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International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol. 3, No.3, pp 1164-1171, July-Sept 2011

Synthesis, Electrochemical and Antimicrobial Studies of 2-Phenylazo-1-naphthol-4-sulphonic acid

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Abstract : 2-Phenylazo-1-naphthol-4-sulphonicacid (PANS) was synthesized and characterized by IR, H^1NMRand C^{13} NMR spectral techniques. The electrochemical behaviour of PANS was studied using cyclic voltammetry and constant current electrolysis. The effect of scan rate and pH on the reduction peaks has been studied. The voltammogram recorded at glassy carbon electrode (GCE) in acidic, neutral and basic media at different scan rates exhibit one or two cathodic peaks depending on the pH of the medium. The kinetic parameters were also calculated and the process was found to be diffusion controlled. The reaction mechanism for the reduction is proposed on the basis of obtained voltammograms and constant current electrolysis data. The antibacterial activity of PANS was tested in vitro against a number of microorganisms.

Key words : 2-Phenylazo-1-naphthol-4-sulphonicacid,Cyclic voltammetry, Constant current electrolysis, Glassy carbon electrode, Antimicrobial activity.

INTRODUCTION

Azo compounds are among the most profoundly explored classes of organic compounds both from theoretical and practical viewpoints. These are the largest group of organic dyes for their widespread applications in many areas of dye-stuff industry, pharmacy and dosimetry due to the presence of azo (-N=N-) linkage [1-3]. Azo dyes have wide interest of application in complexometric titration and in analytical chemistry [4-6]. These compounds are also used in waxes, plastics, oils and polishes [7]. Although some azo dyes have been reported to be toxic, dozens of additional monoazo dyes are permitted in chemotherapeutic drugs and cosmetics [8-10]. In addition, azo compounds and their bioconjugates have attracted clinical interest related to phototherapy and photodiagnosis of tumers and their lesions [11]. They are also of great importance as intermediary products in organic synthesis and as initiators in polymerchemistry [12]. The existence of an azo moiety in different types of compounds has caused them to show antibacterial and pesticidal activity [13.]

In the present work synthesis, characterization, electrochemical and antimicrobial behavior of 2-phenylazo-1-naphthol-4-sulphonicacid (PANS) has been carried out.

EXPERIMENTAL

All chemicals used in the present investigation were of analytical grade. 2-naphthol and dimethylformamide were purchased from Sigma-Aldrich.

The purity of the synthesized compounds was checked by TLC. The melting point was determined in open capillary tubes using Prefit model. The molecular weight was determined by cryoscopic method using glacial acetic acid as solvent. In the present investigation the IR spectra of azo compound was recorded on Schimadzu FTIR spectrophotometer model 8400S in KBr wafer and the NMR spectra was obtained on JEOL AL 300, 300.4 MHz FT NMR spectrometer using $CDCl_3$ as solvent and reported relative to TMS as internal standard.

Synthesis of PANS

Freshly distilled aniline (0.016 M) was dissolved in aqueous acidic medium to get a clear solution. This solution was cooled to 0-5˚C and diazotized with dropwise addition of sodium nitrite solution maintaining the temperature below 5˚C. The diazonium chloride was coupled with an alkaline solution of 4-hydroxy-1-naphthalenesulphonic acid (0.016 mol in 10 % NaOH) and the resulting mixture was stirred at 0-5˚C for additional 60 minutes. The crude was filtered with the help of a suction pump and washed with cold water for several times and recrystallized with methanol-water mixture 60:40 (v/v) to yield shiny red-orange crystals. Yield 72%, M.P. 300ºC.

Cyclic Voltammetry

The completely computer controlled Basic Electrochemistry System model ECDA-001 was used for recording cyclic voltammograms of PANS. Cyclic voltammetric studies were carried out using a glassy carbon working electrode (A = 0.1 mm2) , Ag/AgCl reference electrode and a platinum auxiliary electrode.

1 mM solution of PANS in DMF was used to record the voltammograms. The voltammogram were recorded with an initial potential (Ei) 1200mV and final potential (Es) -1200mV employing scan rates between 50 mv/s to 500 mv/s and at various pH 3.0, 5.0, 7.0 and 9.0. Britton – Robinson buffer was used to maintain the pH of examined solutions which were prepared in doubly distilled water.

The working electrode was polished intensively with aluminium oxide (0.4 μ) on a polishing cloth and degreased in methanol prior to each electrochemical measurement. The solutions were purged with purified clean dry nitrogen for 5 min prior to the experiments in order to remove dissolved oxygen from the media and blanketed thereafter.

