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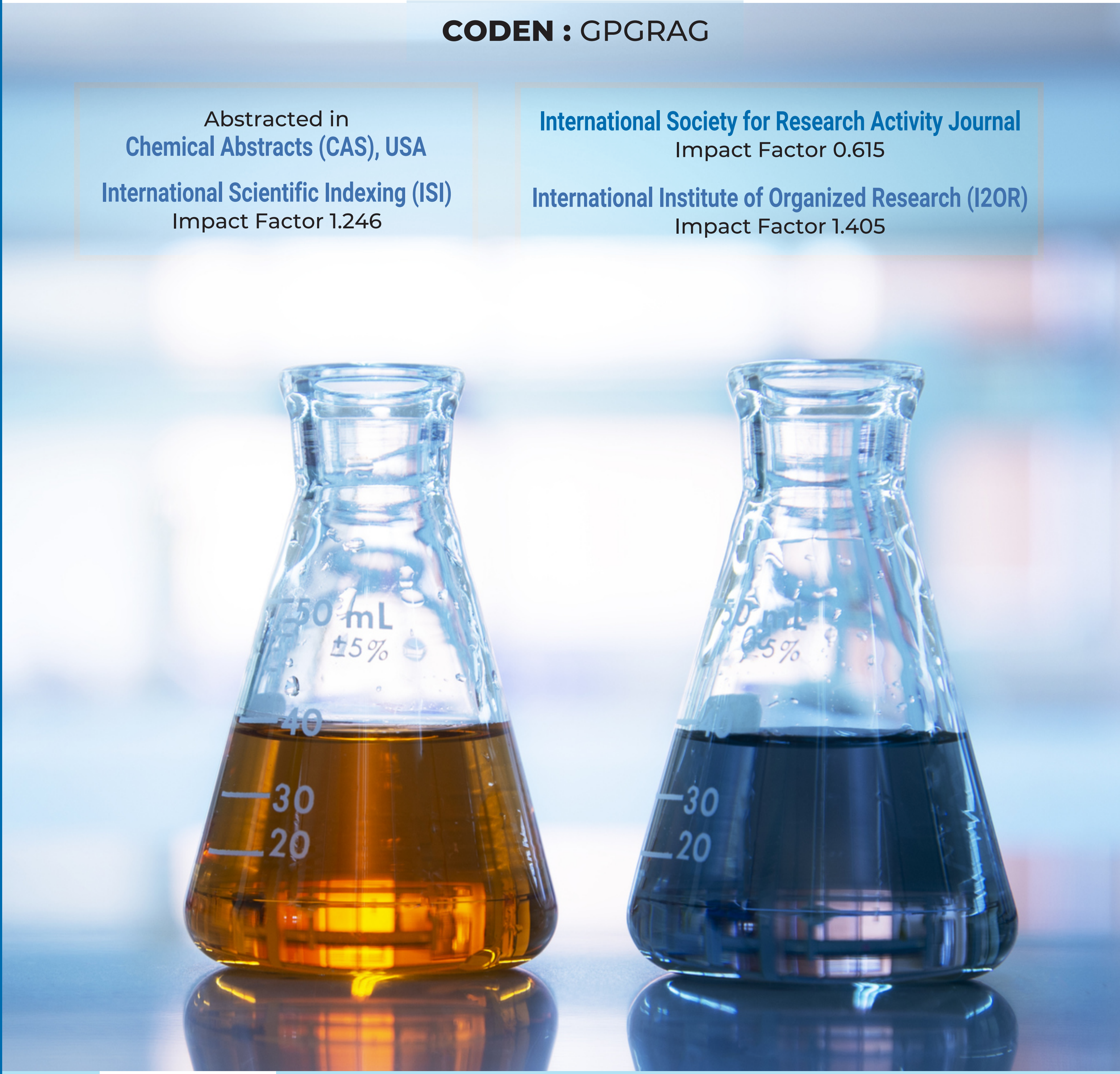

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Ordinary Laboratory Synthesis of Magnetite Nanoparticles

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ABSTRACT

Handy wet chemical methods of synthesizing highly crystalline magnetite nanoparticles (MNPs) in fairly pure state in an ordinary Chemistry laboratory and their characterization using simple methods (complemented by advanced analytical techniques such as X-ray diffraction (XRD), Fourier transmission infrared (FTIR), spectroscopy and optical microscopy (OM)) have been demonstrated. The substance is coated with a primary layer of tetra-methylammonium hydroxide (TMAOH) to increase the stability and dispersibility of the nanoparticles. The XRD results show that the MNPs thus obtained is pure and crystalline. The nanoparticles remain stable for more than a year. The method described in this paper can be easily employed for the synthesis of these interesting materials in an ordinary Chemistry laboratory.

Keywords: *Magnetite nanoparticles, Wet chemical method, Co-precipitation, X-ray diffraction, Electron microscopy*

Introduction

Magnetite, a common iron ore occurring as a rock mineral, is commonly known as ferrous–ferric oxide (chemical formula: Fe_3O_4) and has the IUPAC name Iron (II,III) oxide. It has been long known that the naturally magnetized pieces of Iron (II,III) oxide mineral was named lodestone[37]. It has many attractive properties, attributable to its ferromagnetic behaviour and by virtue of its unique catalytic properties, biocompatibility and superparamagnetic behaviour. It has been widely used in many fields such as in drug delivery systems, as magnetic resonance imaging (MRI) contrast agent, magnetic fluid hyperthermia[10,35] as well as for environmental remediation and in microfluidics[1,29,36]. The substance exhibits crystalline texture with cubic inverse spinel structure (space group $Fd\bar{3}m$) at room temperature where Fe^{3+} ions occupy both octahedral and tetrahedral sites while the Fe^{2+} ions only the octahedral ones[24].

Over the past few decades, with the progress of the nanotechnological revolution in various fields, synthesis and applications of magnetite nanoparticles have attracted enormous academic and technological interest.[3,10,31,37] Various methods have been developed to synthesize magnetite nanoparticles (MNPs), which include thermal decomposition, sol-gel, microemulsion and co-precipitation methods.[2,9,22,31,34] Some methods are so attractive that they offer the control of size as well the dimensionalities of the nanoparticles quite precisely. Among them, co-precipitation is one of the most convenient and economic methods which has the potential to meet the increasing demand for water-dispersible MNPs.[2,28] The co-precipitation method produces monodisperse, highly pure, and stoichiometric nanoparticles of single and multicomponent metal oxide.[5,25,38] Generally, for many applications, MNPs embedded in nonmagnetic matrices such as polymers, and organic and inorganic ligands are required to avoid their macroscopic aggregation and sedimentation.[3,10,20]

Due to the unique magnetic properties and versatile applications of MNPs, it has become necessary to propose a safe method for synthesizing and characterizing these materials in ordinary senior high school laboratories. This would enable the new generation scientists to quickly grasp the essence of applicability of the new materials and guide them in innovative applications pathways. In line with this motto, this study aims at suggesting how stable MNPs can be synthesized and characterized using a simplistic approach and in an ordinary Chemistry laboratory. In this regard, the easy methods of synthesis of different kinds of nanomaterials such as silver[11], hydroxyapatite[27], copper[16], and layered double hydroxides[7] have been explored in our laboratory. In this work, we report the preparation of MNPs by co-precipitation of soluble Iron (II) and Iron (III) salts in the presence of alkali at pH > 9.[6,31,38] We characterized these nanoparticles by ordinary methods using magnets and Tyndall effect and then further confirmed using advanced analytical techniques such as Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and scanning electron microscopy (SEM).

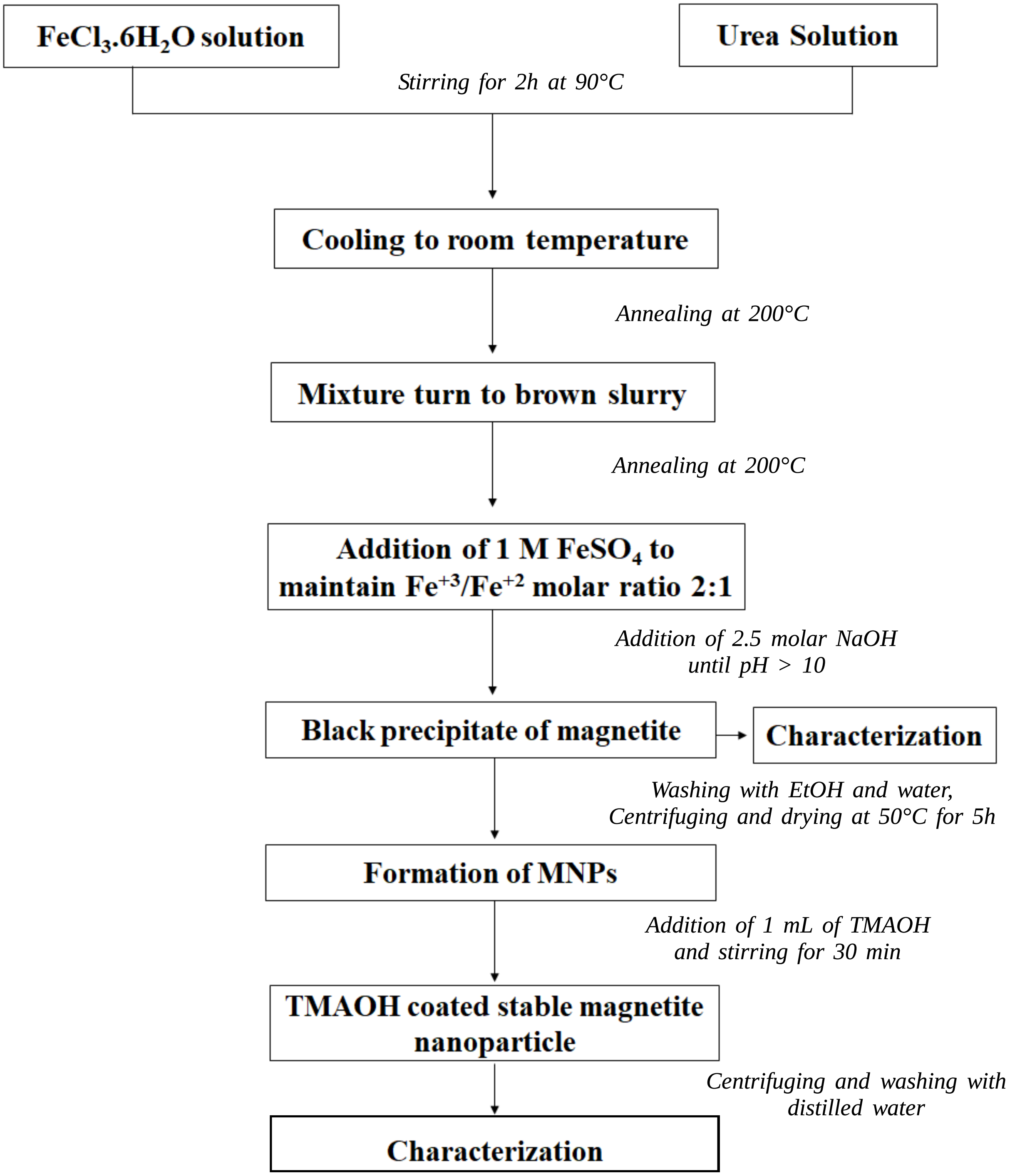
Materials and Methods

Laboratory grade Ferric Chloride ($FeCl_3$), Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7\ H_2O$), urea (N_2H_4CO) and Ferric nitrate [$Fe(NO_3)_3 \cdot 9\ H_2O$], Sodium hydroxide (NaOH), Ammonium hydroxide (NH_4OH), Tetramethylammonium hydroxide (TMAOH), and Ethanol were purchased from Thermofisher Scientific, India and used without further purification.

Synthesis of MNPs by Co-precipitation Method

The MNPs were synthesized by ageing the stoichiometric mixture of ferrous and ferric salts in an aqueous medium by adopting the protocols suggested by different authors[17].

The basic steps and procedures involved in the co-precipitation of magnetite crystals are illustrated in Scheme 1. The changes in the physical appearance of solutions in some of the important steps due to underlying chemical changes are presented in Fig. 1. Briefly, 3.24 g of anhydrous Ferric chloride and 3.6 g of Urea were dissolved in 200 mL of distilled water taken in a 500 mL conical flask. The mixture was thoroughly stirred with heating at about 90°C for 2 hrs till the solution changed to a light brown viscous liquid (Fig. 1a).



Scheme 1. Steps of MNPs synthesis by co-precipitation method and analyses

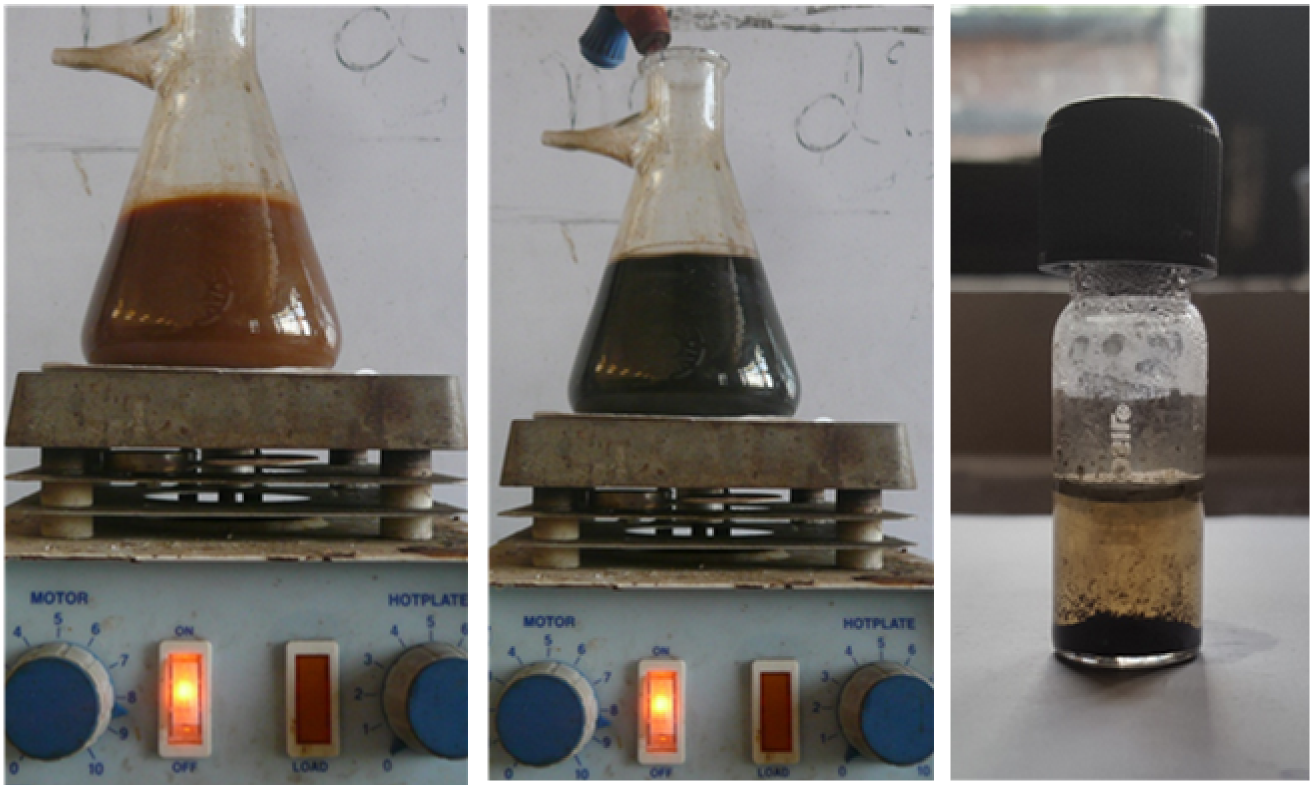


Fig. 1. Photographs showing (a) light brown slurry during co-precipitation reaction (b) black coloured magnetite particles upon addition of NaOH solution to the light brown mixture, and (c) the mass of magnetite particles decanting at the bottom of the glass container

The liquid was then cooled to room temperature to obtain a brown slurry which was further annealed at 200°C. 2.74 g of Ferrous sulphate was dissolved in the mixture with constant magnetic stirring. 2.5 M NaOH solution was added to the mixture drop-wise until the brown solution changed to black colour and reached pH > 10. The black precipitate was allowed to decant and washed several times with distilled water and with ethanol (Fig. 1c). Finally, the solution was filtered off and the precipitate dried at 50°C for 5 h and stored for further study. A part of the powder was redispersed in distilled water by ultrasonication for 15 min and used for further characterization. The overall reaction for the synthesis is $Fe^{2+} + 2\,Fe^{3+} + 8\,OH^{-} \rightarrow Fe_3O_4 + 4\,H_2O$[17].

