IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

DOI: 10.17148/IARJSET.2023.10122

SYNTHESIS OF FORMAZAN DERIVATIVES ANTIMICROBIAL STUDY AND PHYSICOCHEMICAL PROPERTIES IN BINARY SOLVENT SYSTEM

Y S Thakare[*1] and V V Tayade[2]

P.G. Department of Chemistry, Shri Shivaji Science College, Amravati (M.S)[1,2]

Abstract: Formazan derivatives plays vital role in medicinal chemistry because of supreme biological activity. In this study formazan derivatives were synthesized in cold condition. Spectral interpretation was used to characterize the structure of synthesized compounds. Physicochemical properties were studied in binary solvent system using viscometric measurement. Increase in solute-solvent interactions was interpreted from increase in viscosity. Conductivity measurements and antibacterial activity against gram positive and gram negative pathogens were carried out for all derivatives in different solvent systems. E-Coli and S. Aureus shows good activity towards 3 –phenyl –(4 chlorophenyl)-5-phenyl formazan than 3 –phenyl –(4 nitrophenyl)-5-phenyl formazan derivative. The results obtained were interpreted in terms of electrophilic and nucleophilic nature of substituent attached to aryl group.

Keywords: Formazan derivative, Viscosity, Conductivity, Antimicrobial activity.

I. INTRODUCTION

Formazan derivative take over important medical applications because of enormous biological activities such as antiviral [1], antimicrobial [2,3], anti- inflammatory, analgesic [4], antifungal [5], anti-cancer, anti- HIV [6] and anti-fertility [7]. Formazans was first synthesized over a century ago, but still attract attention of chemist, biologist, technologists and other specialists. In recent years anti-fertility, anti tubercular activity and anti- corrosion properties of formazans have been published [8].

A series of phenylsulfanyl and phenylhydrazone substituted formazan derivatives were synthesized by coupling reaction [9-10]. The formazan derivatives were synthesized by the reaction of benzaldehyde phenyl with substituted aromatic and hetroaromatic amines. The synthesized derivatives were screened for anticonvulsant, antimicrobial and antiviral activities [11].

Formazan are known to be characterized by containing very effective functional groups that have an effect on the possession of any compound with pharmaceutical and biological characteristics as antimicrobial for many microbes, as these compounds have proven high resistance to the spread of bacteria and fungi [12]. Formazan derivatives were prepared through reaction of Schiff's base with diazonium salts in presence pyridine as a solvent [13]. Structure of formazan derivatives was first defined in 1892, by Von Pachmann and by Bamberger and Wheelwright independently. Their deep colour and redox chemistry derive from their nitrogen rich backbone [14-15].

Recently Formazan derivatives have been widely used to evaluate the biological activity of formazan derivatives [16-18]. Newly, synthesized formazan-cefixime derivatives found effective drugs against breast cancer cells [19]. Physicochemical properties invoke the pharmacological response on the receptor, which can be a biological molecule or system with which it interacts. The solute drug molecules have a spatial arrangement that defines their physical properties. They have some chemical composition that has some biological or chemical effects on the receptors.

Proposed work explorer synthesis of various formazan derivatives using different aromatic aldehydes and amine groups and study of their physicochemical properties. The viscosity measurement and conductance measurement have been carried out in 70% ethanol – water system and 70% dioxane- water system. Considering drug activity of formazan derivative study have been focus on to study antibacterial activity against some gram positive and gram negative bacteria.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