Constant current electrolysis

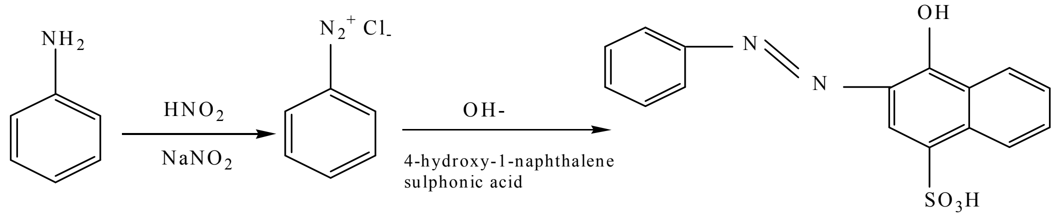
PANS was subjected to constant current electrolysis at constant current at 1.0 amp. for 24 hrs in aqueous dimethylformamide. Potentiostat / Galvanostat supplied by OMEGA type ICVD 60/2 was used to perform the experiment. A Remi hot plate cum magnetic stirrer (2MLH model) was used to stir the solution throughout the electrolysis. A two compartment H- shaped glass cell provided with a fritz glass disc (G-4) was used for electrolysis and rectangular plates of stainless steel (SS-316) each of size (4 cm × 4 cm) was used as cathode as well as anode. The volume of catholyte was reduced by distilling it at reduced pressure and the products obtained after the repetitive extraction with diethyl ether were purified. The products thus obtained were characterized by chromatographic and spectroscopic techniques.

Antimicrobial activity

The synthesized azo compound was screened for the presence of antibacterial constituents against six strains of bacteria i.e. *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa, Enterobacter cloacae*, *Enterococcus faecalis* and one species of fungi i.e. against *Candida albicans* by disc diffusion method. All compounds were dissolved in DMF. Ciprofloxacin (5 mcg/disc for bacteria) and ketoconozole (100 units/disc for fungi) was used as reference antibiotic and DMF as control. The zones of inhibition were determined at the end of an incubation period of 24 hr at 37˚ C. During this period, the test solution diffused and the growth of inoculated microorganism was affected.

RESULTS AND DISCUSSION

In this study, PANS was synthesized according to the scheme (1)



(Scheme 1 Preparative route of PANS)

Spectroscopic characterization

Infrared spectra: In the IR spectra of PANS data were observed due to the following stretching vibrations : 3320 cm^{-1} ($\nu_{O\text{-}H}$), 2820 cm^{-1} ($\nu_{N\text{-}H}$), 1610-1490 cm^{-1} ($\nu_{C=C}$), 1465 cm^{-1} ($\nu_{N=N}$), 1330 cm^{-1} ($\nu_{C\text{-}N}$), 1250 cm^{-1} ($\nu_{S\text{-}O\ asym}$), 1090 cm^{-1} ($\nu_{S\text{-}O\ symm}$), 1150 cm^{-1} ($\nu_{C\text{-}O}$)and 720 cm^{-1} ($\nu_{C\text{-}S}$). Appearance of -N-H stretching vibration in the IR spectrum of PANS can be attributed to –O-H···N tautomeric shift and intramolecular hydrogen bonding [14,15] in the compounds under investigation.

NMR Spectra: : H^1 NMR spectral data of PANS showed two singlets at 12.41 and 3.60 ppm due to one naphthyl O-H proton and one O-H proton of sulphonic acid group respectively. While the aromatic hydrogen peaks resonated as multiplates at 7.29-8.50 ppm.

In examining the ^{13}C NMR spectrum of HPAN, signals for all aromatic carbon atoms were observed in the range 115-145.8 ppm .

Cyclic voltammetry

The electrochemical reduction of PANS takes place in two stages in acidic medium and two irreversible cathodic peaks were obtained in cyclic voltammogram (**fig** 1).The reduction peak corresponds to the formation of a hydrazo compound via 2e$^-$ / 2H$^+$ process and this hydrazo compound reduces in next step to the o-aniline and 3-amino-4-hydroxy-1-naphthalenesulphonic acid via 2e$^-$ / 2H$^+$ process. The whole process involves 4e$^-$ / 4H$^+$

In neutral and basic media reduction takes place in one step and one irreversible cathodic wave was observed in cyclic voltammogram (fig 2). The electrochemical reduction leads to the formation of stable hydrazo compound via 2e$^-$ / 2H$^+$ electrode process [16].