The complete precipitation of MNPs takes place at a pH range of 8–14 with a Fe^{3+}/Fe^{2+} stoichiometric ratio of 2.1 under an inert atmosphere. At the lower pH and in an ordinary atmosphere, the magnetite transforms into maghemite (γFe_2O_3)[14,17].

In order to get the stable nanoparticles dispersed in the aqueous solution, 1 mL of tetra-methylammonium hydroxide (TMAOH), a steric stabilizer, was syringed drop-wise into the flask containing the black precipitate and allowed to coat on the surface of the solid magnetite particles for 30 min with continuous stirring using a magnetic stirrer. The mechanism of the dispersion of the MNPs by TMAOH treatment can be found elsewhere[33].

If more TMAOH free product is desired, the colloidal solution can be centrifuged followed by washing of the solid substance thus obtained, by distilled water. The product can be then redispersed in water under ultrasonication. Similarly, a polar stabilizer such as Salicylic acid and Polyethylene glycol can also be used[5,10,36].

For comparison, the MNPs were also prepared using the sol-gel method by following the standard protocol[12,30]. This method also yielded quite satisfactory results (data is not presented here). Also, the magnetite prepared this way can be coated with TMAOH for colloidal stability.

Preliminary Characterization

The preliminary characterization of the magnetite material was performed using two simple experiments viz. utilizing the concept of the behaviour of the magnetic materials under the influence of external magnetic field; and behaviour of the colloidal dispersion in presence of laser light (Tyndall effect). The experiments are illustrated in Fig. 2.

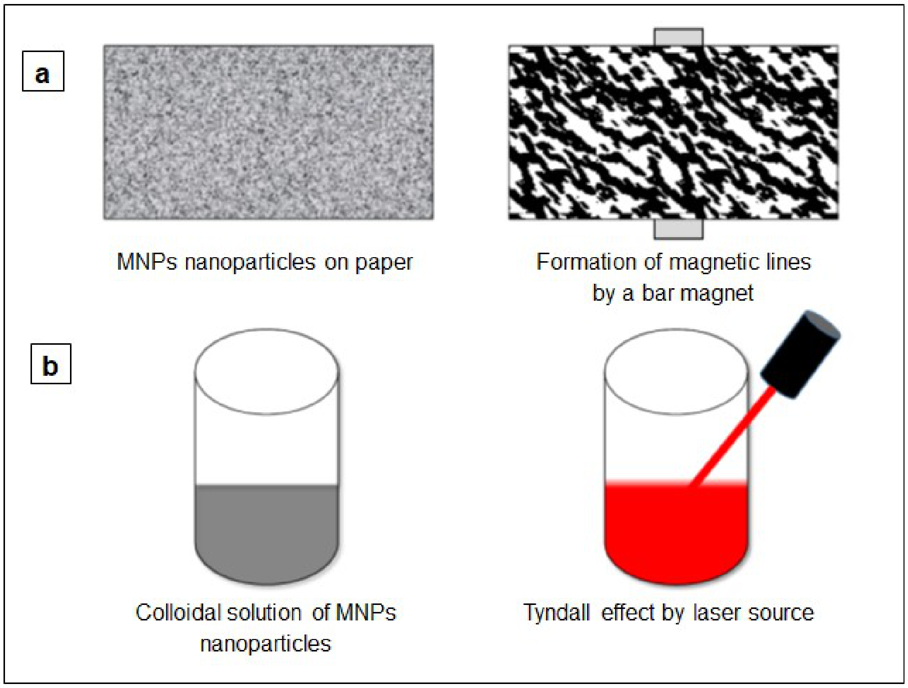


Fig. 2. Schematic diagrams showing the experimental setups for (a) alignment of magnetite powder on a sheet of paper along the magnetic lines of force of a bar magnet placed beneath the paper; and (b) scattering of laser light by a colloidal solution.

Advanced Characterization Techniques

X-ray diffractometer (Bruker D2 Phaser, Germany) with CuKα radiation (wavelength λ = 0.15418 nm) at the scanning rate of 0.1°/step and scatter angle 2θ ranging from 20° to 80° was used to record the powder XRD patterns of the samples. An accelerating voltage of 35 kV and the emission current of 30 mA were used.

Fourier transform infrared (FTIR) analysis was carried out by using the IR Prestige-21 Spectrometer (SHIMADZU, Japan) to identify the functional groups present in the sample. The spectra were collected over the range of 4000–400 cm^{-1} using a KBr pellet technique with a spectral resolution of 4 cm^{-1}.

Scanning electron microscope patterns (SEM) of bare and coated NPs was obtained using Jeol JSM 6300 scanning electron microscope. The specimens were prepared by placing a few drops of colloidal MNPs on a silicon wafer followed by evaporation of water under ambient conditions. The specimens were sputtered with a thin layer of platinum to avoid charging.

Results and Discussion

Preliminary Characterization of MNPs

The preliminary characterization of the synthesized materials by simple methods is demonstrated in Figures 2, 3 and 4. The effect of TMAOH coating on the dispersion and stability of MNPs is shown in Fig. 3. Without the use of coating ligand, the MNPs remain suspended in the solution only for a few hours. They get settled at the bottom of the glass container when stored for months (Fig. 4a). On the other hand, the particles remain stable forming a colloidal solution for several months when they are coated with TMAOH (Fig. 4b). Degradation of MNPs to other products over a longer period is indicated by the complete brown colouration of the mixture, which is attributed to the oxidation of magnetite to hematite and/or maghemite[14,15]. The oxidation process leading to different forms of iron oxide is indeed a complex phenomenon and remains out of the scope of this report.

Fig. 3. Photographs showing different stages of stable precipitate: (a) agglomerated MNPs settling down in the glass vial, and (b) TMAOH-coated MNPs which stayed stable for months

Fig. 4 shows the photographs of the magnetite particles under different conditions. The optical micrograph of the dry magnetite powder is presented in Fig. 4a which shows the formation of elongated patterns. These patterns are formed due to the effect of an external magnet bar placed beneath the paper containing the magnetite powder. The patterns depict simply the magnetic lines of force formed upon the action of an external magnet. The presence of colloidal or nanometric particles can be demonstrated further by a simple experiment illustrating the Tyndall effect, which manifests when the laser light is scattered by the colloidal solution. On passing the red laser light through the dispersion of the magnetite, the light is scattered around giving rise to the glowing appearance of the glass container (Fig. 4b). The Tyndall effect i.e. the scattering of laser light, is the phenomenon exhibited by colloidal solutions and can be thus used for preliminary characterization of the presence of nanoparticles.

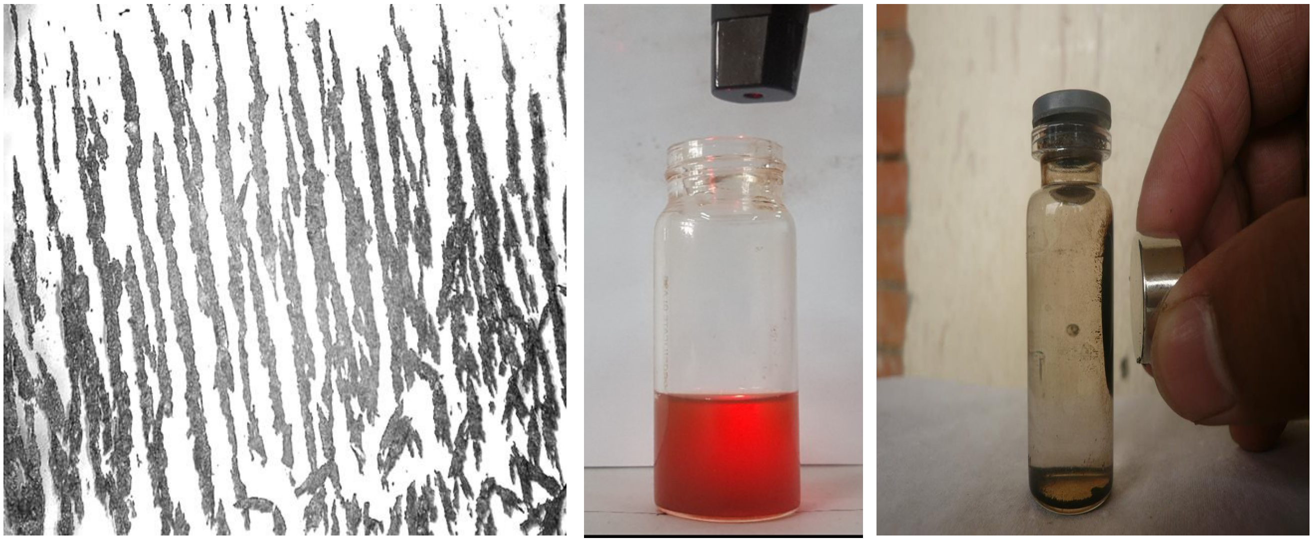


Fig. 4. (a) Optical micrograph showing elongated patterns formed by the magnetite upon the influence of external magnetic field; (b) scattering of laser light (Tyndall effect) by the magnetite particles indicating the presence of nanoparticles; and (c) aggregation of MNPs towards the external magnet

It can be easily confirmed with the help of a common magnet that the product formed is magnetite. The black precipitate is easily attracted towards the magnet when it is brought towards the outer surface of the flask containing the solid product, as shown in Fig. 4c.

The material was subjected to advanced characterization using FTIR spectroscopy, XRD and SEM.

Characterization of MNPs by Advanced Techniques

The FTIR spectra of the bare and TMAOH-coated MNPs are shown in Fig. 5. The FTIR spectra of bare and TMAOH-coated MNPs show the typical bands ca. 609 cm^{-1} and 591 cm^{-1} confirming the presence of Fe–O bonds. The band at 1405 cm^{-1} of TMAOH-coated MNPs corresponds to the asymmetric methyl deformation mode whereas a symmetrical mode of vibration of the methyl group in coated MNPs was found in 1388 cm^{-1}. Similarly, a single band in the coated nanoparticles at 962 cm^{-1} is attributed to the C–N mode of vibration[3]. Similarly, the band between 1675 cm^{-1} to 1600 cm^{-1} is due to the remaining trace amount of H_2O in the sample[21].

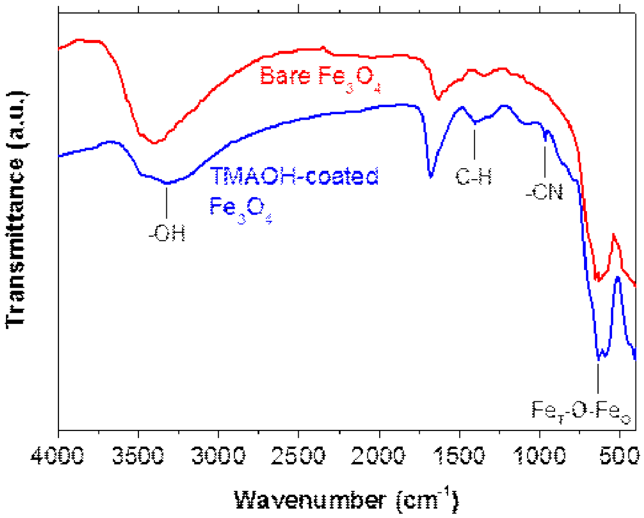


Fig. 5. FTIR spectra of bare Fe_3O_4 and TMAOH-coated MNPs sample

The FTIR spectra provide information about the nature of the chemical bonds in a substance but do not give any information about the structural details of the crystalline solids. Neither does this technique offer evidence about the type and dimensions of the nanoscopic texture of the material. Powder X-ray diffraction and electron microscopy[18] provide structural information on the integral and localized scales.

The XRD patterns of the MNPs synthesized by the co-precipitation and sol-gel methods are shown in Fig. 6. The diffraction patterns are consistent with the standard assigned for the cubic crystalline phase. The characteristic XRD peaks are observed at 2è values ca. 32°, 37°, 44°, 58°, and 63° which corresponds to (200), (311), (400), (511), and (440) Miller reflection planes, respectively. The peaks appearing in Fig. 6 at identical positions can be well indexed to the inverse cubic spinal structures of MNPs[19]. The presence of sharp peaks also suggests that MNPs have well defined crystalline texture. The average crystallite size of the MNPs was calculated using Debye-Scherer's formula which is given in Equation (1).

$$t = \frac{K\lambda}{B \cdot \cos\theta} \tag{1}$$

where, t is the average crystallite size (nm), K the shape factor (K=0.9 for most of the spherical crystals), λ is the wavelength of the X-rays used (λ=0.154056 nm for CuKα radiation), B is the full-width at half maximum (FWHM) and θ is the Bragg's diffraction angle. The crystalline size of the magnetite particles was found to be 13 nm and 8 nm for the co-precipitation and sol-gel methods, respectively. Thus, the XRD analyses confirmed that the materials prepared contained the nanoparticulate structures with grains having a diameter in the order of 10 nm.