DOI: 10.17148/IARJSET.2023.10122

II. EXPERIMENTAL

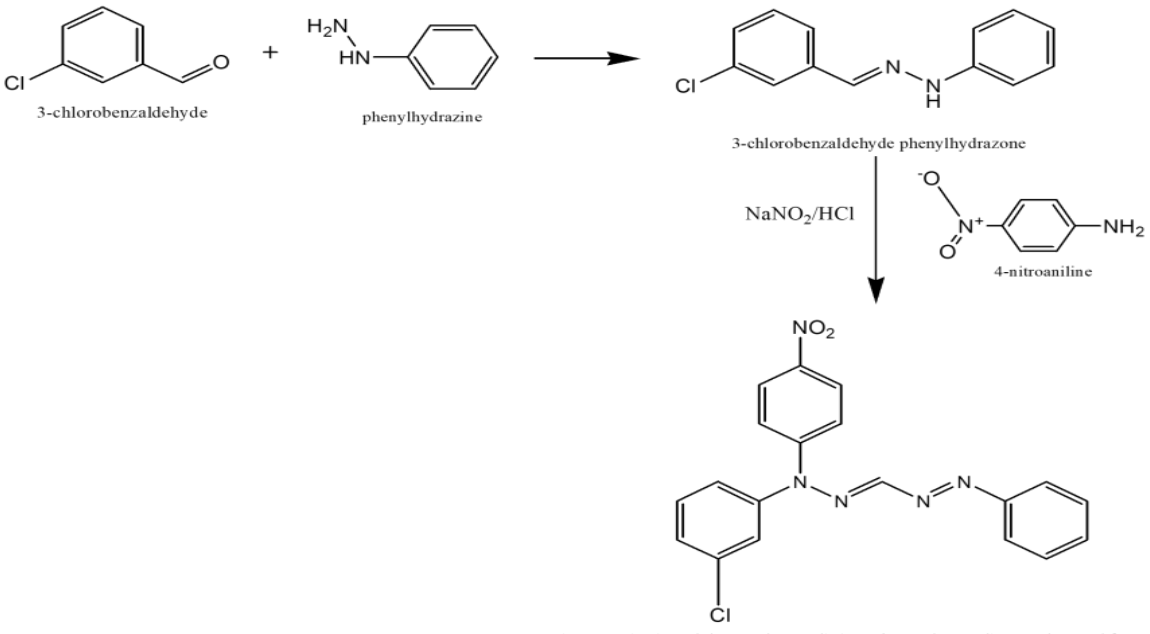
1) Synthesis of 3 –phenyl –(4 nitrophenyl)-5-phenyl formazan :

Step 1 - preparation of 3- chlorobenzaldehyde phenylhydrazone derivatives –0.01 mol of phenyl hydrazine was added drop wise to a well stirred mixture of 0.01 mol of 3- chlorobenzaldehyde dilute in acetic acid (2 ml in 10 ml) in a 100 ml conical flask at room temperature . The reaction mixture was further stirred for 1 hours and kept at room temperature for 30 minutes. The precipitated yellow crystalline mass was filtered and dried in an oven at 60°C. The crude product was crystallized with ethanol. 3- chlorobenzaldehyde phenylhydrazone was obtained as a fine colourless needles.

Step 2 - Synthesis of 3 –phenyl –(4 nitrophenyl)-5-phenyl formazan derivative (PNPF) - 0.01mol of 4 nitroaniline was dissolved in a mixture of 5 ml concentrated hydrochloric acid (HCl) and 5 ml water taken in a 100 ml conical flask, with constant stirring. The reaction mixture was cooled in ice bath until the temperature fall below 5°C. Separately, 1.6 g of sodium nitrite ($NaNO_2$) solution was dissolved in 7.5 ml of water and placed in an ice bath below 5°C. The sodium nitrite solution was filtered and then added drop wise to aniline mixture with vigorous shaking, temperature was not allowed to rise above 10°C. The diazonium salts solution of aryl and heteroaryl amine was filtered then added drop wise with continue stirring to a solution of 3- chlorobenzaldehyde phenylhydrazone (0.01M) in pyridine (20ml), maintain the temperature below 10°C. The reaction mixture was allowed to stand for 4 hours then mixture was poured into 250 ml of ice-cold water with continue stirring, the dark coloured solid which separated out was filtered, washed successively with cold water followed by hot water and finally with methanol. Product obtained was dried in air as well. Melting point 100°C and colour of compound – deep red

1H-NMR : δ 7.94 (d) 1H, 6.95 (d) 1H, 7.50 (s) 1H, 7.3 (d) 1H

IR (KBr) – νmax 752 (C-Cl), 1529 (N-O), 3066 (C-H), 1691 (C=N), 1246 (C-N).



