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Electrochemical analysis of sparfloxacin in pharmaceutical formulation and biochemical screening of its Co(II) complex

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Abstract

Simple and sensitive, direct current polarographic (DCP) and differential pulse polarographic (DPP) methods have been developed for the qualitative as well as quantitative analysis of 5-amino-1-cyclopropyl-7 (*cis*-3,5 dimethyl-1-piperazinyl)-6,8-difluoro–1,4-dihydro-4-oxo-3-quinoline carboxylic acid (sparfloxacin). The developed methods have been standardized for the determination of the drug in pharmaceutical formulation for quality control purposes. The observed data has been subjected to statistical analysis, which revealed high reliability and precision. Sparfloxacin forms a complex with Co(II) which has been characterized on the basis of elemental analysis, IR spectral, polarographic and amperometric analysis. The analytical results indicated a 1:1 (M:L) stoichiometry for the Co(II)–sparfloxacin complex. The antibacterial studies on the drug and its complex were carried out against various pathogenic bacteria. The results revealed that the complex is more potent compared to the pure drug. © 2002 Published by Elsevier Science B.V.

Keywords: Sparfloxacin; Co(II)–sparfloxacin complex; Polarography; Direct current polarographic; Differential pulse polarographic; Pharmaceutical formulation

1. Introduction

Quinolones have been found to possess antibiotic property. Sparfloxacin (5-amino-1-cyclopropyl-7 (*cis*-3,5 dimethyl-1-piperazinyl)-6,8-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid) is a new addition to the class of fluroquinolones which is, used in the treatmennt of cuttaneous allergy, lung infection and urinary tract infection [1].

Although the literature reports the determination of sparfloxacin using various instrumental methods like HPLC, spectrophotometry, colorimetry, etc. [2–5], but polarographic methods which are far better than the existing methods in the field as regards to their extra ordinary detection sensitivity, oligo determination capability, minimum detection limit, low cost, rapidity, accuracy, simplicity and non-destructive nature, have not been used for the said purpose.

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Several trace metals such as Fe, Co, Zn, Cd and Ni are essential for health and for the maintenance of human biological system. The complex of the life essential metals play significant role in biological system, in which enzymes are know to be activated by metals ion [6]. The advantage of complexes of Ni, Zn, Co, etc. with different types of drugs have been discussed by a numbers of workers [7–10]. In continuation to the work reported from our laboratory on the study of electrochemical, bioinorganic and antibacterial behaviour of some drugs and metal–drug complexes [11–14], the authors have standardized direct current polarographic (DCP) and differential pulse polarographic (DPP) methods for the analysis of sparfloxacin and used the developed procedure for the analysis of sparfloxacin in a pharmaceutical formulation, i.e. a sparx-100 tablet.

The characterization of the Co(II)–sparfloxacin complex has been carried out using polarographic and spectral methods. Antibacterial studies on the drug and its complex have also been carried out. The results of which have been reported in the paper.

2. Experimental

2.1. Apparatus

All the polarograms/voltammograms were recorded on an Elico (India) DC polarograph, model CL-357 and an Elico (India) pulse polarograph model CL-90, respectively, coupled with an x–y polarocard model LR-101 and LR-108P.

The polarographic cell consisted of an electrode assembly having a dropping mercury electrode (working electrode), a saturated calomel electrode (reference electrode), and a coiled platinum wire (auxiliary electrode). A Systronics digital pH meter-335 was used for the pH measurements. The amperometric titrations were performed on a manually operated set up, equipped with a polyflex galvanometer (sensitivity 8.1×10^{-9} amp./div.) and an AJCO vernier potentiometer. The capillary characteristic of the DME had a $m^{2/3}$, $t^{1/6}$ value of 2.5 $mg^{2/3}$ $s^{-1/2}$ at 60 cm effective height of mercury column. The IR spectrum of solid complex was recorded using KBr pellets on a Shimadzu, Japan. Model 470, IR spectrophotometer.

2.2. Chemicals and reagent

The chemicals used were of AnalaR/BDH grade. Free gift sparfloxacin ($C_{19}H_{22}F_2N_4O_3$) sample was supplied to us by Ranbaxy, Laboratories Gurgaon, India. Doubly distilled water and absolute ethanol (55:45 v/v) was used as solvent. A sparfloxacin solution (2.5 mM) was prepared by dissolving the requisite amount in (55:45 v/v) distilled water:ethanol. Stock solutions of 1 M potassium chloride and ammonium tartrate were prepared by dissolving a requisite quantity of each compound in distilled water. pH adjustments were made using dilute solutions of HCl, NaOH whenever necessary. The test solution was deaerated by bubbling purified hydrogen gas for 10 min before recording the polarogaram/voltammogram.