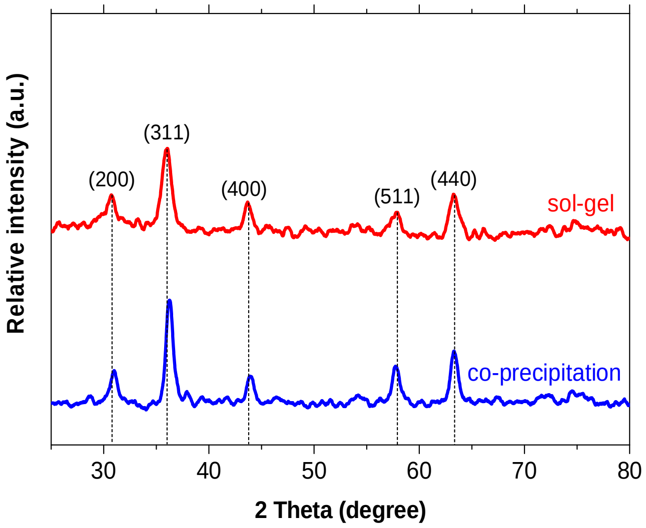


Fig. 6. Powder XRD patterns of MNPs synthesized by co-precipitation and sol-gel methods

As already mentioned, the XRD method gives an integral idea of the crystalline morphology of the materials. A slight change in the diffraction peak to a lower value of 2θ is due to the slightly higher Fe^{+4} content in the sample[8]. However, the local morphology of the same material may show a different picture. To study the microscopic texture of the MNPs, the specimens were imaged by a scanning electron microscope equipped with the field emission gun cathode which offers the structural analyses of the materials in the native state with a pretty high resolution. The results obtained for the MNPs prepared by the co-precipitation method are presented in Fig. 7.

The micrographs presented in Fig. 7 show that in contrast to the XRD results, there is a wide disparity in particles size, ranging from about 100 nm to several microns in diameter. The formation of large particles is the result of the agglomeration of the primary nanoparticles during the synthesis of MNPs as no capping agents were used. It can be further revealed that both the bigger and the smaller particles are composed of granular structures which indeed represent the primary nanoparticles with the diameter in the order of 10 nm thus correlating with the XRD result.

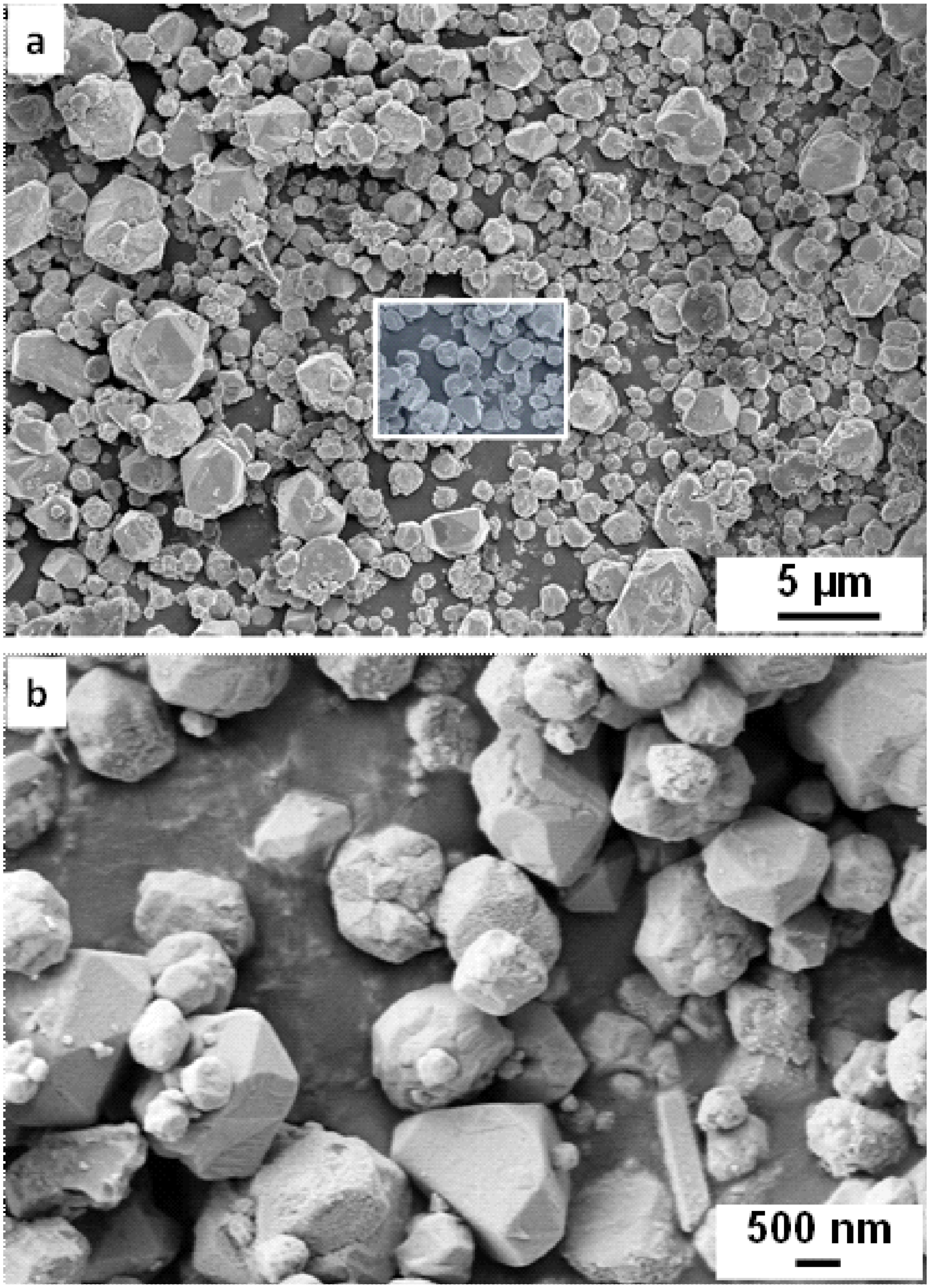


Fig. 7. Lower (a) and higher (b) magnification FESEM images of the MNPs synthesized by co-precipitation method; the area indicated by the white rectangle in the top image is magnified in the lower image.

Another interesting feature observed in Fig. 7 is that almost all of the particles exhibit sharp-edged cubic-like geometric texture[26], which is also in coherence with the XRD results. Fig. 7 shows different projections of the cubic crystalline texture of the magnetite.

Conclusions

The present work aims at investigating the chemical pathways towards synthesizing the pure MNPs using a simple, efficient and economic method. The MNPs were successfully synthesized via the chemical co-precipitation route by using soluble Ferrous and Ferric salts.

Handy wet chemical methods for synthesizing highly crystalline MNPs in a fairly pure state in an ordinary chemistry laboratory and their characterization using simple methods (complemented by advanced tools) are demonstrated.

The substance was coated with a primary layer of tetramethylammonium hydroxide (TMAOH) to increase the stability and dispensability of the nanoparticles. The nanoparticles remained stable for more than a year.

The XRD results show that the MNPs thus obtained were pure and crystalline. The electron microscopic results demonstrated the cubic-like texture of the materials with agglomerated texture.

The method discussed in this work can be employed for the synthesis of MNPs in an ordinary Chemistry laboratory, such as in senior high schools for enhancing the enthusiasm of new generation scientists towards nanoscience and nanotechnology.

References

1. Ain, Q. U., Zhang, H., Yaseen, M., Rasheed, U., Liu, K., Subhan, S. and Tong, Z. 2020), *Journal of Cleaner Production*, **247**, 119088. https://doi.org/10.1016/j.jclepro.2019.119088
2. Al-Alawy, A. F., Al-Abodi, E. E. and Kadhim, R. M., 2018, *Journal of Engineering*, **24(10)**, 60. https://doi.org/10.31026/j.eng.2018.10.05
3. Andrade, Â. L., Fabris, J. D., Ardisson, J. D., Valente, M. A. and Ferreira, J. M. F., 2012, *Journal of Nanomaterials*. https://doi.org/10.1155/2012/454759
4. Andrade, Â. L., Fabris, J. D., Ardisson, J. D., Valente, M. A. and Ferreira, J. M. F., 2012, *Journal of Nanomaterials*. https://doi.org/10.1155/2012/454759
5. Cheng, F. Y., Su, C. H., Yang, Y. S., Yeh, C. S., Tsai, C. Y., Wu, C. L., Wu, M. T. and Shieh, D. Bin. 2005, *Biomaterials*, **26(7)**, 729–738. https://doi.org/10.1016/j.biomaterials.2004.03.016
6. Daniel-da-Silva, A. L., Trindade, T., Goodfellow, B. J., Costa, B. F. O., Correia, R. N. and Gil, A. M., 2007, *Biomacromolecules*, **8(8)**, 2350–2357. https://doi.org/10.1021/bm070096q
7. Dhakal, K. N., Aryal, G. M., Adhikari, H. S. and Adhikari, R., 2020, Facile Synthesis of Magnesium-Aluminium Layered Double Hydroxides (Mg-Al LDHs).
8. Duan, Y., Fu, N., Liu, Q., Fang, Y., Zhou, X., Zhang, J. and Lin, Y., 2012, *Journal of Physical Chemistry C*, **116(16)**, 8888–8893. https://doi.org/10.1021/jp212517k
9. Eastoe, J., Hollamby, M. J. and Hudson, L., 2006, *Advances in Colloid and Interface Science*. https://doi.org/10.1016/j.cis.2006.11.009
10. Gahrouei, Z. E., Labbaf, S. and Kermanpur, A., 2020, *Physica E: Low-Dimensional Systems and Nanostructures*, **116**, 113759. https://doi.org/10.1016/j.physe.2019.113759
11. Ghimire, S., Lebek, W., Godehardt, R., Lee, W. I. and Adhikari, R., 2016, *Journal of Nanoscience and Nanotechnology*, **16(5)**, 5200–5206. https://doi.org/10.1166/jnn.2016.12155

12. Hamed Sadabadi, A. A., 2015, *Journal of Petroleum & Environmental Biotechnology*, **06(01)**, 1–3. https://doi.org/10.4172/2157-7463.1000200

13. Harold P. and Klug, L. E. A., 1974, X-Ray Diffraction Procedures: For Polycrystalline and Amorphous Materials, 2nd Edition - NASA/ADS.

14. Khan, U. S., Amanullah, Manan, A., Khan, N., Mahmood, A. and Rahim, A., 2015, *Materials Science- Poland*, **33(2)**, 278–285. https://doi.org/10.1515/msp-2015-0037

15. Kumari, M., Gupta, R. and Jain, Y., 2019, *Applied Organometallic Chemistry*, **33(11)**, 1–11. https://doi.org/10.1002/aoc.5223

16. Lamichhane, R., Ghimire, S. and Adhikari, R., 2020, Facile Method of Synthesis and Characterization of Antimicrobial Copper Nanoparticles in Ordinary Laboratory.

17. Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L. and Muller, R. N., 2008, *Chemical Reviews*, **108(6)**, 2064–2110. https://doi.org/10.1021/cr068445e

18. Lin, P. C., Lin, S., Wang, P. C. and Sridhar, R., 2014, *Biotechnology Advances*, **32(4)**, 711–726. https://doi.org/10.1016/j.biotechadv.2013.11.006

19. Lopez, J. A., González, F., Bonilla, F. A., Zambrano, G. and Gómez, M. E., 2010, *Revista Latinoamericana de Metalurgia y Materiales*, **30(1)**, 60–66. https://doi.org/10.1016/j.matchemphys.2008.10.039

20. M. Awwad, A. and M. Salem, N., 2013, *Nanoscience and Nanotechnology*, **2(6)**, 208–213. https://doi.org/10.5923/j.nn.20120206.09

21. Ma, M., Zhang, Y., Yu, W., Shen, H., Zhang, H. and Gu, N., 2014, *Applied Mechanics and Materials*, **618**, 24–27. https://doi.org/10.4028/www.scientific.net/AMM.618.24

22. Malik, M. A., Wani, M. Y. and Hashim, M. A., 2012, *Arabian Journal of Chemistry* **5(4)**, pp. 397–417). https://doi.org/10.1016/j.arabjc.2010.09.027

23. Mascolo, M. C., Pei, Y. and Ring, T. A., 2013, *Materials*, **6(12)**, 5549–5567. https://doi.org/10.3390/ma6125549

24. Noh, J., Osman, O. I., Aziz, S. G., Winget, P. and Brédas, J. L., 2014, *Science and Technology of Advanced Materials*, **15(4)**. https://doi.org/10.1088/1468-6996/15/4/044202

25. Nsom, M. V., Etape, E. P., Tendo, J. F., Namond, B. V., Chongwain, P. T., Yufanyi, M. D. and William, N., 2019, *Journal of Nanomaterials*. https://doi.org/10.1155/2019/4576135

26. Onyenwoke, R. and Wiegel, J., 2014, Magnetite/ : Structure, Properties and Applications.

27. Pandey, G., Dhakal, K. N., Singh, A. K., Dhungel, S. K. and Adhikari, R., 2020, *BIBECHANA*, **18**, 96–103.

28. Petcharoen, K. and Sirivat, A., 2012, *Materials Science and Engineering B: Solid-State Materials for Advanced Technology*, **177(5)**, 421–427. https://doi.org/10.1016/j.mseb.2012.01.003

29. Rashid, M., Price, N. T., Gracia Pinilla, M. Á. and O'Shea, K. E., 2017, *Water Research*, **123**, 353–360. https://doi.org/10.1016/j.watres.2017.06.085

30. Sara Shaker., Shirzad Zafarian., Shilpa Chakra and Venkateswawa Rao, 2013, *International Journal of Innovative Research in Science, Engineering and Technology*, **2(7)**, 2969–2972. https://doi.org/10.1016/j.arabjc.2010.06.061

31. Sayahi, H., Mohsenzadeh, F., Darabi, H. R. and Aghapoor, K., 2019, *Journal of Alloys and Compounds*, **778**, 633–642. https://doi.org/10.1016/j.jallcom.2018.11.186

32. Schwaminger, S. P., Bauer, D., Fraga-García, P., Wagner, F. E. and Berensmeier, S., 2017, *CrystEngComm*, **19(2)**, 246–255. https://doi.org/10.1039/c6ce02421a

33. Sen, T., Magdassi, S., Nizri, G. and Bruce, I. J., 2006, *Micro & Nano Letters*, **1(1)**, 39. https://doi.org/10.1049/mnl:20065033

34. Sun, S. and Zeng, H., 2002, *Journal of the American Chemical Society*, **124(28)**, 8204–8205. https://doi.org/10.1021/ja026501x

35. Tian, Y., Yu, B., Li, X. and Li, K., 2011, *Journal of Materials Chemistry*, **21(8)**, 2476–2481. https://doi.org/10.1039/c0jm02913k

36. Vats, M., Kumar, R., Sharma, J. and Luwang, M. N., 2020, *Indian Journal of Pure and Applied Physics*, **58(1)**, 31–35.