(2*E*,4*E*)-(3-chlorophenyl)(4-nitrophenyl)-5-phenylformazan

2) Synthesis of 3 –phenyl –(4 chlorophenyl)-5-phenyl formazan:

Step 1 - Preparation of 3- chlorobenzaldehyde phenylhydrazone derivatives – 0.01 mol of phenyl hydrazine was added drop wise to a well stirred mixture of 0.01 mol of 3- chlorobenzaldehyde dilute in acetic acid (2%) in a 100 ml conical flask at room temperature. The reaction mixture was further stirred for 1 hour and kept at room temperature for 30 minutes. The precipitated yellow crystalline mass was filtered and dried in oven at 60°C. The crude product was crystallized with ethanol. 3- chlorobenzaldehyde phenylhydrazone was obtained as a fine colourless needle.

Step 2 - Synthesis of 3 –phenyl –(4 chlorophenyl)-5-phenyl formazan derivative (PCPF) - 0.01 mol of 4 - chloroaniline was dissolved in a mixture of 5 ml concentrated hydrochloric acid (HCl) and 5 ml water taken in a 100 ml conical flask, with constant stirring. The reaction mixture was cooled in ice bath until the temperature fall below 5°C. Separately, 1.6

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

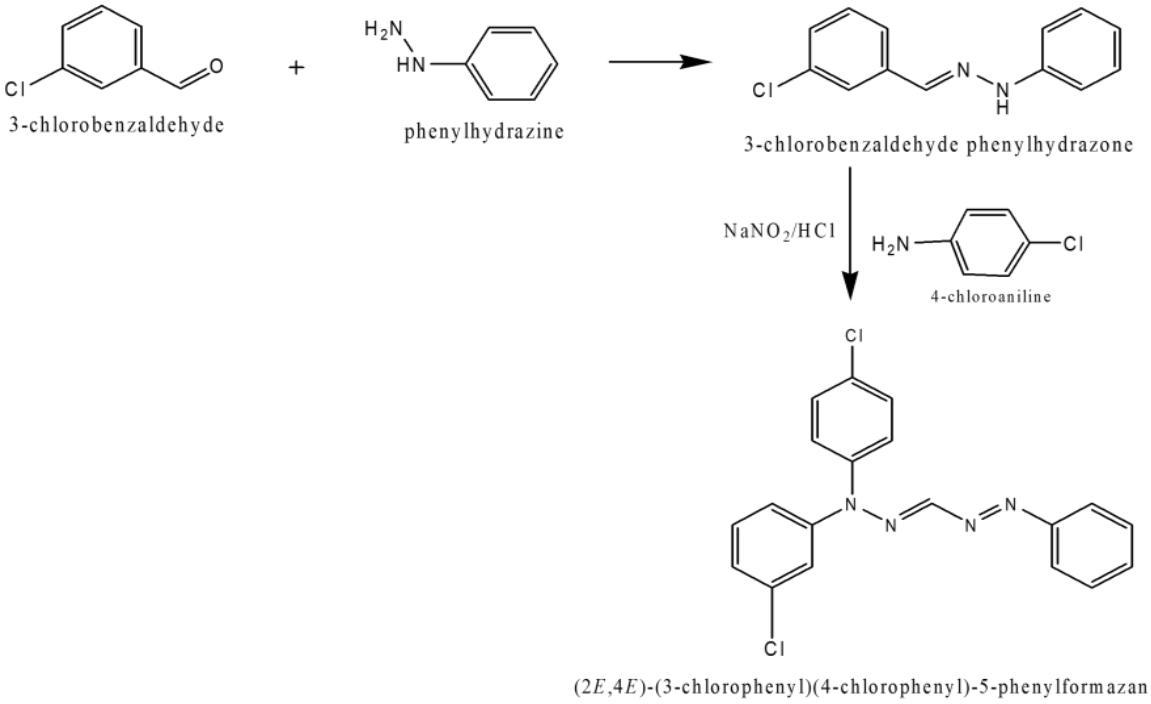
DOI: 10.17148/IARJSET.2023.10122

g of sodium nitrite ($NaNO_2$) solution was dissolved in 7.5 ml of water and placed in ice bath below 5°C. The sodium nitrite solution was filtered and then added drop wise to aniline mixture with vigorous shaking, temperature was not allowed to rise above 10°C. The diazonium salts solution of aryl and heteroaryl amine was filtered then added drop wise with continuous stirring to a solution of 3- chlorobenzaldehyde phenylhydrazone (0.01M) in 20 ml pyridine, maintain the temperature below 10°C.