2.3. Determination of sparfloxacin

Electrochemical behaviour of sparfloxacin has been studied using DCP and DPP [15,16]. For polarographic studies, experimental sets were prepared, taking 10 ml of 1 M ammonium tartrate and different concentrations of sparfloxacin in the polarographic cell. The total volume of the analytes was made to 100 ml. The pH of test solution was adjusted to 6.0 and the polarogram/voltamogram was recorded as earlier. The analysis of sparfloxacin in a pharmaceutical formulation, i.e. sparx-100 [tablet] has been done following similar experimental conditions.

For the analysis of sparfloxacin in the pharmaceutical formulation, i.e. sparx-100 (Tablet) was dissolve in (55:45 v/v) water: ethanol. The solution was then transferred into polarograhic cell containing 10 ml of 1 M ammonium tartrate and the volume was made up to 100 ml. The pH of test solution adjusted to 6.0 and the polarogram/voltamogram was recorded as earlier.

2.4. Preparation of complex

For the analysis of metal:ligand (M:L) complexation equilibrium experimental sets were prepared by keeping Co(II) and potassium chloride (supporting electrolyte) concentration fixed at 1.0 mM and 0.1 M, respectively, and varying the concentration of ligand. The volume was made to 100 ml and the pH of each set was adjusted to 5.0. The test solution was deaerated for 15 min, before recording the polarogram/voltammogram.

2.5. Amperometric titration

Experimental sets, each having different but known amount of Co(II) were prepared in appropriate quantity of supporting electrolyte (potassium chloride) and pH was adjusted to 5.0 and titrated separately against the standard solution of the titled sparfloxacin whose pH was also adjusted to that of the titrate (5.0) at -1.50 V SCE, (the plateau potential of Co(II)). The current after each addition of the titrant was read and a curve was plotted between current against volume of titrant added.

2.6. Synthesis procedure of solid complex

Cobalt(II) chloride and sparfloxacin solutions were prepared separately in distilled water and ethyl alcohol (55:45 v/v) and were mixed in 1:1 molar ratio. The mixture was then refluxed in a round bottom flask for 2 h. The residue (complex) was filtered and washed thoroughly to remove any unreacted material. The complex was dried at low temperature and stored over P_4O_{10}.

The results of elemental, C, H and N analysis on the drug and its Co(II)–sparfloxacin complex were furnished by the CDRI, Lucknow, India. The complexometric method was used for the estimation of cobalt in the complex [17].

2.7. Biological activity of sparfloxacin and Co (II)–sparfloxacin complex

Biochemical appilications have greater demand nowadays. The activity of bacteria on several compounds give more important information about the complex. Therefore, this prompted us to screen the complex and precursors to find out which part of the molecule is actually responsible for its physiological activity.

2.8. Antibacterial studies

Raper's paper disc method [18,19] was followed for the antibacterial screening of the compounds (0.5 mM conc.) against some pathogenic bacteria, viz. *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtalis* and *Escheria coli*. The numbers of replicates in each case were three. The percentage inhibition was calculated.

3. Results and discussion

3.1. Electrochemical analysis of sparfloxacin

Under the above mentioned experimental condition sparfloxacin are easily reducible at DME surface. In 0.1 M ammonium tartrate (pH 6.0), sparfloxacin produces a well defined polarographic wave/peak with $E_{1/2}/E_p = -1.45/1.49$ V versus SCE. The wave height was found to be proportional to the concentration of sparfloxacin (Fig. 1). The results of the polarographic analysis of the sparx-100 tablet showed the presence of 99.95 mg of sparfloxacin per tablet. Quantitative analysis of the sample has been done by wave height and the statistical analysis of the results by external spiking method. The standardized method was found to be accurate for the determination of these compounds in pharmaceutical formulations.