37. Wasilewski, P. and Kletetschka, G., 1999, *Geophysical Research Letters*, **26(15)**, 2275–2278. https://doi.org/10.1029/1999GL900496

38. Zhao, F. Y., Li, Y. L. and Li, L. H., 2014, *Applied Mechanics and Materials*, **618(7)**, 24–27. https://doi.org/10.4028/www.scientific.net/AMM.618.24

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Synthesis, Characterization and Biological Activity of Zirconium Metal Complexes Derived from Novel Salens

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ABSTRACT
A new series of Zirconium metal complexes were synthesized with different hydrazone derivatives which was prepared[1] by condensation of 2-Hydroxy benzoyl hydrazide with substituted aromatic aldehydes, viz. Salicylaldehyde, 5-Bromo Salicylaldehyde, 5-Chloro Salicylaldehyde, 5-Methoxy Salicylaldehyde and 5-Hydroxy Salicylaldehyde. The Zirconium metal complexes were characterized by using different physico-chemical techniques like elemental analysis, molar conductance, magnetic susceptibility, electronic absorption, IR, XRD and TG analysis. Their biological activities against some bacterial and fungal strains have been studied.

Keywords: *Zirconium, hydrazide, XRD, aromatic aldehyde, biological activity.*

Introduction

Coordination compounds are very important to the chemist and there has been rapid development in Coordination Chemistry due to its versatile applications. Several coordination complexes have been synthesized and investigated. Transition metal complexes with soft and hard donor groups[2] have been used extensively in Coordination Chemistry. The Coordination Chemistry of hydrazones has been a subject of research because of their physiological activity, coordination capability and wide range of applicatons[3-6] in biological, pharmaceutical, analytical, catalytic and industrial fields, especially those derived from aldehydes and ketones. Hydrazone ligands and/or their metal complexes have a wide range of applications because of their antimicrobial, antitubercular, antitumour, analgesic and antiinflammatory, anti-HIV, anthrax lethal factor inhibitory, antidiabetic and antimalarial properties[7].

Materials and Methods

Materials

All the chemicals used were of the analytical grade (AR) and with of highest purity. Zirconium Nitrate was obtained from Sigma-Aldrich Chemicals and used directly without further purification. Liquids like Ethanol whenever used were distilled and purified according to standard procedure[8].

Methods

Preparation of Zirconium Metal Complexes

The synthesis of metal complexes was done by using 1:2 proportion of Zirconium Nitrate (0.01M) and hydrazone ligand (0.02M) in ethanol. The resulting reaction mixture was refluxed for 3 hrs and then cooled at room temperature. To the clear solution, an aqueous NH_3 solution was added drop wise with constant stirring till the pH was in between 8.5-9.0. The mixture was further refluxed for 1hr and cooled to room temperature. The product was isolated by filtration. The complexes thus obtained were washed repeatedly with solvent ethanol and finally dried in an oven at 110°C. The yield was above 80%.

Antimicrobial and antifungal activity

The invitro antimicrobial screening effects of the synthesized Hydrazones and their metal complexes were studied against three bacterial strains namely *Bacillus, E. coli, S. aureu* and two fungal strains namely *Candida and Aspergillus* by paper disc diffusion method using nutrient agar medium for antimicrobial activity and Sabouraud dextrose agar for antifungal activity.

Results and Discussions

The Zirconium complexes synthesised are pale yellow solids, soluble in solvents like DMF and DMSO. The complexes are non-hygroscopic and thermally stable above 200°C indicating a strong metal-ligand bonding. The physical and elemental analysis data of the metal complexes is shown in Table-1. The values observed for molar conductance of all Zirconium metal complexes fall between 0.0005-0.0011 $mhos.cm^2mol^{-1}$ in 10^{-3}M solution which indicates that all the complexes are non-electrolyte in nature.[9]

Zirconium metal chelates are diamagnetic in nature, as expected due to the absence of unpaired electrons in the outermost orbital of Zirconium according to its electronic configuration $4d^0$, $5s^0$ and also reduction of Zirconium ion does not takes place under influence of the ligand. The electrons are paired because of strong conjugation in the chelate ring.

The electronic spectra of Zirconium complexes were recorded in DMF solution (10^{-3}M). The electronic absorption studies of Zr(IV) complexes do not show any characteristic transitions due to the absence of unpaired electron. The complexes of Zr(IV) shows only charge transfer transitions between metal and ligand (22630, 21593 in cm^{-1}) and intra-ligand transition bands (32645, 28789, 25742 in cm^{-1}) which is in accordance with the results revealed by magnetic susceptibility studies i.e. diamagnetic nature of the complexes. The electronic spectra of Zr(IV) chelates in comparison with ligand spectra shows that the peaks of the complexes are deep and intense and hence support complex formation.

Table 1: Physical and analytical data of Metal Complexes

Complexes	Molecular Formula (Formula Wt.)	% Elemental analysis Found (calculated)				Electrical conductance $mhos.cm^2mol^{-1}$
		Zr	C	H	N	
Zr_1	$[C_{28}H_{20}N_4O_6Zr]$ (599.70)	15.21 (15.18)	56.08 (56.09)	3.36 (3.33)	9.34 (9.35)	0.0005
Zr_2	$[C_{28}H_{18}Br_2N_4O_6Zr]$ (757.49)	12.04 (12.01)	44.40 (44.38)	2.40 (2.42)	7.40 (7.37)	0.0010
Zr_3	$[C_{28}H_{18}Cl_2N_4O_6Zr]$ (668.59)	13.64 (13.63)	50.30 (50.26)	2.71 (2.73)	8.38 (8.36)	0.0008
Zr_4	$[C_{30}H_{24}N_4O_8Zr]$ (659.75)	13.83 (13.84)	54.61 (54.60)	3.67 (3.69)	8.49 (8.47)	0.0011
Zr_5	$[C_{28}H_{20}N_4O_8Zr]$ (631.70)	14.44 (14.41)	53.24 (53.20)	3.19 (3.21)	8.87 (8.85)	0.0011

The FTIR spectrum of the chelating ligand shows four characteristics bands around 3600-3142 cm^{-1}, 3246-3000cm^{-1}, 1650-1623 cm^{-1}, and 1618-1614 cm^{-1} assignable to (-NH), free (-OH) stretching, amide carbonyl (-C=O) and azomethine (-C=N) stretching mode respectively. In the FTIR spectrum (Table-2), the absence of a weak broad band in the region 3197-3042 cm^{-1}, in the spectra of the metal chelate indicates deprotonation of -OH group during complex formation and subsequent coordination of the oxygen of amide carbonyl to the metal ion[10]. The υ(C=N) band is shifted to lower wavenumber with respect to free ligand, indicating that the nitrogen of azomethine group is coordinated to metal ion, which was further confirmed by observation of the red shift in the υ(N-N) stretching frequency from 813 to 976 cm^{-1} region[11-13]. The spectra of metal complexes showed new bands at 545 to 656 cm^{-1} and 556 to 567 cm^{-1} for υ(Zr-O) and υ(Zr-N) vibrations respectively[6]. Hence, coordination takes place via oxygen of amide, nitrogen of azomethine group and oxygen of hydroxyl group of ligand molecules and support coordination number 8 around Zirconium in the present complexes[13-15].

Table 2: Important IR Spectral Bands (cm^{-1}) of the Complexes

Assignment	Zr_1	Zr_2	Zr_3	Zr_4	Zr_5
ν (C=N)	1552	1549	1543	1525	1548
ν (C=O)	1606	1608	1607	1602	1607
ν (Zr-O)	645	654	656	650	654
ν (Zr-N)	560	556	558	564	567

The powder X-ray diffraction pattern of Zirconium metal chelates are shown in Figures **1a** to **1e**. The $d_{h k l}$ and 2θ values of difractograms were compared with the values recorded in literature (JCPDS). The XRD pattern confirms single phase crystalline nature of all metal chelates of Zirconium. The sharp and highly intense peaks suggest that the prepared chelates are crystalline in nature.[16]

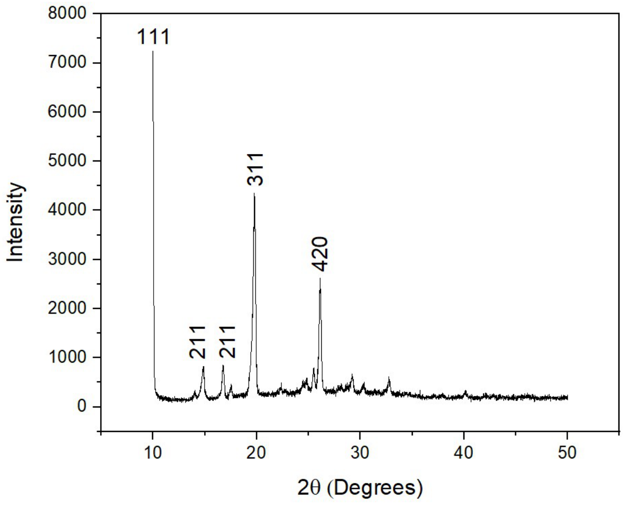


Fig.1a: XRD of metal complex Zr_1 [$C_{28}H_{20}N_4O_6Zr$]

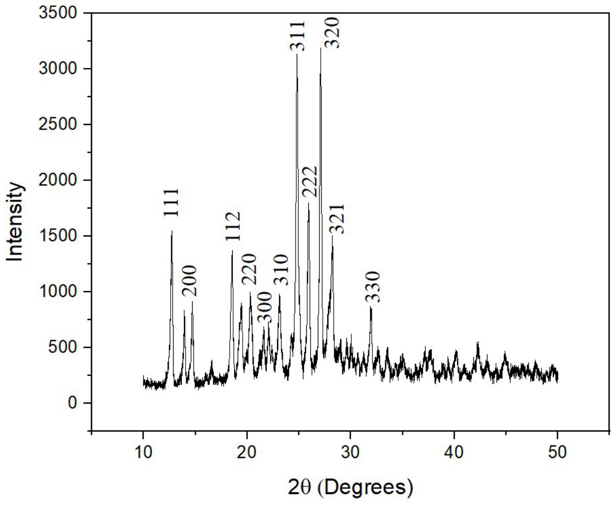


Fig.1b: XRD of metal complex Zr_2 [$C_{28}H_{18}Br_2N_4O_6Zr$]

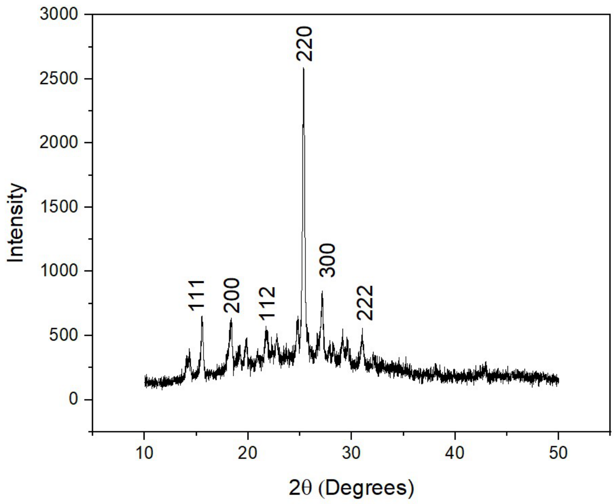


Fig.1c: XRD of metal complex Zr_3 [$C_{28}H_{18}Cl_2N_4O_6Zr$]

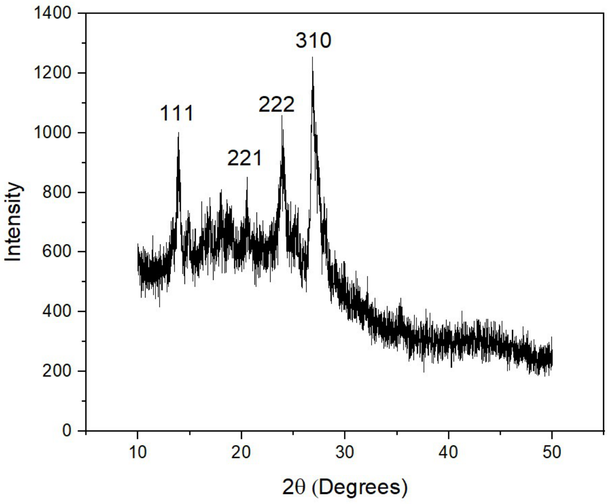


Fig.1d: XRD of metal complex Zr_4 [$C_{30}H_{24}N_4O_8Zr$]

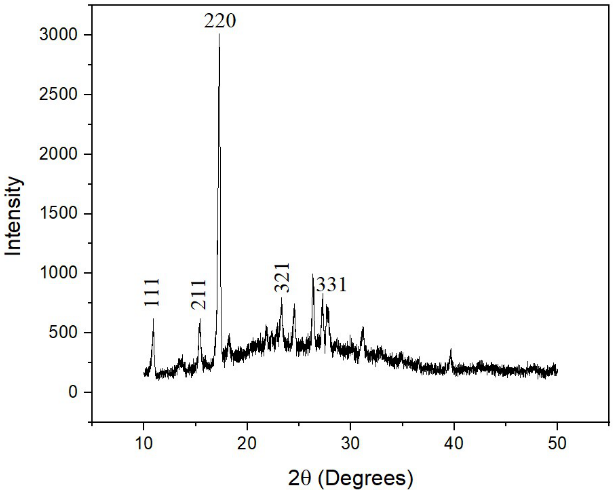


Fig. 1e: XRD of metal complex Zr_5 [$C_{28}H_{20}N_4O_8Zr$]

The Thermo gravimetric (TG) analysis of the present metal complexes was carried out in the inert atmosphere of nitrogen with a heating rate of 10°C per minute. The Thermographs (Figures **2a-2e**) are single step indicating continuous mass loss of the ligand part with increasing temperature. The metal chelates are thermally stable and decompose above 200°C and gives Zirconium oxide as the ultimate product of heating above 850°C.