The reaction mixture was allowed to stand for 4 hours then mixture was poured into 250 ml of ice-cold water with continue stirring, the dark coloured solid which separated out was filtered, washed successively with cold water followed by hot water and finally with methanol. Product obtained was dried in air as well. Melting point 60°C and colour of compound – deep red

1H-NMR : δ 6.63 (d) 1H, 6.4 (d) 1H, 7.3 (d) 1H, 7.50 (s) 1H.

IR (KBr) – 777-833 (C-Cl), 1512 (N-O), 3061 (C-H), 1689 (C=N), 1240 (C-N).



(2*E*,4*E*)-(3-chlorophenyl)(4-chlorophenyl)-5-phenylformazan

III. RESULTS AND DISCUSSION

Viscosity Measurement

Viscometric study of synthesized formazan derivatives, PNPF and PNPC at different concentrations using 70% ethanol-water system and 70% dioxane-water system was carried out. Relative viscosity of each prepared system was determined. Jones- Dole equation ; $\eta r - 1/\sqrt{c} = A + B \times \sqrt{c}$ is used to determine solute-solvent and solute-solute interaction. The graphs are plotted between $(\eta r - 1)/\sqrt{c}$ verses $\sqrt{c}$ for binary solvent system 70% ethanol-water and 70% dioxane-water system for formazan derivative PNPF (Fig. 1 and Fig. 2) and PNPC (Fig. 3 and Fig. 4) which gives straight line showing validity of Jones-Dole equation.

Where, 'A' is the coefficient which measure of solute-solute interaction and 'B' measure of solute-solvent interaction. The slope of the graph shows the value of 'B' coefficient and intercept gives the value of 'A' coefficient for different formazan derivative PNPF and PCPF as shown in Table 1 and Table 2 respectively in various binary solvent system.

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

DOI: 10.17148/IARJSET.2023.10122

TABLE I RELATIVE AND SPECIFIC VISCOSITY AT DIFFERENT CONCENTRATIONS OF PNPF

Medium	Conc 'C'(mole/lit)	√C	Density	Time flow (sec)	Viscosity (η)	Relative Viscosity (ηr)	Specific Viscosity(ηsp)	Coefficient 'A'	Coefficient 'B'
70% ethanol-water system	0.01 0.005 0.0025	0.1 0.070 0.05	0.532 0.52 0.516	87 60 57	0.7284 0.4910 0.4629	0.7004 0.4721 0.4451	-0.2995 -0.5278 -0.5548	-0.8912	4.0511
70% dioxane-water system	0.01 0.005 0.0025	0.11 0.070 0.05	0.852 0.847 0.817	43 40 33	1.0111 0.9351 0.7441	0.5081 0.4699 0.3739	-0.4918 -0.5301 -0.6260	-0.7351	3.8012