3.2. Polarographic study of M:L complexation equilibrium

The Co(II) and its complex with sparfloxacin ligand were found to be reversibly reduced in 0.1 M KCl at pH 5.0 involving two electrons which was evidenced by the linear plots of $\log i/(i_d - i)$ versus E. The reduction was found to be diffusion controlled, which was evidenced by plot of i_d versus $\sqrt{h}$ corr. On gradual increase of sparfloxacin concentration the half wave potential of

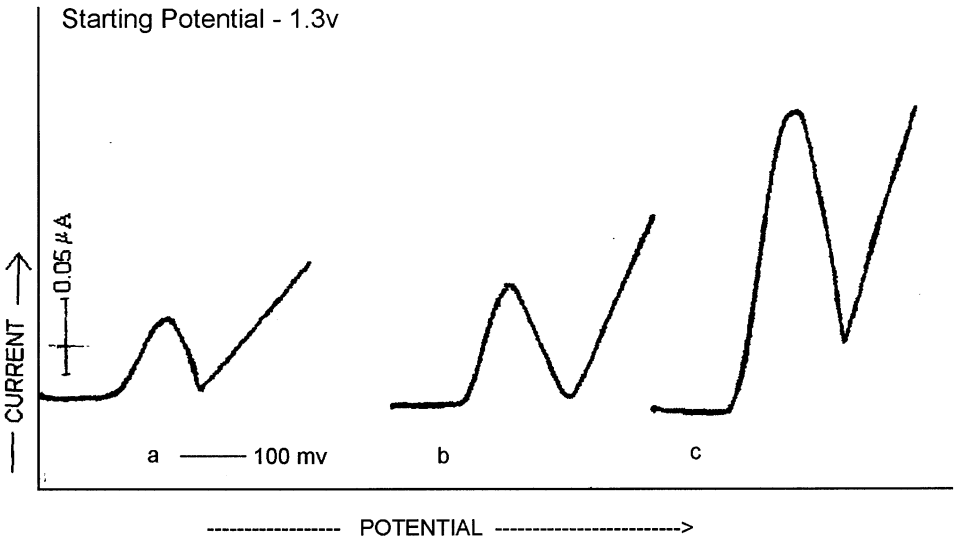

Fig. 1. Differential Pulse Polarogram of sparfloxacin in 0.1 M ammonium tartrate at pH 5.0 ± 0.1: (a) 0.125 mM; (b) 0.250 mM; (c) 0.50 mM.

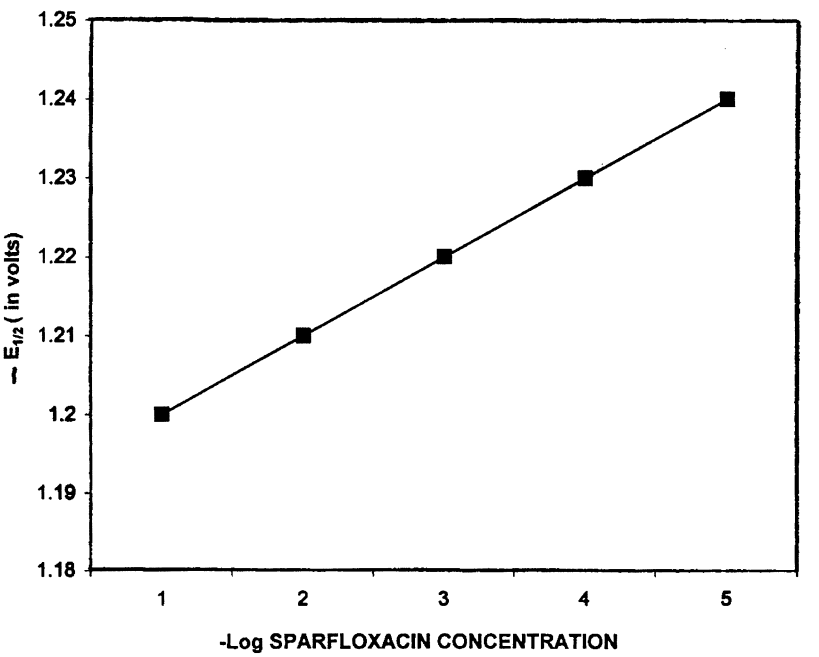

Fig. 2. Plot of $-E_{1/2}$ vs. log C_x for Co(II) with the sparfloxacin complex.