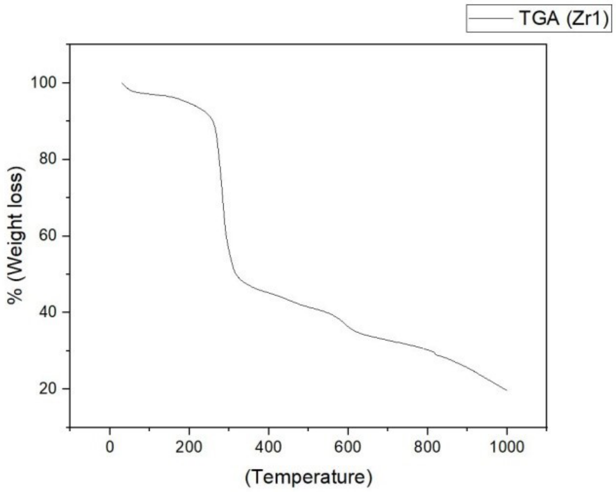


Fig 2a: TG of metal complex Zr_1 [$C_{28}H_{20}N_4O_6Zr$]

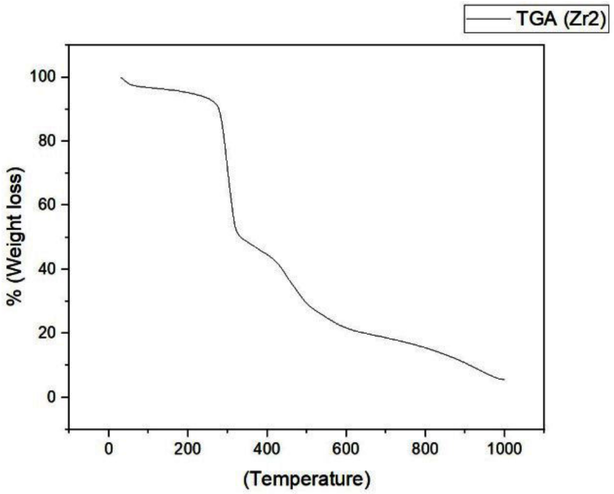


Fig 2b: TG of metal complex Zr_2 [$C_{28}H_{18}Br_2N_4O_6Zr$]

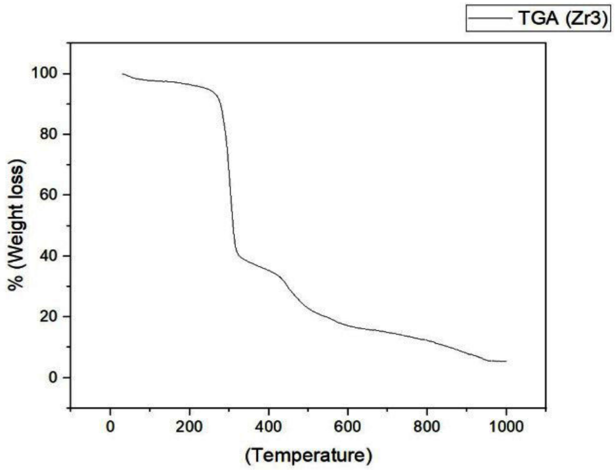


Fig 2c: TG of metal complex Zr_3 [$C_{28}H_{18}Cl_2N_4O_6Zr$]

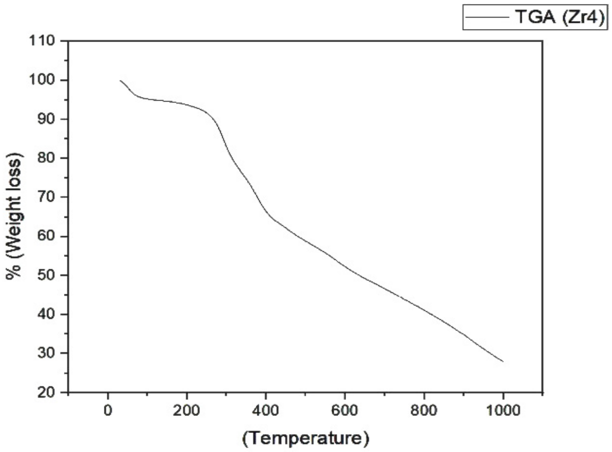


Fig 2d: TG of metal complex Zr_4 [$C_{30}H_{24}N_4O_8Zr$]

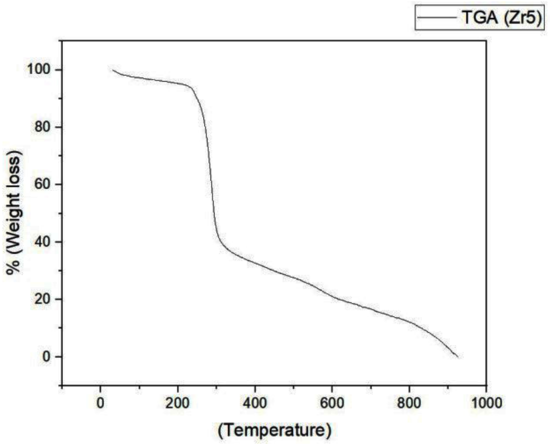
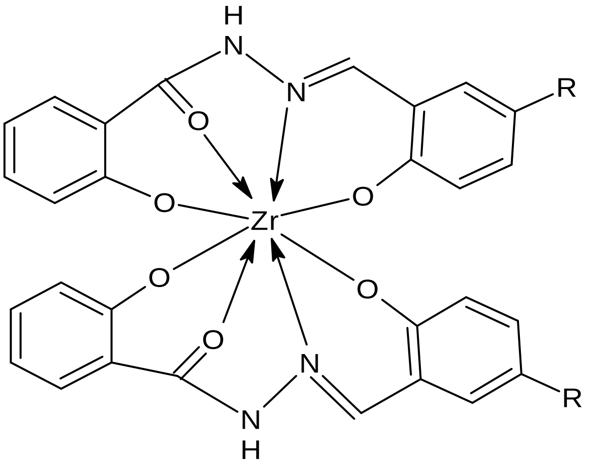


Fig 2e: TG Pattern of metal complex Zr_5 [$C_{28}H_{20}N_4O_8Zr$]

On the basis of the above results the following bonding structure was proposed for the Zirconium metal complexes.



where, R= Zr_1= -H, Zr_2= -Br, Zr_3=-Cl, Zr_4= $-OCH_3$, Zr_5=-OH

Fig. 3: The proposed structure of Zirconium metal complexes

Table 3: Biological activity of hydrazone derivatives and their Zirconium metal complexes

Compound	Antibacterial activity (Zone of inhibition in mm)			Antifungal activity (Zone of inhibition in mm)	
	1	2	3	4	5
L_1	6	9	4	18	4
L_2	4	9	5	8	4
L_3	-	4	4	10	6
L_4	-	6	4	5	7
L_5	15	8	9	4	4
Zr_1	6	7	10	6	4
Zr_2	11	7	22	8	6
Zr_3	9	8	9	7	4
Zr_4	6	9	8	9	5
Zr_5	6	10	8	4	4
Tetracycline	16	26	18	-	-
Amphotericin	-	-	-	19	13

[Where, 1= *E. coli*, 2- *S. aureu*, 3- *Bacillus*, 4- *Candida*, 5- *Aspergillus*]

The in vitro antimicrobial screening results are shown in Table-3. The antimicrobial activity of all the hydrazone ligands and their Zirconium metal chelates is less that of standard tetracycline and amphotericin. On the basis of the observed zone of inhibition, it was found that some zirconium metal chelates are more active than their respective hydrazone ligands and also that hydrazone ligands and zirconium metal chelates both shows good antimicrobial activities.

References

1. Lad, M. N., Patil, R. M. and Sathe, G. B., 2020, *Res. J. Chem. Envi.*, **24(6)**, 6.
2. Sarkar A. R. and Mandal, S., 2000, *Synth. React. Inorg. Met-Org Chem*., **80**, 1477.
3. Heravi, M. M., Ranjbar, L., Derikvand, F., Oskooie, H. A. and Bamoharram, F. F., 2007, *J. Mol. Catal.* A: Chem., **265**, 186.
4. Narang, K. K. and Singh, V.P., 1997, *Synth. React. Inorg. Met.-Org. Chem.*, **27**, 721.
5. Naik, V. M., Sambrani, M. I. and Mallur, M. B., 2008, *Indian. J. Chem.*, **47**, 1793.
6. Shelke, V. A., Jadhav, S. M., Shankarwar, S. G., Munde, A. S. and Chondhekar, T. K., 2011, *Chem. Soc. Ethiop.*, **25(3)**, 381.
7. Harinath, Y., Harikishore Kumar Reddy, D., Naresh Kumar, B., Lakshmi K. and K. Seshaiah, 2011, *J. Chem. Pharm. Res.*, **3(1)**, 698.
8. Furnis, B. S., Hannaford, A. J., Smith P. W. G. and Tatchell, A. R., 1989, Vogel’s Textbook of Practical Organic Chemistry, 5th Ed. ELBS Longman London.
9. Geary, W. J., 1971, *Coord. Chem. Rev*. **7(1)**, 81.
10. Baliger R. S. and Revankar, V. K., 2006, *J. Serb. Chem. Soc*., **71**, 1301.
11. Hueso-Urena, F., Illan-Cabeza, N. A., Moreno-Carretero M. N. and Penans Chamorro, A. L., *Acta Chem. Solv*., **47**, 481.
12. Narang K. K. and Aggarwal, A., 1974, *Inorg. Chim. Acta,* **9**, 137.
13. Chandra, S., Tyagi M. and Refat, M., 2009, *J. Ser. Chem. Soc*., **74**, 907.
14. Nakamoto, K., 1986, Infrared and Raman spectra of Inorganic and Coordination Compounds, 4th Ed. John-Wiley and Sons, N.Y.
15. Nakamoto, K., Morimoto Y. and Martell, A. E., 1961, *J. Am. Chem. Soc.;* **83**, 4528.
16. Cullity, B., 1978, Elements of X-ray Diffraction, second ed., Addison-Wesley, Reading, MA.

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Screening of Green Energy (Biofuel) Implementation and Future Prospective in India: An Overview

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ABSTRACT
Energy security looms as one among the vital challenges of this century for scientists and users. For a developing country like India, sustainable sources of energy are the backbone of economical growth. India is the fifth biggest power user globally, and by 2030, it is predicted to overtake Russia, Brazil, and Japan to be the third highest. Also, increasingly India is reliant on fossil fuel consumption, which results in greenhouse emissions and other issues. Conventional resource planning has also increasingly crumbled, leading to disparities in energy resource utilization. Therefore, we need to focus on new sources of energy, which has led to the exploration of renewable, environment friendly, socially accepted to the communities and non-conventional fuel sources. Through the advent of bioenergy technology development, it has become feasible to combat climatic changes without relying on non-renewable coal and oil resources. The Indian government is indeed assisting research and academic institutions, companies, and non-governmental organisations in exploring and implementing technical developments that would increase the production of biofuel. The present paper critically reviews the current situation, technologies involved, biofuel policy in India, and future perspectives.

Keywords: *India, Biofuel, Energy, Environment, R&D Technology, Policy.*

1. Introduction

According to the Energy Technology Perspectives Report[1] of International Energy Agency released in the year 2008, the BLUE Map scenario aims to achieve a half reduction in world carbon dioxide diffusion before 2050. The IPCC estimates that the pollutant diffusion must be cut by 50–85% to maintain temperature rise globally around 2°C and 2.4°C[2]. According to the scenario in 2050, biomass contributes for roughly 23% of global total predominant energy and becomes a significant source of energy. Solar and wind energy will account for 11% and 12% of worldwide electricity output respectively. With the rise in anthropogenic Green House Gas (GHG) discharge, owing primarily to the widespread use of fossil energy, heat energy generation production, and transportation fuels, it is becoming increasingly vital to build abatement methods and guidelines for mitigating the consequences of these emissions[3]. Climate change forecasts might have substantial ramifications for both environment and human functions, raising doubts about the long-term viability of present non-renewable source of energy usage, mostly because of resource finiteness, and also due to the detrimental impacts of CO_2 emissions[4]. Since pre-industrial ages (past 420,000 years), scientists have observed a significant rise in global GHG levels, caused by human actions, primarily the fossil fuel combustion and, deforestation to a smaller extent[2]. Following USA, Japan, and China, India is the 5th largest main energy user and 4th biggest gasoline consumer globally. As a result, it should concentrate on the production of new fuel origins, which might lead to the investigation of environmentally benign renewable energy origins. With worries about high energy costs, oil supply unpredictability, and greenhouse gas emissions, energy generated from biological entities, particularly biofuels, has gained a lot of interest in the last several years[5, 6].

The attention given to biofuels by researchers, non-governmental organizations (NGOs), industries and media is directly reflecting the level of concern over the impact of biofuel policies and production on people in developing countries. This concern has also been incorporated into regulations for renewable fuel targets, use of biofuels and social impacts under the Renewable Energy Directive (RED) European Commission[7].

First generation biofuels rely on consumable feedstock like sugar and starch, which raises production costs and results in depletion of natural resources and resources consumed in crop cultivation. Consumable feedstock, in particular, conflicts with food plants, necessitates the use of a lot of fertilizer and water, and takes up a lot of acreage. The second generation of biofuels is focused on more sustainable renewable sources, like switch hay, sawdust, low-cost timber, agriculture wastes, and sludges, which are indigestible hemicelluloses biomass. Third generation biofuels employ aquatic biomass like algae that are photosynthetic organisms collecting large amounts of CO_2 and produce both O_2 and oil[8]. To boost biofuel output, the fourth generation biofuel employs genetically altered algae. Despite the fact that genetically modified algae biofuel would be a familiar alternate form to fossil fuels, the possible health and environmental dangers remain a major issue. An assessment of these problems and, as a result, the development of effective mitigation techniques to address them is critical to commercialization. While much study was done on genetic alteration and some other methods aimed at increasing the yield of algal strains, only a few of them have addressed the legal restrictions placed on the genetically altered algae harvest and process[9]. Various generation of biofuel production, the different feedstocks used in the particular generation and the impacts of each generation is depicted in Figure 1[10].

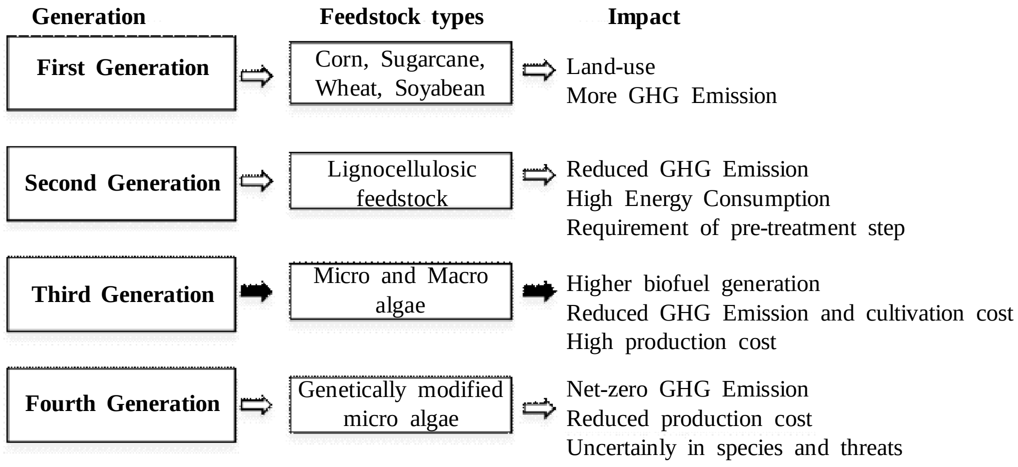

Figure 1. Different generations, feedstocks and the impact of Biofuel production

Technically speaking, Biodiesel is a naturally “oxygenized” biofuel that is created entirely of edible oil and contains over 10% oxygen. It is produced by the transestrification method in presence of a catalyst. Burning fuels made from edible oils does not add any more CO_2 to the environment. Using Green oils (Bio-diesel) is therefore more beneficial to the environment and economy of a developing country like India due to its nature-portability, easy access, renewability, improved combustion efficiency, reduced sulphur and aromatics, higher point of flash, greater biodegradability, and less reliance on imported gasoline[11]. Therefore, the present study focusses on the infancy of biodiesel industry, demand and production, R&D technological breakthroughs require policy support, community and local interest, stimulus packages, future and cost-effective feedstock alternatives in India.