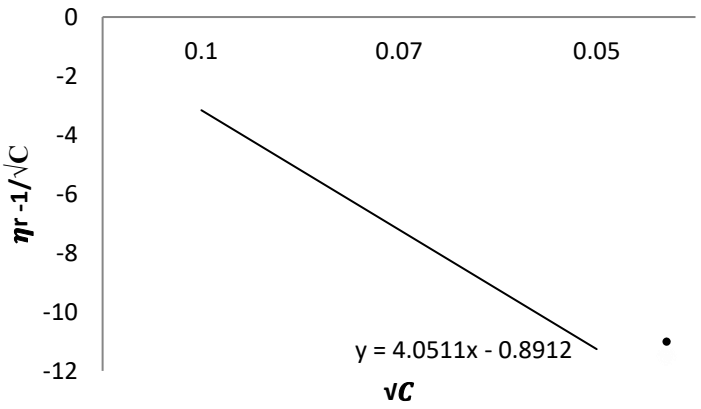


Fig. 1 Plot of (ηr - 1)/√c verses √c for PNPF in 70% ethanol-water

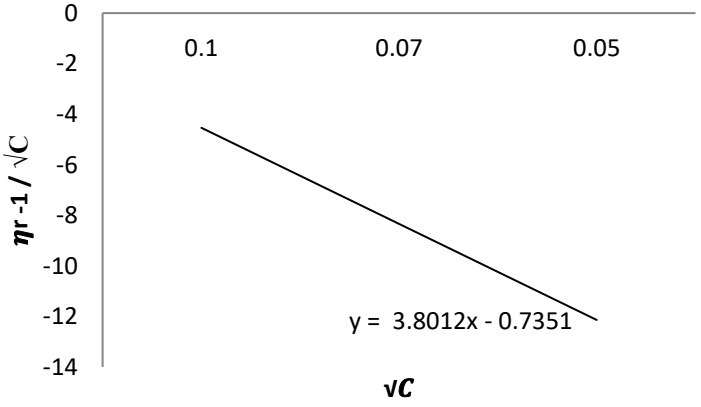


Fig. 2 Plot of (ηr - 1)/√c verses √c for PNPF in 70% dioxane-water

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

DOI: 10.17148/IARJSET.2023.10122

TABLE II RELATIVE AND SPECIFIC VISCOSITY AT DIFFERENT CONCENTRATIONS OF PCPF

Medium	Conc 'C' (mole/lit)	√C	Density	Time flow (sec)	Viscosity (η)	Relative Viscosity (ηr)	Specific Viscosity (ηsp)	Coefficient 'A'	Coefficient 'B'
70% ethanol-water system	0.01 0.005 0.0025	0.1 0.070 0.05	0.54 0.516 0.528	95 75 68	0.8074 0.6091 0.5651	0.7763 0.5856 0.5433	-0.2236 -0.4143 -0.4566	-1.1339	3.4489
70% dioxane-water system	0.01 0.005 0.0025	0.1 0.070 0.05	0.822 0.817 0.812	50 46 30	1.1343 1.0372 0.6723	0.5700 0.5212 0.3378	-0.4299 -0.4787 -0.6621	-0.8159	4.4716

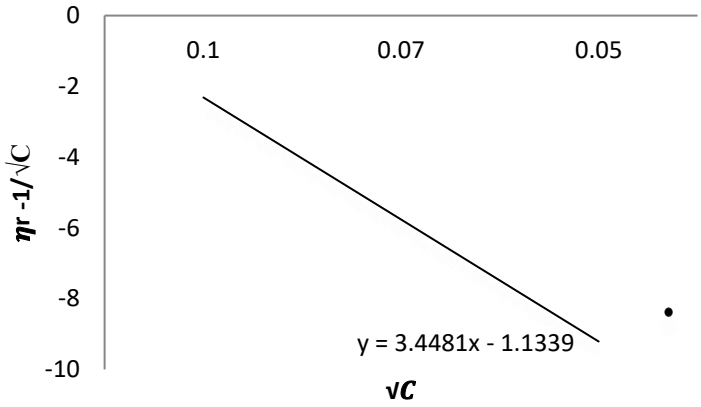


Fig. 3 Plot of (ηr - 1)/√c verses √c for PCPF in 70% ethanol-water

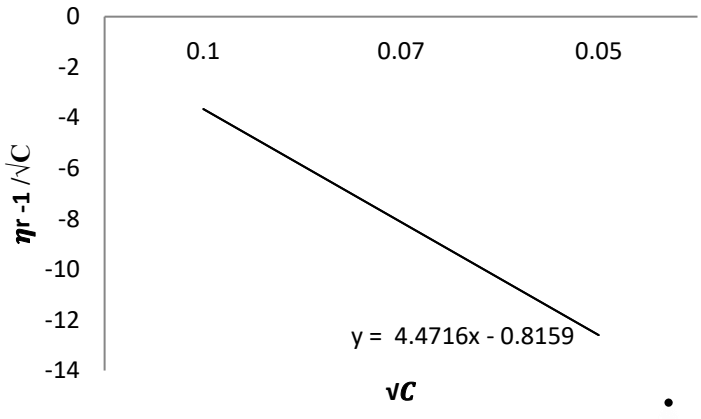


Fig. 4 Plot of (ηr - 1)/√c verses √c for PCPF in 70% ethanol-water

From above it was conclude that the coefficient. 'B' is positive, shows strong solute-solvent interaction of formazan derivative in 70% ethanol-water and 70% dioxane-water system. Also results show negative values for coefficient 'A' in binary solvent system for PNPF and PCPF which shows weak solute-solute interaction. These facts also supported decrease in relative viscosity for all the tested ligand in different solvent system. Variation of density, viscosity, relative viscosity and the ionic interaction of formazan derivative may be due to electrophilic and nucleophilic nature of substituent attached to aryl group. Results also shows more negative value of coefficient 'A' in 70% ethanol-water as