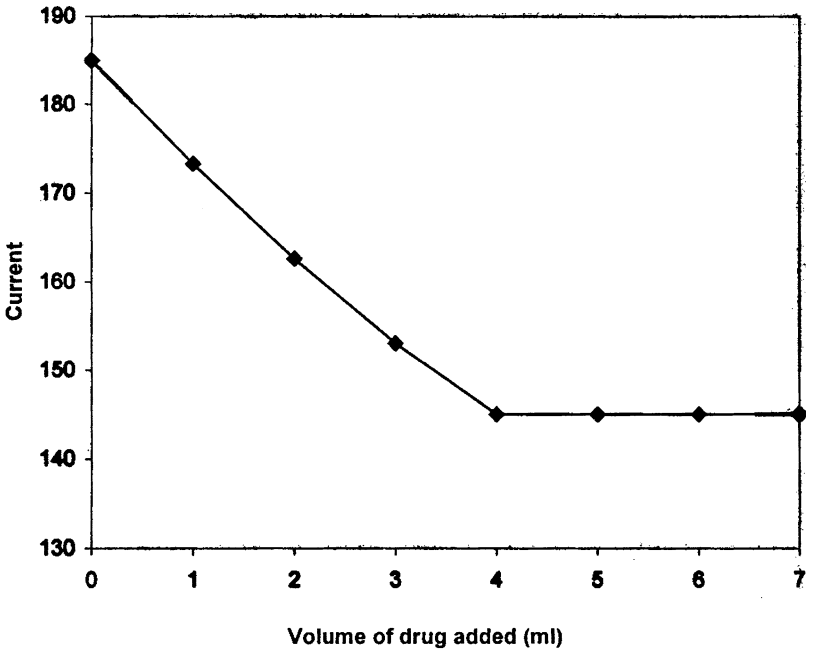

Fig. 3. Amperometric titration OF 10 μm/50 ml analyte Co(II) with 2.5 μm/ml sparfloxacin in 0.1 M KCl.

Table 1
Analytical data of sparfloxacin and Co(II)–sparfloxacin complex

Compound	Found (calculated) (%)					
	M(Co)	C	H	N	O	F
Sparfloxacin	–	58.1 (58.5)	5.65 (5.68)	9.69 (9.7)	14.28 (14.35)	12.23 (12.23)
Complex	13.00 (13.04)	50.6 (50.0)	4.91 (4.94)	8.42 (8.45)	10.6 (10.63)	12.48 (12.51)

Co(II) metal ion shifted to more electronegative value and the diffusion current also decreased, there by showing complex formation between Co(II) with sparfloxacin.

The composition and formation constant of the complex was studied by the plots of $\Delta E_{1/2}$ (shift in the $E_{1/2}) = (E_{1/2})_c - (E_{1/2})_s$ against log C_x (logarithm of the concentration of the ligand). The plots were linear showing the formation of single complex species in solution (Fig. 2). Lingane [20] method was therefore applied. It showed 1:1 (M:L) complex formation with formation constant log $\beta_1 = 4.0$.

The analyte was found to be fairly stable, as it is clear from the reproducibility of the polarogram. The presented data has been compared with that observed using spectrophotometric method.

3.3. Amperometric determination of sparfloxacin with Co(II)

Co(II) gives a well defined polarographic wave in 0.1 M KCl at pH 5.0. The diffusion current was found proportional to its concentration. The sparfloxacin does not produce any wave under said experimental conditions.

The plateau potential for the polarographic wave of Co(II) -1.4 V versus SCE, was applied on the potentiometer for carrying out amperometric titration. Co(II) was taken as the titrate and

the drug solution was taken as titrant. The current volume plots resulted in an L-shaped curve (Fig. 3). The end point as located by the graphical method revealed a metal-to-drug ratio of 1:1, which is in agreement with the author's observations on the metal:ligand complexation equilibrium using the polarographic method.

3.4. Characterization of Co(II)–sparfloxacin complex

The colour of the complex is dark blue. It is highly soluble in water. The results of elemental analysis on the drug and its complex with Co(II) are depicted in Table 1. The table revealed a 1:1 metal–drug ratio in the complex, which supports authors findings using polarographic and amperometric methods.

Table 2
Principal IR signals (cm^{-1}) and their assignments for sparfloxacin and its Co(II) complex

Sparfloxacin	Assignments	Co(II)–sparfloxacin complex
920	N–H wagging vibration	920
1025	Region of C–F absorption	1025
1435	C–N ring stretching	1435
1630	C=C stretching in ring six member vibration	1630
1710	C=O stretching cyclic (COOH)	1720
3300–3400	Symmetrical N–H stretching	3150–3400

Table 3
Antibacterial screening data of sparfloxacin and its Co(II)–sparfloxacin complex

Bacterial species	Zone of inhibition		% Inhibition
	Drug(a)*	Complex(b)*	
Staphylococcus aureus	15	18	−20.6
Salmonella typhi	17	16	−17.6
Escherchia coli	14	18	−28.5
Bacillus subtilis	16	18	−12.5

3.4.1. IR spectra

Some structurally important features of the IR spectra of the drug and its complex, which are particularly important in assigning the position of metal–ligand bonding are tabulated in Table 2. A critical comparison of the IR spectra of sparfloxacin and its Co(II) complex shows a band at 1710 cm^{-1} due to the C=O stretching vibration and a doublet between 3300 and 3450 cm^{-1} due the N–H stretching vibration [21,22] in the IR spectrum of the sparfloxacin, which appears as a reduced band at 1720 cm^{-1} and a broad band at 3150–3400 cm^{-1} in the IR spectrum of Co(II)–sparfloxacin complex. These observations clearly indicate the involvement of oxygen of carboxylic group and nitrogen of N–H group in complex formation.