Kinetic parameters evaluated from cyclic voltammograms are given in Table 1. To determine α.n$_a$ values the following expression was used [17, 18]

$$Ep_{1/2} = [\ Ep \pm 0.048/\ \alpha.n_a]$$

Where α is reversibility criterion and n is number of electrons.

The influence of pH on reduction process was examined. As can be seen in fig.3 the reduction peakd shifts towards more negative values with increase in pH. The observed shift in E$_{½}$ with decreasing pH to more positive values indicates that there is a proton transfer in the electrode reactions [19].

Table 1 Effect of sweep rate on voltammetric parameters of 1.0 mM PANS in aqueous Dimethylformamide solution containing BR buffer (pH 3.0, 5.0, 7.0, and 9.0)

pH	ν (mV/s)	1st Cathodic Wave			2 nd Cathodic Wave				
		E_{pc}(mV)	I_{pc}(μA)	Ipc/√ν	E_{pc}(mV)	$E_{p1/2}$ (mV)	I_{pc}(μA)	I_{pc}/√ν	α.n$_a$
3.0	100	-22	83	11.73	-568	-497	241	24.1	.000676
	200	-20	162	11.50	-574	-500	363	25.7	.000645
	300	-35	195	11.31	-577	-502	334	21.65	.000637
	400	-47	248	12.43	-580	-507	457	22.85	.000654
	500	-62	282	12.65	-584	-510	569	25.44	.000645
5.0	100	-37	146	14.60	-579	-522	291	29.10	.000838
	200	-40	189	13.36	-596	-523	406	28.71	.000654
	300	-43	226	13.04	-597	530	485	28.00	.000713
	400	-45	239	11.95	-599	-545	574	28.70	.000885
	500	-54	268	11.85	-607	-555	648	29.00	.000919

pH	ν (mV/s)	E_{pc}(mV)	$E_{p1/2}$ (mV)	I_{pc}(μA)	I_{pc}/√ν	α.n$_a$
7.0	50	-666	-602	227	32.10	.000746
	100	-681	-625	290	29.00	.000853
	200	-692	-634	395	27.93	.000824
	300	-697	-637	489	28.23	.000796
9.0	100	-707	-653	393	39.3	.000885
	200	-711	-662	355	35.50	.000975
	300	-715	-665	505	29.15	.000937
	400	-722	-673	617	30.80	.000975
	500	-744	-687	778	34.79	.000713

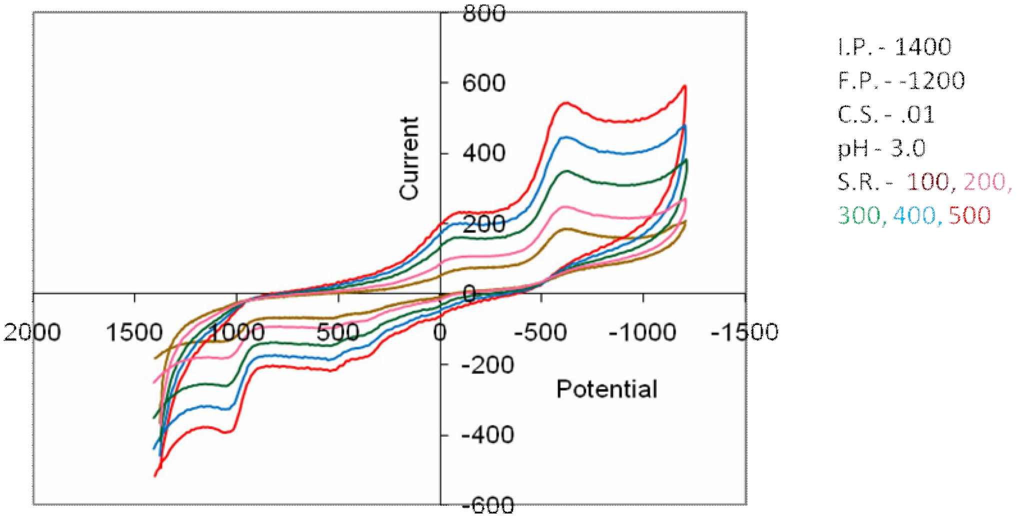


Fig 1. Cyclic Voltammograms of 1mM PANS in aqueous dimethylformamide medium containing BR buffer (pH 3.0)