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

DOI: 10.17148/IARJSET.2023.10122

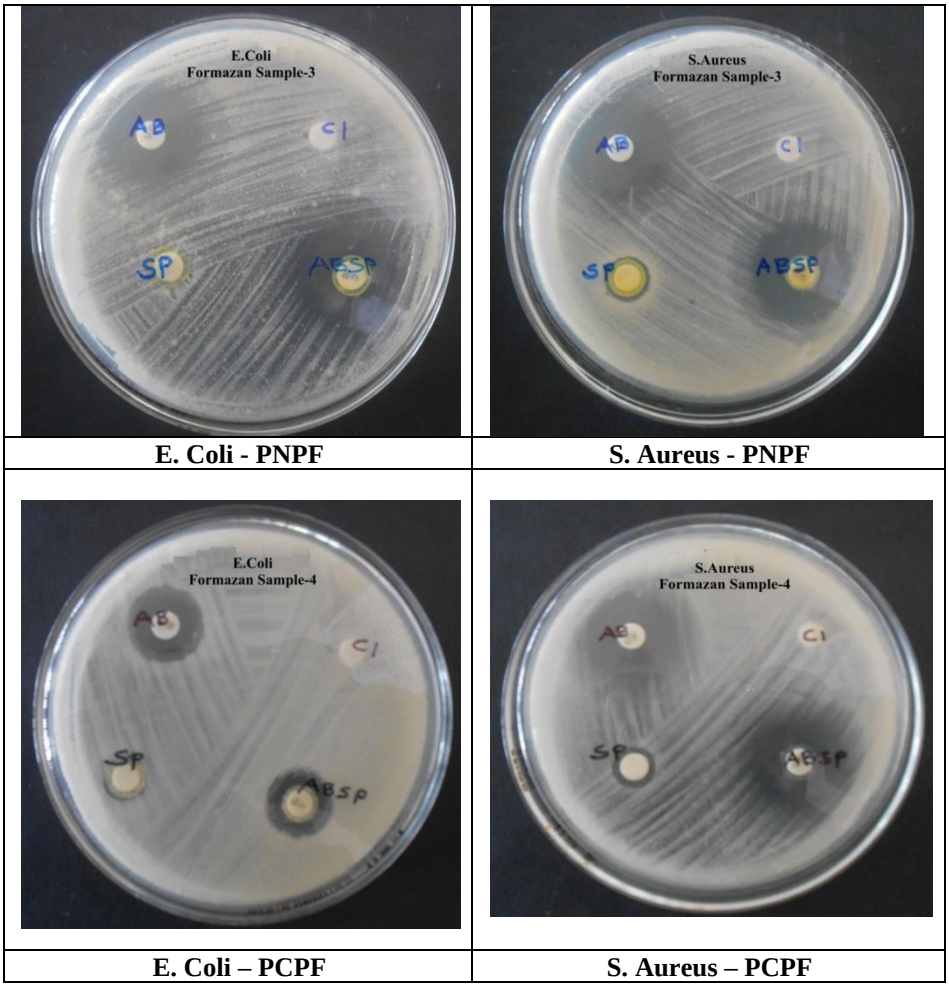
compared to 70% dioxane-water system for both ligands. The value of coefficient 'B' for PNPF is more in polar than non polar which exactly contradict for PCPF. These diverse results for formazan derivatives due to its directing nature and assorted polarity index of binary solvent system.

Antimicrobial activity of formazan derivatives

The antibacterial activities of synthesized compounds were tested on against gram positive and gram negative microorganisms using disc diffusion method. The bacteria used in the present investigations included -Escherichia coli, Staphylococcus aureus. The Minimum inhibitory concentration (MIC) was evaluated for compounds which showed higher antibacterial activity. Tetracycline was used as standard antibiotics. The diameter of zone of inhibition and the MIC results for the compounds are compiling in Tables-4. Zone of inhibition and MIC of all compounds is illustrated in images of antibacterial screening discs shown in fig. 5.