3.5. Biological studies

3.5.1. Antibacterial studies

Sparfloxacin is a broad spectrum antibiotic and very effective against a number of bacteria. A study of the results depicted in Table 3 indicates that the complex of Co(II) with sparfloxacin exhibit increased activity against pathogenic bacteria as compared to sparfloxacin drug. The complex of Co(II)–sparfloxacin shows variable response with the test pathogens. Among the test pathogens under study it shows a maximum inhibition of 28.5% over the control drug against *Escherchia coli*.

4. Conclusion

The standardized methods were found be highly accurate for the determination of sparfloxacin in pharmaceutical formulation. Quantitative analysis of the sample was carried out by wave height and external spiking methods.

The estimation of sparfloxacin in pharmaceutical formulation by polarographic method and

Co(II)–sparfloxacin complex has not been reported in literature.

Looking at the sensitivity, accuracy, simplicity, rapidity and reliability of the results, the polarographic/amperometric methods could be used successfully for the qualitative and quantitative analysis of sparfloxacin in pharmaceutical formulation and may be recommended for its use for the quality control purpose in drug industry. The final analysis results have been compared with those claimed by the manufacturer. Besides, looking at the increased potency of Co(II)–sparfloxacin complex, it may be recommended to the therapeutic experts to ascertain its possible use as a more potent antibiotic drug in lieu of sparfloxacin.

References

[1] J. Martindale, The Extra Pharmacopoeia, 29th edn., The Pharmaceutical Press, London, 1989.
[2] K. Bhavani, C.M.R. Srivastava, Eastern Pharmacist 456 (1997) 161–162.
[3] C. Tekchandani, Indian Drugs 35 (1998) 229–233.
[4] S.N. Meyyanathan, S.N. Sebastian, B. Suresh, Eastern Pharmacist 11 (1998) 129–130.
[5] A.V. Mithai, A.V. Tipre, A.V. Kasture, Indian Drugs 35 (1998) 239–240.
[6] A. Sigel, H. Sigel, Metal Ions in Biological Systems, Marcel Dekker, New York, 1971–1997.
[7] A.B. Khare, R.K. Goutam, O.P. Shrivastava, J. Indian Chem. Soc. 63 (1986) 660.
[8] A. Mastrolorenzo, C.T. Supuran, Metal-Based Drugs 7 (1) (2000) 49–54.
[9] M.S. Nair, M.A. Neelkantan, J. Indian Chem. Soc. 77 (2000) 373–375.
[10] G.N. Mukherjee, T.K. Ghosh, Indian J. Chem. 30A (1991) 1033–1037.
[11] S. Narad, N.N. Mishra, P. Pandey, A. Kumar, K.S. Pitre, Indian J. Physiol. Pharmacol. 39 (1995) 166.
[12] J. Shukla, S. Pitre, Indian J. Exp. Biol. 37 (1999) 429.
[13] A.V. Trivedi, K.S. Pitre, Indian J. Exp. Biol. 36 (1998) 1125.
[14] R. Das, K.S. Pitre, J. Indian Chem. Soc. 78 (2001) 257–258.
[15] L. Mathew, M.L.P. Reddy, T.P. Rao, C.S.P. Iyer, Indian J. Chem. 37A (1998) 1027.
[16] I. Siddiqui, K.S. Pitre, J. Pharm. Biomed. Anal. 26 (2001) 1009.
[17] J. Basset, R.C. Denny, G.H. Jeffery, J. Medham, Vogel's Text Book of Quantitative Analysis, ELBS IV, London, 1978, p. 324.
[18] K.B. Raper's, D.F. Alexander, R.P. Coghill, J. Bacterial. 48 (1944) 693.
[19] R. Kumar, S. Giri, Nizamuddin, J. Indian Chem. Soc., 65, (1988) 571.
[20] J.J. Lingane, Chem. Rev. 1 (1941) 29.
[21] R. Dyer, Application of Absorption Spectroscopy of Organic Compounds, Practice/Hall of India Pvt. Ltd, New Delhi, 1974.
[22] K. Nakamoto, IR and Raman Spectra of Inorganic and Co-ordination Compounds, vol. III, Interscience, New York, 1977.