2. The infancy of biofuel industry in an India

India’s crude oil requirements are mainly reliant on foreign markets. India now imports 82% of its crude oil needs, making the nation vulnerable to global price changes. India’s energy strategy calls for a shift to more alternative energy sources. Increased usage of domestically generated biofuels helps to save considerable amounts of foreign currency while also rejuvenating the domestic economy via economic possibilities across the supply chain[12].

Biofuel programme in India serves as a model for other countries looking to develop renewable and sustainable energy sources. The demand for feedstock and rising production costs are the two biggest roadblocks to achieving the goal of ethanol production. There are two strategies to boost ethanol output. One method is to employ lignocellulosic feedstock that is already abundant and inexpensive. Another method is to use biochemical or genomic engineering to change the content of the feedstock. Furthermore, an incorporated systematic approach necessitates a review of biomass supply as well as changes in biomass content for a consistent supply of feedstock[13].

The availability of a large area of degraded land in India might be utilized to grow energy crops. The agroforestry leftovers are abundant and offer tremendous energy and bioenergy potential. Agroforestry leftovers have their own significance that cannot be overlooked, since they are now the only true biomass resources accessible. The exploitation of biofuel capability would almost certainly benefit India, since it is expected that increased local production of the alternative renewable diesel substitute will cut oil imports and emissions[14].

A large number of industrial plants are present that can process bio-ethanol, but very few are capable of producing biodiesel. Europe, U.S.A, Brazil, China, and India are the major countries involved in the research and development of biofuels. Biodiesel 2020 analyses worldwide biodiesel market expansion metrics and estimates of biodiesel output for the next 5 to 10 years. Proprietary projections are also available to create “circumstances” for European countries, the United States, China, India, and Brazil in 2020. Different outlooks and circumstances are also presented for the migration from first to second generation biofuel programs in developing and developed nations, as well as growing markets[15]. Commercial manufacture and distribution of Jatropha-based biofuel is still limited in India, around 1,400 and 3,000 lakh liters every year. This modest amount of biofuel is supplied to pilot programs run by automotive manufacturers and transportation businesses, as well as to the underfunded sector, which includes mobile antennas, furnaces, irrigation pumps, agricultural applications, diesel generator proprietors etc. However, according to reports from industry and research surveys, no commercial supply of biofuel to state-owned transportation firms has been documented, with the exception of experiments[16, 17].

3. Demand and production of biofuel in India

The Planning Commission, Government of India has a model for the establishment of biofuel generation in two stages. A demo project was worked out in stage I during 2003 and 2007. The advancement of Jatropha oilseed seedlings, the growth of 4 Lakh hectares of Jatropha, the establishment of seed acquisition and Jatropha oil abstraction hubs, and the construction of eighty thousand Mt/year transesterification plants to generate biofuels from Jatropha oil were included in this mission. Stage II included an extension of the programme that will result in the creation of biofuel to fulfil 20% of the nation's diesel needs by 2011-12[18]. However, according to the 2014 India Gain Report, local Indian ethanol output in 2015 is expected to reach 21000 million liters, with three fourth of that going to the consumable spirits sector, a modest increase from over 2000 million liters in 2014[17].

4. Research and Development technologies involved in biofuel production

4.1. Algal based

Researchers around the world identified algae as a feedstock for biofuel production over 40 years back. But the actual usage started around ten years ago. A lot of advancement has taken place in this field by the development of an integrated system for large scale algae culture production with industrial effluents and fertilizers input. However, major challenges for economical production of biofuel are high cost of fertilizers, continuous production with culture integrity, better understanding of anti-flocculation and purification processes to name a few. Christenson et al. tried to investigate the applications of sewage water[19], as raw material for the production of algal. Woertz et el., reported and stu-died the use of dairy and municipal waste[20], to tackle high fertilizer cost for cost effective biofuel production. They reported 14-29% peak lipid content, relying on sewage water aggregation. Highest volumetric capacity corresponding to 11,000 lakhs per hectares per year, if continued through the year has been reported by them. Orthophosphate and ammonium removal from the waste has also been reported. Genetic manipulations and recombinant DNA technology have enabled researchers to use an improved variety of algal cultures to tackle culture integrity.

Algal productions methods available are

i. Suspended Cultures
    a. Open ponds
    b. Closed reactors

ii. Immoblized cultures
    a. Matrix- immobilized microalgae
    b. Algal biofilms

At present in India, large scale production is only possible with suspended algal culture methods. Among them, open raceways ponds are being successfully implemented. However, closed reactors are also being employed for pilot level production. Immobilized culture methods are in infancy stage but, most of the academic and research laboratories are trying to explore ways for improvement of efficiency and cost effectiveness.

4.2 Pyrolysis

Pyrolysis is the term referring to the thermic degradation of biomass in the absence of oxygen or combustion deficient materials[21]. Ideally, solid biomass conversion into biofuel through a single step process within a short residence time is generally preferred. Pyrolysis and gasification are different processes. By properly managing the quantity of oxygen available, biomass is decomposed into syngas. Currently, the term "pyrolysis" is used to designate operations wherein oils are the chosen end product.

Feasible pyrolytic process requirements are as given below:

- To raise the temperature within the pyrolytic chamber from a heat source.
- At this high temperature, the major pyrolysis events begin to liberate volatiles and generate char, followed by autocatalytic subsidiary pyrolysis process.
- Further thermal decomposition, reforming, purification, and further dehydration, by keeping residence time/temperature/pressure profile parameters in optimized range.

The usage of lignocellulosic biomass, microalgae biomass, sewage sludge, dairy waste, and agricultural waste is being currently employed for thermal decomposition. Various types of pyrolysis methods exist depending on the variations in the heat source, catalyst and residence time. Microwave assisted pyrolysis exploits the use of microwaves as heat source along with a short residence time. Beneroso et al. reported high syngas production via microwave pyrolysis of microalgae at 400°C to 800°C[22]. Salema et al. demonstrated the use of an overhead stirrer for improvement of biofuel production efficiency by increasing heat transfer rate by using microwaves as a heat source[23]. However, the use of electrical conventional furnace is a quite well established technique among researchers. A comparative analysis of the use of electrical furnace and microwave has been done and it is found that both the techniques are well suited for the present needs along with slight differences in the products obtained[24]. The use of an inorganic catalyst has also been studied by researchers to reduce residency time for fast pyrolysis. One such study was conducted by Carlson et al, for rapid pyrolysis of solid biomass in the existence of zeolite based stimulant at 200°C for 15 minutes[25].

4.3 Trans-esterification

The use of transesterification reaction for biodiesel preparation from edible and non-edible vegetable oil is the most common method employed till date. Due to cost effectiveness, non-edible oils are the only option left for bulk biofuel production. The triglycerides in oil combine with alkyl alcohol in the presence of a catalyst, like sodium hydroxide (NaOH), sodium methoxide (CH_3ONa), potassium hydroxide (KOH), and potassium methoxide (CH_3OK), to produce biodiesel and glycerol as a residue in trans-esterification processes[26]. At present, homogeneous acid stimulants like hydrochloric and sulphuric acids, homogeneous alkaline stimulants like NaOH, KOH, and CH_3ONa[27] and metallic oxides like zinc oxide, calcium oxide and magnesium oxide are also utilized[28]. Although, both forms of catalysts have drawbacks, acid catalysts probably take longer to produce biofuel than alkaline catalysts and also are caustic in nature. Even if alkaline catalysts require less time, they raise the pH of the biofuel, necessitating a complete cleaning, leading to sewage water formation and the removal of methyl esters (MEs), resulting in yield loss. Complete cleaning causes side effects such as glyceride and methyl ester saponification and demineralization of pufa through a catalyst[28]. Metal oxide catalyst is the most suited candidate till date for trans-esterification process with the highest efficiency. Wang et al articulated the use of M_gO nanoparticles[31] as stimulant for the biofuel production[29]. Similar work was done by Wen and Yang[30], and Veriansyah et al.,[31] by using KF/CaO nano-catalyst and surface modified zinc oxide nanoparticles respectively and they reported the increase in efficiency[30, 31].

4.4 Fermentation

- **Anaerobic digestion**

The biogas generation through the anaerobic absorption of natural wastes was achieved in this process. Methane and CO_2 are mainly present in biogas. It also contains other gas residues including hydrogen disul-phide. It entails the decomposition of organic materials by methanogens, which produces an energy content gas of around 20–40% of the additional raw material. The whole process involves primarily three stages:

- **Hydrolysis:** At this stage, complex chemicals are transformed into simple sugars that can be dissolved.

- **Fermentation:** These simple carbohydrates are fermented by microorganisms into methyl esters, acetic acid, furfurals, and a gas comprising H_2 and CO_2.
- **Methanogenesis:** This is then further metabolized mainly into 70 to 60% CH_4 and 30 to 40% CO_2 (30–40%) by methanogens[32].

The remaining nutrient rich residues were utilized as fertilizers[33]. At present, in India, the household waste, cow dung, and other cattle waste are exploited for large scale production of biogas. Microalgae anaerobic digestion has a rich protein level, resulting in lower C/N proportions. This can also increase in high production of by-product such as ammonia, which can be toxic to methanogens. But through co-digestion with other waste products, this problem can be tackled.

- **Alcoholic fermentation**

Alcoholic fermentation is the process of producing alcohols, most often ethanol, but also butanol, and propanol from biomass sources like cellulose or glucose. Ethyl alcohol is the most common biofuel used worldwide particularly in those countries which produce wheat, sugar beet, sugar cane, corn etc. in bulk quantities. The blending of alcohol is more common in countries like India and Brazil to cater to energy needs. This conversion of starch into sugars is done in large tanks called fermenter at slightly warmer temperature with the help of yeast. The conversion of sugar into ethanol is done by yeast. A multi stage fractional distillation process is used to drain the moisture and other contaminants from the 10-15% diluted ethyl alcohol[34]. The solid material remaining over from the filtration process could be utilised for cow feed or combustion for additional syngas generation. It is a wide accepted approach worldwide for the bulk production of bioethanol. However, a lot of controversies have taken place regarding the usage of edible sources such as sugarcane juice for the production of bioethanol and its consequent use as biogasoline but the usage of agricultural waste and other organic waste for the same purpose is termed as a better alternative.

4.5 Direct combustion

It is a process in which the biomass is combusted directly in the presence of air to transform chemical energy stored into combustion gases and get stored thermal energy[35]. Generally, an electric fired kiln, container, or vapor turbine at above 800°C is employed for this process. The main drawback of this method is that it usually needs pre-treatment activities like drying, grinding and chopping for direct burning, which adds to energy consumption and costs[35]. Also, a low moisture content of <50% dry weight is required but, any type of biomass can be burnt. The generated heat should be used without delay since storing it is not a feasible choice[36]. Owing to a greater moisture content in biomass, large scale power production is not technically feasible, even though the conversion efficiency of combustion of biomass for heat is comparable to that of thermal power plant generation of combined heat and power (CHP). During the co-combustion of biomass in thermal power plants, higher efficiencies are obtained but the net energy exchange effectiveness is in between 20% and 40%[34]. At present, rural areas in India exploit the direct combustion method for small household needs to meet their daily energy requirements (direct surveys).

4.6 Gasification

It is the biomass semi oxidation process involving a flammable gas mixture known as syngas at extreme temperatures around 800°C and 1000°C[36]. A wide variety of feedstock i.e. any organic matter can be used for gasification process for the production of syngas. Syngas is a combination of CO_2, CO, N, H_2, and CH_4 produced by a conventional gas production process in which biomass combines with water and oxygen to produce syngas, a lower nutritional gas that may be burned directly. It may also be utilized as a fuel for energy generation in gasoline engines or turbines.

Beneroso et al[25] articulated a microwave supported microalgae pyrolysis for the syngas production. They studied the microalgae pyrolysis at 400ºC and 800ºC of *Scenedesmus almeriensis*. They achieved an exceptional production of syngas and higher yield of gas around 94% by volume at 800ºC. Additionally, they found a lesser CO_2 production and lesser hydrocarbons than at a similar temperature mentioned earlier[22].

Table 1. Different process and technology of biofuel production

Process	Technology	Algae type	Produced Biofuel	Ref.
Thermochemical Conversion	Pyrolysis	Micro algae Macro algae	Bio-oil Syngas Char	37
	Liquefaction	Micro algae	Bio-oil	38
	Gasification	Micro algae Macro algae	Syngas	39
	Direct Combustion	Micro algae	Electricity, H_2	40
Biochemical Conversion	Anaerobic digestion	Micro algae	Methane, H_2	37
	Fermentation	Micro algae	Ethanol	
	Photobiological H_2 Production	Micro algae	Hydrogen	
Chemical Conversion	Trans-esterification	Micro algae Macro algae	Bio-diesel	41

The technology and process employed with respect to the phylum of algae for biofuel production is summarized in Table 1.

5. Government of India policies

The Ethyl alcohol policy of India indicates a 5% merger permission and tax benefits to sugarcane mills[42]. Policies are needed to announce a Minimum Support Price (MSP) for non-edible oilseeds used for the production of biodiesel. The formation of a National Biofuel Working Committee, which would report to the Prime Minister, and a Biofuel Advisory Board, which would report to the Secretary of Cabinet and monitor implementation of policy will be helpful. A National Biofuel Initiative has been established to provide economic incentives for the development of novel and second generation biofuels. Biodiesel and bioethyl alco-hol are expected to be classified as “declared products” by the authorities in order to allow unfettered circulation of biofuels inside and outside the regions[42].

5.1 Role of Indian Ministries for implementation of biofuel production

- **Ministry of New and Renewable Energy:** For promoting biofuel research and development and technology improvement for the production of biofuels.
- **Ministry of Petroleum and Natural Gas:** For advertising, expansion, and pricing implementation and acquisition policies for biofuels.
- **Ministry of Agriculture:** For R&D encouragement for the biofuel feedstock plant production.
- **Ministry of Rural Development:** For Jatropha plantation promotion on wastelands.
- **Ministry of Science & Technology:** For support in research of biofuel plants, particularly in the biotechnology field.
- **National Biofuel Coordination Committee (NBCC) headed by the honorable Prime Minister:** To offer sophisticated organization and guidance/review of policy on several biofuel development aspects, usage and promotion.