TABLE IV ANTIBACTERIAL ACTIVITY OF COMPOUNDS (CONC. 10 MG/DISC)

Sr. No.	Compound Code	Zone of Inhibition in mm	
		E. Coli	S. Aur
1	PNPF-Sample 3	07 ±0.5	12 ±0.2
2	PCPF-Sample-4	10.00	15 ±0.6
Tetracycline		**28**	**30**



E. Coli - PNPF | **S. Aureus - PNPF**

E. Coli – PCPF | **S. Aureus – PCPF**

Fig. 5 Antimicrobial activity of PNPF and PCPF against E. Coli and S. Aureus bacteria

IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 7.12 ⁜ Vol. 10, Issue 1, January 2023

DOI: 10.17148/IARJSET.2023.10122

The 3 –phenyl –(4 nitrophenyl)-5-phenyl formazan compound showed moderate activity against Staphylococcus aureus, whereas it showed poor activity against Escherichia coli bacteria. On the other hand formazan derivative 3 –phenyl –(4 chlorophenyl)-5-phenyl formazan compound showed moderate activity against Staphylococcus aureus and Escherichia coli bacterial strains.

Conductivity Measurement

Microbial growth can be capture by using conductivity measurement. It has a great potential as a research tool of predictive microbiology [20]. In present study, observed conductance of formazan derivatives was determined in binary solvent system shown in Table 3.

TABLE III DETERMINATION OF OBSERVED CONDUCTANCE OF FORMAZAN DERIVATIVES

Sr no.	Formazan derivatives	Observed Conductance in 70%ehtanol-water system	Observed Conductance in 70%dioxane-water system
1	3-phenyl-(4-nitrophenyl)-5-phenyl formazan-PNPF	0.038 0.029 0.015	0.024 0.014 0.007
2	3-phenyl-(4-chlorophenyl)-5-phenyl formazan-PCPF	0.044 0.031 0.025	0.025 0.021 0.013

Conductance depends on number of ionic species in the given system. It was perceive from above observation that for given solvent system observed conductivity reveals distinct outcome for PNPF and PCPF. Observed conductance decrease with decrease in concentration for PNPF and PCPF. In binary polar solvent system 70% ethanol-water the value of observed conductivity is high as compare to 70% dioxane-water system for formazan derivative. The study was evaluating the ion-solvent and ion-ion interaction.

IV. CONCLUSION

Formazan derivatives have been found to possess important role in medicinal chemistry because of various biological activities. Synthesized formazan derivative subjected to viscometric measurements to study ionic interaction. It was observed that variation of density, viscosity, relative viscosity and solute-solute and solute-solvent interaction of formazan derivative may be due to electrophilic and nucleophilic nature of substituent attached to aryl group. Negative values of coefficient 'A' shows weak solute-solute interaction and positive value of B shows strong solute-solvent interaction which further depends up on polarity of binary solvent system. Conductometric study was evaluating the ion-solvent and ion-ion interaction which depends on directing nature of the substituted group. Formazan derivative PNPF showed moderate activity against Staphylococcus aureus, whereas poor activity against Escherichia coli bacteria. On the other hand PCPF showed moderate activity against both the pathogens.