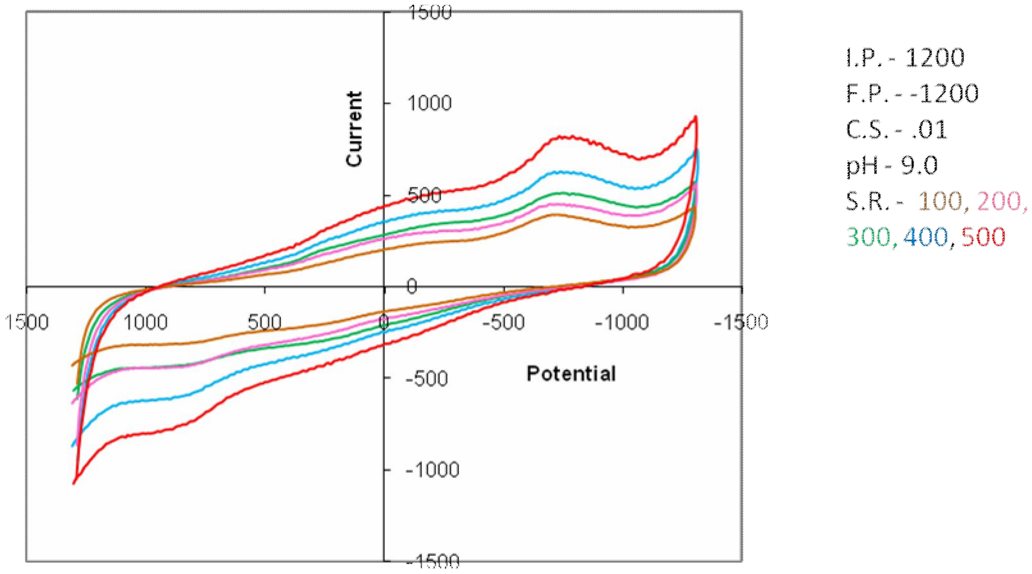


Fig 2. Cyclic Voltammograms of 1mM PANS in aqueous dimethylformamide medium containing BR buffer (pH 9.0)

The effect of scan rate on peak current potential (E_{pc}) was also studied. Fig 1 and 2 shows the effect of scan rate on E_{pc} at pH 5.0 and 9.0 respectively. The peak current potential (E_{pc}) does shift to more negative potentials on increasing the scan rate indicating an irreversible electron transfer process. The slopes obtained are larger than that expected for a reversible process. The dependence of the voltammetric peak current (I_{pc}) of the wave on the square root of scan rate ($v^{1/2}$)is linear with correlation coefficients close to unity (fig 4) at all the pH [20].

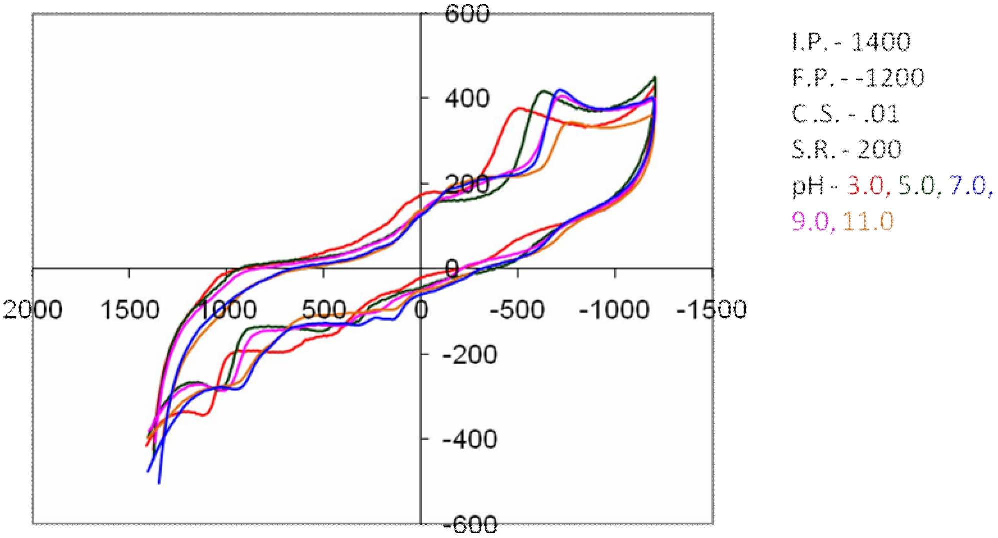


Fig 3 Cyclic Voltammogram of 1mM MPANS in aqueous dimethylformamide medium containing BR buffer (pH 3.0, 5.0, 7.0 and 9.0)