Current production of biofuel in India relies on both usage of edible organic sources and as well as non-edible organic sources. Our sugarcane business entities have been given permission for ethanol generation and processing. However, no specific provisions are made for the treatment of other bio-ethanol fuel sources other than sugarcane and molasses[43]. Rao et al[44] debated on the use of alternative feedstock such as sugar beet and sweet sorghum, which were also quoted in the policy however, there is no detailed roadmap recommended for their endorsement or demotion[44]. Biodiesel production from non-edible oilseeds such as *Jatropa curcas* need to be promoted. Use of wastewater for the production of micro algal based biofuel is a promising method. Sewage sludge, agricultural waste and other organic waste can also be good candidates for biofuel manufacture.

In India, biofuel scheme provides an exemption from central taxes and duties for our biofuel sector. Currently, as biodiesel is totally ruled out from duty charges, bio-ethyl alcohol has a concessional rate of 16% excise duty. For the biofuel production plant and machinery and bioethyl alcohol, additional customs and excise duty concessions are also provided[45]. The policy does not rule out sales tax, license charge, permit charges and taxes on import, thus hindering the expansion and development of the industrial production process. There is a need to establish and formulate policies regarding transportation, sales, permit and entry taxes.

6. Future of biofuel production in an India

As per the 55th Round Table Conference of the National Sample Survey of India in 1999–2000, biomass is used as the major fuel for cooking by 86% of rural families and 24% of urban families out of a total of 120,000 families[46]. Biomass gasification processes rely on wastelands that are thought to offer the most potential to address India's rural energy demands. The use of forest residues as a power generating feedstock can give a financial incentive to convert wastelands into fuel forests[47]. As per the Department of Land Resources (DoLR) and the Government of India, approximately 63.9 million acres of arable in India are now unfit for agriculture. Conversely, the Asian Development Bank (ADB) predicted in 2011 that 32 million acres of wastelands needed to be devoted to biofuel crops, with certain growth promotion, to reach the Indian biofuel scheme's 20% oil-blending objective. Presently, the biofuel plants, Jatropha curcas and Pongamiapinnata seem to be the most popular, with necessary financial returns to sustain their growth[48].

Balooni[49] found that planting trees with surrounding natural root system or perfectly adapted plants like *Prosopis Juliflora, Acacia Nilotica, Sesbania Egyptia, and Casuarina Equisetifolia*, and diverse Eucalyptus species is economically beneficial for the harvest of fuel wood, fodder, timber, and other forest products[49] In Kakinada, Andhra Pradesh, Natural Bioenergy Limited (NBL), a joint venture with Energea Gmbh (Austria) and Fe Clean Energy (United States), plans to establish a 300 t/day (about 90,000 t/year) biodiesel facility. Energea is a significant biodiesel technology provider with many sites around Europe[50]. The project would be funded by Fe Sustainable, a private equity fund committed to clean energy initiatives. The project's cost, which is nearing completion, is projected to be Rs.1,400 million. The Industrial Development Bank of India (IDBI) has decided to invest Rs.330 million after doing thorough research. Other institutions interested in debt funding the project are Infrastructure Development and Finance Company, Andhra Bank, the National Bank for Agriculture and Rural Development, and the State Bank of India (SBI).

7. Stimulus packages

The Indian government has started a Nationwide Biofuels Program with the goal of attaining a 20% biofuel blend[51]. Railway Authorities of India is planning to build four biofuel facilities for Rs. 120 crore as part of one of the nation's biggest biofuel ventures. Two biofuel esterification units will be constructed in Raipur and Chennai over the next 2 years, with the remaining two plants to be built later. Each facility will cost roughly Rs 30 crore and generate 30 tonnes of biodiesel per day, totaling over 9,000 tonnes per year. Besides lowering reliance on fuel imports, the project hopes to provide a number of additional local advantages, such as job creation for the literate rural population, wasteland rehabilitation, and emission reductions from energy consumption, all of which can contribute to good economic transformation[42, 52, 53].

The search for an ideal feedstock is a challenging and motivational task for researchers. At present, microalgae, dairy and cattle waste, agricultural waste, sugarcane, corn stover, and various other organic biomass are being used for biofuel production. One such study investigated biofuel production by green algae through the processing of cattle farm and civic wastewaters. They treated wastewater from dairy in an outdoor batch culture[20]. Only utilizing oil containing plants and residue oils for biofuel production is not enough to meet the current demands for fuel[54, 55]. Even though Ethanol production using corn directly competes with the food supply, still it the most dominant feedstock. However, microalgae appear to be a more promising feedstock option. Also, it can be potentially cultured using wastewater[56].

8. Discussion

The present review investigates one of the prime considerations in the recent biofuel discussion on the impact of biofuel status and future prospective in India. The paper also annotates that it is impossible to assess the trade policy effects. The above mentioned R&D technologies are presently being employed for large scale as well as small scale production of biofuel depending on the feasibility and yield of the technology concerned. In India, all the technologies are being employed but in the initial development stages.

Currently it is impossible to direct the production of biofuel feedstock into areas that will not cause oblique changes in usage of land that may counteract any GHG biofuel incentives. Non-edible oil crops such as Rapeseed oil, Jatropha oil, Mahua oil, Pongamia oil, Rice Bran oil, Palm oil, Olive oil, Linseed oil, Cotton seed oil, Soya bean oil, Guang-Pi, Beef Tallow and Lard are commonly available in India and are more cost-effective for the production of biodiesel rather than edible oils[11]. Encouraging the usage of biofuels from these feedstocks might be an alternative to boost the GHG potential savings from biofuel usage while lowering their effects on farm products market and agricultural prices once their social and environmental effects are well understood[57].

Lastly, in the light of increased prices of fossil fuel, policy makers will have less impact over the quantity of biofuel that is employed in the future. If rising rates of oil (and attractive prices of feedstock) boost the market competitiveness of biofuels, the role of policy, whether in the form of benefits, regulations, or duty exemptions, lessens. Additional study is being undertaken in the field of sources of renewable energy all over the world; however there is a necessity for further study in this area, which must be performed on a big scale. Production cost of biofuel is now too high, and innovative methods are needed to lower production costs.

9. Conclusions

India is known for emphasizing the social elements of sustainability, and its endeavour to build a "green power economy" via biofuels is no exception. India's biofuel regulations are said to be targeted at both local sustainable energy generation and regional development co-benefits such as job creation and poverty alleviation. India's place in the world biofuel map is presently not remarkable. However, the country has plans to expand the biofuel sector, which can provide a great opportunity for enhancement of environment sustainability, improve economic conditions, infrastructure development and also foreign investment in the Indian biofuel industry.

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References

1. IEA Strategies and Scenarios to 2050. International Energy Agency; Paris, 2008, p 1-14.
2. IPCC Climate change impacts, adaptation, and vulnerability. In: A report of working group II of the Intergovernmental Panel on Climatic Change; IPCC: Cambridge, 2001, p 1-94
3. Wang, J. and Chameides, B., 2005, Global Warming's Increasingly Visible Impacts. *Environmental Defense*.
4. Wang, B., Li, Y., Wu, N. and Lan, C. Q., 2008, CO(2) bio-mitigation using microalgae. *Applied Microbiology and Biotechnology,* **79(5)**, 707-18.
5. IEA World Energy Outlook, 2004, International Energy Agency, Paris, France, 2004b.
6. Dufey, 2006, A. Biofuels production, trade and sustainable development, emerging issues; International Institute for Environment and Development: London.
7. Commission, E., 2009, The Promotion of the Use of Energy from Renewable Sources and Amending and Subsequently Repealing Directives 2001/77/EC and 2003/30/EC'. Directive 2009/28/EC of the European Parliament and of the Council; European Commission..
8. Alalwan, H.A., Alminshid, A.H. and Aljaafari, H.A., 2019, Promising evolution of biofuel generations. Subject review. *Renewable Energy Focus* **28**, 127-139.
9. Abdullah, B., Syed Muhammad, S.A., F.A., Shokravi, Z., Ismail, S., Kassim, K.A., Mahmood, A. N. and Aziz, M.M.A., 2019, Fourth generation biofuel: A review on risks and mitigation strategies. *Renewable and Sustainable Energy Reviews* **107,** 37-50.
10. Mat Aron, N.S., Khoo, K.S., Chew, K.W., Show, P.L., Chen, W.H. and Nguyen, T.H.P., 2020, Sustainability of the four generations of biofuels – A review. *International Journal of Energy Research* **44 (12),** 9266-9282.
11. Sekhar, M., Mamilla, V., Reddy, D. and Rao, G., 2010, Synthesis of biodiesel. *International Journal of Engineering Science and Technology*, 2.
12. Chigullapalli, S., Rao, A.B. and Patwardhan, A., 2019, Biodiesel Blending in India—Analysis of National Biofuel Policy., 247-253.
13. Packiam, M., Subburamu, K., Desikan, R., Uthandi, S., Subramanian, M. and Soundarapandian, K., 2018, Suitability of pearl millet as an alternate lignocellulosic feedstock for biofuel production in India. *J Appl Environ Microbiol*, **6(2)**, 51-58.
14. Usmani, R. A., 2020, Potential for energy and biofuel from biomass in India. *Renewable Energy*, **155**, 921-930.
15. Biodiesel 2020: 2008, Global Market Survey, Feedstock Trends and Forecasts. 2nd ed., Emerging Markets Online: Houston, TX USA, p 685.

16. Aradhey, A., 2012, Gain Report no. IN2018, 2012 on India Biofuel Annual., p 1-16.

17. Aradhey, A., 2014, Gain Report no. IN4055, 2014 on India Biofuel Annual., p 1-14.

18. Commission, P., 2003, Report of the Committee on Development of Biofuel. Planning Commission, G. o. I., Ed., p 1-214.

19. Christenson, L. and Sims, R., 2011, Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology advances,* **29(6)**, 686-702.

20. Woertz, I., Feffer, A., Lundquist, T. and Nelson, Y., 2009, Algae Grown on Dairy and Municipal Wastewater for Simultaneous Nutrient Removal and Lipid Production for Biofuel Feedstock. *Journal of Environmental Engineering,* **135 (11)**, 1115-1122.

21. Mohan, D., Pittman, C.U. and Steele, P.H., 2006, Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review. *Energy & Fuels***, 20(3)**, 848-889.

22. Beneroso, D., Bermudez, J. M., Arenillas, A. and Menendez, J. A., 2013, Microwave pyrolysis of microalgae for high syngas production. *Bioresource technology,* **144**, 240-6.

23. Salema, A.A. and Ani, F.N., 2012, Microwave-assisted pyrolysis of oil palm shell biomass using an overhead stirrer. *Journal of Analytical and Applied Pyrolysis,* **96,** 162-172.

24. Ferrera-Lorenzo, N., Fuente, E., Bermudez, J. M., Suarez-Ruiz, I. and Ruiz, B., 2014, Conventional and microwave pyrolysis of a macroalgae waste from the Agar-Agar industry. Prospects for bio-fuel production. *Bioresource technology,* **151,** 199-206.

25. Carlson, T.R., Vispute, T.P. and Huber, G.W., 2008, Green gasoline by catalytic fast pyrolysis of solid biomass derived compounds. *ChemSusChem*, **1(5)**, 397-400.

26. Meher, L.C., Kulkarni, M.G., Dalai, A.K. and Naik, S.N., 2006, Transesterification of karanja(Pongamia pinnata) oil by solid basic catalysts. *European Journal of Lipid Science and Technology,* **108(5)**, 389-397.

27. Sanli, H. and Canakci, M., 2008, Effects of Different Alcohol and Catalyst Usage on Biodiesel Production from Different Vegetable Oils. *Energy & Fuels,* **22(4)**, 2713-2719.

28. Veljkoviæ, V.B., Stamenkoviæ, O.S., Todoroviæ, Z.B., Laziæ, M.L. and Skala, D.U., 2009, Kinetics of sunflower oil methanolysis catalyzed by calcium oxide. *Fuel,* **88(9)**, 1554-1562.

29. Wang, L. and Yang, J., 2007, Transesterification of soybean oil with nano-MgO or not in supercritical and subcritical methanol. *Fuel*, **86(3)**, 328-333.

30. Wen, L., Wang, Y., Lu, D., Hu, S. and Han, H., 2010, Preparation of KF/CaO nanocatalyst and its application in biodiesel production from Chinese tallow seed oil. *Fuel,* **89(9)**, 2267-2271.

31. Veriansyah, B., Kim, J.-D., Min, B. K., Shin, Y. H., Lee, Y.-W. and Kim, J., 2010, Continuous synthesis of surface-modified zinc oxide nanoparticles in supercritical methanol. *The Journal of Supercritical Fluids,* **52(1)**, 76-83.

32. Cantrell, K.B., Ducey, T., Ro, K.S. and Hunt, P.G., 2008, Livestock waste-to-bioenergy generation opportunities. *Bioresource technology,* **99(17)**, 7941-53.

33. Phang, S.M., Miah, M.S., Yeoh, B.G. and Hashim, M.A., 2000, Spirulina cultivation in digested sago starch factory wastewater. *Journal of Applied Phycology,* **12(3/5),** 395-400.

34. Demirbaº, A., 2001, Biomass resource facilities and biomass conversion processing for fuels and chemicals. *Energy Conversion and Management,* **42(11),** 1357-1378.

35. Goyal, H.B., Seal, D. and Saxena, R.C., 2008, Biofuels from thermochemical conversion of renewable resources: A review. *Renewable and Sustainable Energy Reviews,* **12(2)**, 504-517.

36. James H. Clark, 2008, F. E. I. D., Introduction to Chemicals from Biomass. John Wiley & Sons.

37. Pourkarimi, S., Hallajisani, A., Alizadehdakhel, A. and Nouralishahi, A., 2019, Biofuel production through micro- and macroalgae pyrolysis – A review of pyrolysis methods and process parameters. *Journal of Analytical and Applied Pyrolysis,* **142,** 104599.

38. Djandja, O.S., Wang, Z., Chen, L., Qin, L., Wang, F., Xu, Y. and Duan, P., 2020, Progress in Hydrothermal Liquefaction of Algal Biomass and Hydrothermal Upgrading of the Subsequent Crude Bio-Oil: A Mini Review. *Energy & Fuels,* **34(10)**, 11723-11751.

39. Jayaraman, R.S., Gopinath, K.P., Arun, J., Malolan, R., Adithya, S., Ajay, P. S., Sivaramakrishnan, R. and Pugazhendhi, A., 2021, Co-hydrothermal gasification of microbial sludge and algae Kappaphycus alvarezii for bio-hydrogen production: Study on aqueous phase reforming. *International Journal of Hydrogen Energy,* **46(31)**, 16555-16564.

40. Lam, M.K., Loy, A.C.M., Yusup, S. and Lee, K. T., 2019, Biohydrogen Production From Algae., 219-245.

41. Ghedini, E., Taghavi, S., Menegazzo, F. and Signoretto, M., 2021, A Review on the Efficient Catalysts for Algae Transesterification to Biodiesel. *Sustainability,* **13(18)**, 10479.