REFERENCES

[1] V. S. Misra, S. Dhar, and B. L. Chowdhary, " Synthesis of some newer formazan and tetrazolium salt as antiviral agents", Pharmazie, vol. 33 (12), pp. 790-792 , Dec. 1978.
[2] R. M. Desai, J. M. Desai, and V. H. Shah, " Synthesis and antimicrobial activity of some new formazan derivatives", Indian J. Heterocycl. Chem., vol. 8 (4), pp. 329-331, 1999.
[3] J. M. Desai, and V. H. Shah, " Synthesis and antimicrobial profile of 5-imidazolinones, sulphonamides, azomethines, 2-azetidinones and formazans derived from 2-amino-3-cyano-5-(5-chloro-3-methyl-1-phenyl pyrazol-4-yl vinyl)-7,7-dimethyl-6,7-dihydrobenzo (b) thiophenes", NISCAIR-CSIR , India., pp. 631-635, Mar. 1999
[4] R. Kalsi, K. Pande, T. N. Bhulla, S. S. Parmar, and J. P. Barthwal, " Novel formazans as potent anti- inflammatory and analgesic agent", Pharmacology, vol. 37 (4), pp. 218-224, 1998.
[5] K. G. Desai, and K. R. Desai, " Microbial screening of novel synthesized formazans having amide linkage", J. Heterocycl. Chem., vol. 43 (4), pp. 1083-1089, 2006.
[6] S. D. Bhardwaj, and V. S. Jolly, " Synthesis, anti- HIV and anti-cancer activities of some new formazan", Asian J. Chem., vol. 9 (1), pp. 48-51, 1997.
[7] M. Kidwai, N. Negi, and S. D. Gupta, " Synthesis and anti-fertility activity of 1,5-diaryl-3-(3`-indolyl) formazans", Chem. Pharm. Bull, vol. 42 (11), pp. 2363-2364, 1994.

DOI: 10.17148/IARJSET.2023.10122

[8] Y. H. Al-Araji, J. K. Shneine, and A. A. Ahmed, " Chemistry of formazan" Int. J. Res. Pharm. Chem., vol. 5 (1), pp. 41-76, 2015.
[9] G. Turkoglu, and H. Berber, " Novel formazan derivatives containing phenylsulfanyl and carbonyl units: synthesis, optical and electrochemical properties, RSC Adv, vol. 6 (98), pp. 96065-96078, 2018.
[10] G. Turkoglu, H. Berber, I. Kani , " Synthesis, crystal structure, optical and electrochemical properties of novel diphenylether- based formazan derivatives", New J Chem., vol. 39 (4), pp. 2728-2740, 2015.
[11] TabarkEmad Al-Faham, Dr.Nagham Mahmood Alijamali, "Synthesis and Spectral Indentification Of formazan-phenylenediamine Derivative", Res. Rev. Drug. Drug. Dev., Vol. 3(1) , pp.14-24, Jan. 2021). ISSN: 2582-5720.
[12] Hussein Ali Ahmed, Nagham Mahmood Alijamali. "Preparation, Characterization, Antibacterial study, toxicity study of New Phenylene diamine Formazan Derivative". Indian J. Forensic Med. Toxicol. 15 (2), 3102-3112, 2021.
[13] ShimaIbraheemChyad Al-khazraji, Hussain AbboodIdham,"Synthesis of Some Formazan Derivatives from Schiff's Bases and Studying of Biological Activity". Diyala J. Pure Sci., Vol. 13, 2017.
[14] Wolfrom, M.L.; R. S. Tipson, Advances in Carbohydrate Chemistry. ISSN. Elsevier Science. ISBN 978-0-08-056272-8, 1958.
[15] A.W. Nineham, "The Chemistry of Formazas and Tetrazolium Salts." Chem. Rev., 55(2) pp. 483-355, 1955
[16] G. Mariappan, R. Korim,N. M. Joshi, F. Alam, R. Hazarika, D. Kumar, and T. Urih, "Synthesis and biological evaluation of formazan derivatives", J. Adv. Pharm. Technol.Res., Vol. 1(4), pp. 396-400, 2010
[17] V. D. Saharan, S. S. Mahaja, "Development of gallic acid formazans as novel enoyl acyl carrier protein reductase inhibitors for the treatment of tuberculosis", Bioorg. Med. Chem. Lett., Vol. 27(4), pp. 808-815, 2017.
[18] P. Mukherjee, and A.R. Das "One-flask synthesis of pyrazolone thioethers involving catalyzed and uncatalyzed thioetherification pathways of pyrazolones" Org. Biomol. Chem. Vol. 15(35) pp. 7267-7271, 2017.
[19] N. M. Aljamali and H. M. Azeez, "Synthesis and Characterization of Some New Formazan- Cefixime and Study of Against Breast Cancer Cells", Annals of R.S.C.B Vol. 25(3), pp. 8562 – 8578, March 2021
[20] E. Szigeti and J. Farkas, Use of Conductometric technique for data capture in predictive microbiology, Acta Alimentaria, Vol. 29(3), pp.307–314, 2000.

