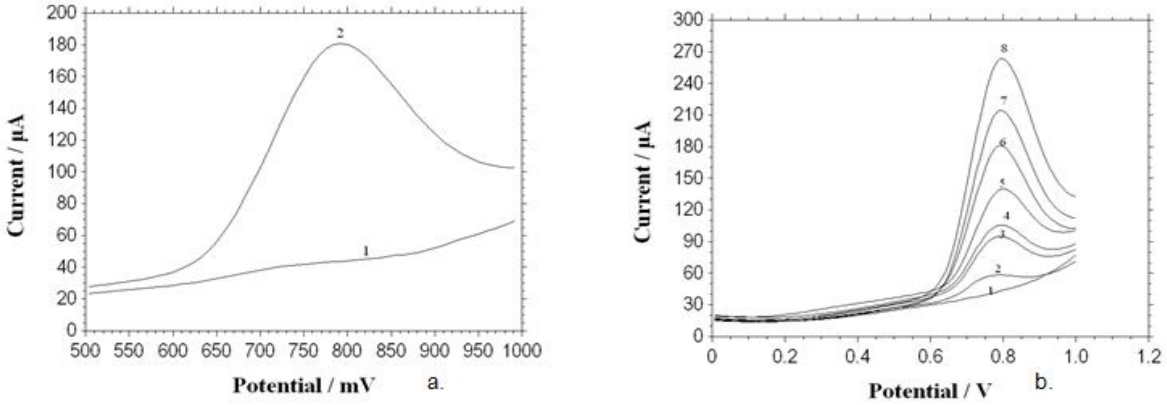

Fig-7

a. Square wave stripping voltammogram of Sulphamethoxzole at ILCE as a function of concentration of the drug: (1).blank; (2). $2x10^{-5}$ mol L^{-1} in 0.04mol L^{-1} BR buffer at pH - 9

b. Square wave stripping voltammogram for the determination of Sulphamethoxzole at ILCE as a function of concentration of the drug: (1).blank; (2). $2x10^{-8}$; (3). $4x10^{-8}$; (4). $2x10^{-7}$; (5). $5x10^{-7;}$ (6).$3x10^{-6;}$ (7). $6x10^{-6;}$ & (8). $3x10^{-5}$ mol L^{-1} in 0.04mol L^{-1} BR buffer at pH - 9