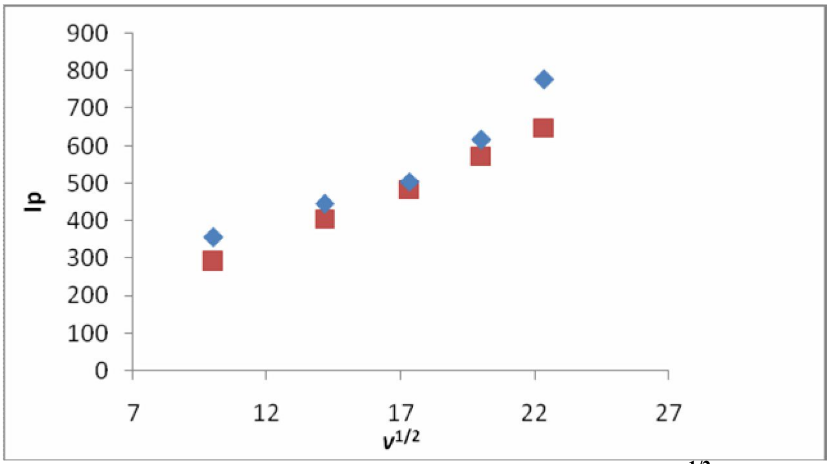


Fig 4 Variation of the cathodic peak currents (Ip) with $v^{1/2}$ for 1mM of PANS at pH 5.0 and 9.0

Constant current electrolysis

o-Aniline and 3-amino-4-hydroxy-1-naphthalene sulphonic acid were obtained after bulk elctrolyssis of PANS at pH 5.0 and 4-Hydroxy-3-(N-phenylhydrazino)- naphthalen-1-sulphonic acid was obtained at pH 9.0. IR and 1H NMR data of these products are given in Table 2.

It is evident from the data (Table 2) that the reduction process in acidic medium takes place via 4e$^-$ /4H$^+$ process and in neutral and basic media via 2e$^-$/2H$^+$ process. On the basis of above results, mechanism may be proposed for the reduction of MPANS in acidic, neutral and in basic media and is given in scheme 2.

Antimicrobial activity

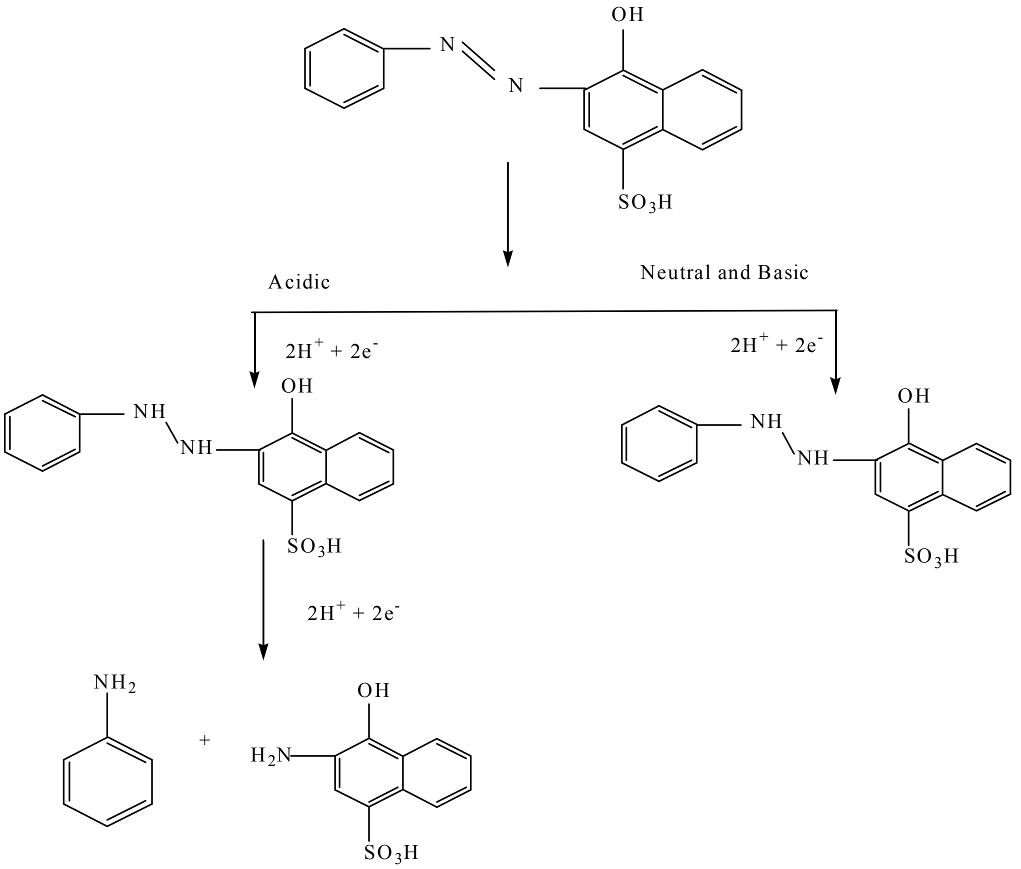
The antimicrobial activities of PANS were evaluated in vitro using disc diffusion method. The antimicrobial activities data are given in Table 3. PANS showed remarkable activity against all species but was inactive against fungal species. Results were compared with standard drugs. The variation in the effectiveness against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells [21].

Table 2 IR and ^{1}H NMR data of products obtained after constant current electrolysis of PANS

pH	Products obtained after controlled current electrolysis	IR data	^{1}H NMR
5.0	o- aniline	3370 cm^{-1} ($\nu_{\text{N-H asymm}}$), 3290 cm^{-1} ($\nu_{\text{N-H symm}}$), 3050 ($\nu_{\text{Ar-H}}$), 2930 cm^{-1} ($\nu_{\text{C-H}}$), 1630 cm^{-1} ($\delta_{\text{N-H}}$), 1620-1430 cm^{-1} ($\nu_{\text{C=C}}$ in plane skeletal vibrations), 1250 cm^{-1} ($\nu_{\text{C-N}}$).	δ 6.3 (NH_2 protons) δ 7.3 (aryl protons)
5.0	3-amino-4-hydroxy-1-naphthalenesulphonic acid	3400 cm^{-1} ($\nu_{\text{O-H}}$), 3330 cm^{-1} ($\nu_{\text{N-H asymm}}$), 3280 cm^{-1} ($\nu_{\text{N-H symm}}$), 3050 cm^{-1} ($\nu_{\text{Ar-H}}$), 1590-1440 cm^{-1} ($\nu_{\text{C=C}}$ in plane skeletal vibrations), 1230 cm^{-1} ($\nu_{\text{C-O}}$), 1210 cm^{-1} ($\nu_{\text{C-N}}$), 1250 cm^{-1} ($\nu_{\text{S-O asymm}}$), 1050 cm^{-1} ($\nu_{\text{S-O symm}}$).	δ 4.3 (NH_2 protons) δ 5.1 (OH protons) δ 7.12-7.85 (aryl protons) δ 2.0 (SO_2-O-H protons)
9.0	4-Hydroxy-3-(N-phenylhydrazino)-naphthalen-1-sulphonic acid	3340 cm^{-1} ($\nu_{\text{O-H}}$), 2950 cm^{-1} ($\nu_{\text{N-H}}$), 1610-1490 cm^{-1} ($\nu_{\text{C=C}}$ in plane skeletal vibrations), 1360 cm^{-1} ($\nu_{\text{C-N}}$), 1250 cm^{-1} ($\nu_{\text{S-O asymm}}$), 1090 cm^{-1} ($\nu_{\text{S-O symm}}$), 1140 cm^{-1} ($\nu_{\text{C-O}}$), 720 cm^{-1} ($\nu_{\text{C-S}}$)	δ 4.0 (N-H protons) δ 5.0 (aromatic O-H protons) δ 2.0 (SO_2-O-H protons) δ 6.64-7.89 (aryl and naphthyl protons)

Table 3 Antimicrobial screening data (zone of inhibition in mm) of PANS

Compound	***E.coli* (Gram negative)**	***P.aeruginosa* (Gram negative)**	***S.aureus* (Gram positive)**	***B. subtilis* (Gram positive)**	***E. cloacae* (Gram positive)**	***E. faecalis* (Gram positive)**	***C. albicans***
PANS	12	11	12	7	11	8	-
Standard	22	19	25	21	22	19	18



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Scheme 2 Proposed reaction mechanism for the reduction of PANS at different pH

ACKOWLEDGEMENT

The authors are thankful to Head, Department of Chemistry, University of Rajasthan, Jaipur and the Principal, S.M.S. Medical college, Jaipur, for the antimicrobial screening of the compounds. One of the authors, Ms. Swati, gratefully acknowledges CSIR, New Delhi, for providing Fellowship.

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