42. MNRE, National Biofuel Policy. Ministry of New Renewable Energy, I., Ed. 2009.

43. Kumar, B., Hiremath, R.B., Balachandra, P. and Ravindranath, N.H., 2009, Bioenergy and food security: Indian context. *Energy for Sustainable Development,* **13(4)**, 265-270.

44. P. Srinivasa Rao., B.V.R., Michael Blümmel, Gv Subbarao, Raraj K.P., Sanjana Reddy and P Parthasarathy Rao, 2009, Sweet Sorghum as a Biofuel Feedstock: Can there be Food-Feed-Fuel Tradeoffs?

45. Shinoji, P., Raju, S.S. and Joshi, P.K., 2011, India's biofuels production programme: need for prioritizing the alternative options. *Indian J. Agr. Sci.,* **81(5)**, 391-397.

46. Balakrishnan, K. M., Sumi, Kumar, Priti, Ramaswamy, Padmavathi, Sambandam, Sankar, Kumar, Kannappa Satish and Smith, Kirk, R, 2004, Indoor Air Pollution Associated with Household Fuel Use in India. World Bank: Washington, DC.

47. Ravindranath, N.H., Somashekar, H.I., Dasappa, S., Reddy and C. N. J., 2004, Sustainable biomass power for rural India: Case study of biomass gasifier for village electrification. *Curr Sci India***, 87 (7)**, 932-941.

48. Gunatilake, 2011, H. Financial and Economic Assessment of Biodiesel Production and Use in India.

49. Balooni, K., 2003, Economics of wastelands afforestation in India, a review. *New Forests,* **26(2)**, 101-136.

50. Uppal, J., 2004, In Emerging Employment Opportunities in Biofuels Sector, International Conference on Biofuels in India.

51. Commission, P., Report of the Committee on Devel-

opment of Biofuels. Planning Commission, G. O. I., Ed. 2003.

52. Times, E., Railways to set up four biodiesel plants. *The Economic Times* Feb 06, 2011, 2011.
53. Annual, I. B. *India Biofuels Annual*, 7/1/2010, 2010.
54. Chisti, Y., 2007, Biodiesel from microalgae. *Biotechnology advances*, **25(3)**, 294-306.
55. Chisti, Y., 2008, Biodiesel from microalgae beats bioethanol. *Trends in biotechnology,* **26(3)**, 126-31.
56. Hirano, A., Ueda, R., Hirayama, S. and Ogushi, Y., 1997, CO_2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation. *Energy,* **22(2-3)**, 137-142.
57. Hertel, T.W., McDougall, R.A, Narayanan G. B. and Aguiar, A.H., 2005, Behavioral Parameters. In GTAP 7 Data Base Documentation, Center for Global Trade Analysis, Department of Agricultural Economics, Purdue University: USA, p 1-18.

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Remediation Potential of Select Wetland Plants

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ABSTRACT
Phytoremediation refers to the ability of certain plants to bioaccumulate, degrade, or render harmless the contaminants in soil, water, etc. Wetlands function as the kidneys of a landscape as plants in wetlands act as sinks by uptake of nutrients and heavy metals. The current study focuses on estimating the phytoremediation potential of some selected wetland plants. Plant samples were collected from inlets and outlets of Jakkur wetlands, Bengaluru, India on a weekly basis. The plants were identified using standard keys based on taxonomic literature. Plants identified were Typha *sp.,* Cyperus *sp.,* Ludwigia *sp.,* Pistia stratiotes, Polygonum *sp.,* Alternanthera philoxeroides *and* Spirodela *sp., which are perennial. Among these,* Typha *sp.,* Cyperus *sp.,* Pistia stratiotes *and* Spirodela *sp. are monocots, and* Ludwigia *sp.,* Polygonum *sp. and* Alternanthera philoxeroides *are dicots. These plants showed the uptake of the heavy metals viz., cadmium, zinc, nickel, copper, chromium and lead. During the first week, uptake of Cu and Zn was more by young* Typha *sp. at inlet and Cr was more at outlet of sampling. The uptake of Cu, Pb, Zn and Ni was 1.4, 9.0, 9.4 and 10.0 mg/kg respectively during the second week of sampling in young* Typha *sp. shoot. Also, the Pb and Zn uptake was 13.6 and 10.4 mg/kg respectively in the mature* Typha *sp. during the third week at outlet of sampling. Ni uptake was 4.0 mg/kg during the third week of sampling by mature* Typha *sp. at inlet. The uptake of Pb, Zn and Cd was 12.2, 23.0 and 1.4 mg/kg respectively during the fourth week of sampling in the mature* Typha *sp. at outlet. The uptake of Pb, Ni, Cr and Cd was 5.2, 2.4, 12 and 0.8 mg/kg respectively during the first week of sampling in the medium* Polygonum *sp. The uptake of Zn was 10.2 mg/kg during the second week in the mature* Alternanthera philoxeroides *at outlet of sampling. The uptake of Cr was 19.6 mg/kg during the third week was found to be more by mature* Alternanthera philoxeroides *at outlet of sampling. The uptake of Cu, Pb, Zn and Cr was 2.2, 9.8, 8.6 and 10.2 mg/kg respectively during the fourth week in young* Alternanthera philoxeroides *at inlet. Similarly, the uptake of Cr was 13.6 mg/kg at outlet during the second week in* Spirodela *sp. The uptake of Cu was 2.4 mg/kg during the third week in medium* Ludwigia *sp. at outlet.*

Keywords: *Phytoremediation, Macrophytes, Physiology, Wetland, Nutrients, Perennial*

Introduction

Metallic elements that have a high atomic weight, relatively high density and are toxic at low concentrations such as mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), lead (Pb), etc. are referred to as heavy metals. Applications of metals include domestic, agricultural, medical and technological with wider distribution in the environment. The potential effects on human health and the environment have been a concern, which necessitates suitable methods which can help in the remediation to minimise harmful effects. Soil and water are increasingly getting contaminated with heavy metals[1] due to sustained flow of untreated effluents which is damaging the environment. Sources of heavy metals in the environment are geogenic, industrial, agricultural, pharmaceutical, domestic effluent, etc. Escalated health concerns associated with environmental contamination due to heavy metals have necessitated remediation measures that are economical and technically feasible. Remediation is to avoid all such environmental damage. Remediation can be achieved with the help of various physico-chemical processes viz., ion-exchange, precipitation, reverse osmosis, ultrafiltration, flocculation, electro dialysis, etc. But these methods are costly, time-consuming, and are also not very effective[2].

Bioremediation is a viable and cost effective approach using microorganisms (bacteria, actinobacteria, fungi, algae and yeasts), plant residues and plants to remove heavy metals from waste water, industrial effluents, etc.[3,4]. Bioremediation is emerging as a green approach for the removal of heavy metals and other pollutants from contaminated land, water, etc.

Phytoremediation is one of the most eco-friendly, economical, simple and effective bioremediation options to remove heavy metals from waste water, industrial effluents, etc. In phytoremediation, the contaminated soils and water are treated *in-situ* with the help of plants[5]. Aquatic plants are of importance in this regard due to uptake mechanisms and rates of uptake of metals of these plant.[6,7].

Aquatic plants (macrophytes) are important component of wetlands[8], which have the special ability of adaption to the constant contact with surface water and groundwater[9]. Macrophytes have thin outer tissues and aerenchyma, through which air is distributed to the parts of plant below the surface of the water. Macrophytes vegetation has been used in wetlands to treat wastewater by uptake nutrients and heavy metals[10]. Macrophytes have a metabolic role in wastewater treatment due to their potential to release oxygen into the rhizosphere which helps in nitrification and direct uptake of nutrients[11] as in *Typha* spp. Which has a considerably high nutrient uptake capacity[12].

Aquatic macrophytes are appropriate for metal removal as they can tolerate adverse conditions and are easier to harvest[13]. Wetland plants are divided into emergent, submerged and floating types. The emergent plants are rooted in the soil with basal portions, and leaves, stems and reproductive organs are aerial[14]. The examples of emergent plants are *Phragmites australis*, *Typha domingensis*, *Typha latifolia*, *Phragmites karka*, *Juncus pallidus*, *Empodisma minus*, *Phalaris arundinacea*, *Scirpus cyperinus*, *Aster novae-angliae*, *Limonium carolinianum*, *Cephalanthus occidentalis* and *Rhizophora mangle*. Submerged plants are below the surface of water for their entire life cycle. The examples of submerged plants are *Ceratophyllum demersum*, *Vallisneria americana*, *Myriophyllum spicatum*, *Hydrilla verticillata*, *Heteranthera dubia*, etc. Submerged species provide more biomass for the uptake and sorption of the contaminants through phytoextraction. Submerged plants have the ability to accumulate metals in their tissues in comparison to rooted emergent plants. In floating plants, the leaves and stems float on the surface of water. The examples of floating plants include *Eichhornia crassipes*, *Pistia stratiotes*, *Salvinia herzogii*, *Wolffia columbiana*, *Lemna valdiviana*, *Nymphaea* spp., *Nuphar advena*, *Juncus effusus*, *Phyllanthus fluitans*, etc.[14].

The common aquatic plant species (*Typha latifolia*, *Myriophyllum exalbescens*, *Potamogeton epihydrus*, *Sparganium angustifolium*, *Myriophyllum spicatum* and *Sparganium multipedunculatum*) have been used for aluminium (Al) removal[15]. Macrophytes such as Parrot feather (*Myriophyllum aquaticum*), creeping primrose (*Ludwigia palustris*), and water mint (*Mentha* sp.) have been used for removal of iron (Fe), zinc (Zn), copper (Cu), and mercury (Hg) from water[16]. The *L. minor* has been used for removing copper (Cu) and cadmium (Cd) from contaminated soils[17].

Aim of the study:

The main aim of this research work was to assess the remediation potential of select macrophytes. This involved:

i) Weekly sampling of macrophytes

ii) Identification of macrophytes samples

iii) Assessment of remediation potential of selected wetland plants.

Materials and Methods

Study Area

Sampling of wetland plants

Wetland plants were sampled from inlets and outlets of Jakkur Lake, Bengaluru on a weekly basis (0.25 m^2 area). The plant samples were collected in triplicates. Collected macrophyte samples were washed, labelled and the plant species were identified using standard morphological keys based on taxonomic literature.[18]

Remediation potential of wetland plants

All the dried plant samples were powdered using mortar and pestle, sieved (1 mm) to get fine powders and labelled properly. The powdered plant sample (0.5 g) were acid digested[19] and analysed for six heavy metals viz., cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) using reagent blanks and suitable standards using Flame Atomic Absorption Spectrophotometry (GBC Avanta version 1.31).

Results and Discussion

Diversity of the wetland plants

Total seven wetland plant species were collected from Jakkur Lake, Bengaluru. The identified plant species were *Typha* sp. (Figure 1), *Cyperus* sp. (Figure 2), *Ludwigia* sp. (Figure 3), *Pistia stratiotes* (Figure 4), *Polygonum* sp. (Figure 5), *Alternanthera philoxeroides* (Figure 6) and *Spirodela* sp. (Figure 7). Among these five are emergent (*Typha* sp., *Cyperus* sp., *Ludwigia* sp., *Alternanthera philoxeroides* and *Polygonum* sp.) and two are floating wetland plant species (*Spirodela* sp. and *Pistia stratiotes*).

Fig. 1. *Typha* sp.

Fig. 2. *Cyperus* sp.

Fig. 3. ***Ludwigia*** **sp.**

Fig. 4. ***Pistia stratiotes***

Fig. 5. ***Polygonum*** **sp.**

Fig. 6. ***Alternanthera philoxeroides***

Fig. 7. *Spirodela* sp.

Remediation potential of the wetland plants

Copper replaces other co-factors in key enzymes and disrupts photosynthetic activity and other cellular processes[20]. Lead toxicity causes swollen, bent, short and stubby roots[21]. Excess Zn will alter the physiological, ultra structural and biochemical parameters of the plants. Excess of Ni causes various physiological alterations and diverse toxicity symptoms such as chlorosis and necrosis in the plant species[22]. Copper concentration exceeding 20 mg/kg in the shoot is injurious, though copper is an essential element for growth[23]. The remediation potential of the wetland plants collected on weekly basis is represented in Tables 1, 2, 3 and 4 respectively.

Table 1. Uptake of heavy metals by the wetland plants (sampling done on 17/5/2017)

Plant samples	***Inlet**						***Outlet**					
	Heavy metals (mg/kg)						**Heavy metals (mg/kg)**					
	Cu	Pb	Zn	Ni	Cr	Cd	Cu	Pb	Zn	Ni	Cr	Cd
young *Typha* sp.	**17.2**	0.0	14.4	1.4	5.0	0.0	1.2	6.0	4.2	1.4	13.2	0.2
mature *Typha* sp.	-	-	-	-	-	-	0.0	3.2	3.2	0.6	10.0	0.0
young *Alternanthera philoxeroides*	0.0	0.0	2.8	2.0	0.6	0.0	0.0	7.6	3.0	**5.8**	10.8	0.0
medium *Polygonum* sp.	1.4	5.2	9.0	2.4	12.0	0.8	-	-	-	-	-	-
mature *Polygonum* sp.	0.8	0.0	3.2	0.2	8.8	0.2	-	-	-	-	-	-
medium *Ludwigia* sp.							0.6	8.4	6.4	**6.2**	10.6	0.6
mature *Pistia stratiotes*	-	-	-	-	-	-	2.6	8.2	7.0	**6.2**	9.6	0.2
Spirodela sp.	-	-	-	-	-	-	0.8	7.6	8.8	**6.8**	10.6	0.0
Normal range[24]	1-5	0.2-20	1-400	0.02-5	0.03-14	0.1-2.4						
Critical range[24]	5-30	30-300	100-400	10-100	5-30	5-30						
Threshold effect level[25]	35.7	18.0	123.0	35.0	37.3	0.596						

Table 2. Uptake of heavy metals by the wetland plants (sampling done on 30/5/2017)

Plant samples	***Inlet**						***Outlet**					
	Heavy metals (mg/kg)						Heavy metals (mg/kg)					
	Cu	Pb	Zn	Ni	Cr	Cd	Cu	Pb	Zn	Ni	Cr	Cd
young *Typha* sp. shoot	1.4	9.0	9.4	**10.4**	11.2	0.4	0.0	7.8	5.4	**5.8**	8.2	0.4
medium *Typha* sp. shoot	-	-	-	-	-	-	0.6	9.8	6.4	9.0	10.2	0.2
mature *Typha* sp. shoot	0.0	8.0	7.6	**10.0**	14.0	0.6	0.0	7.2	5.2	**8.0**	12.6	0.0
mature *Alternanthera philoxeroides*	-	-	-	-	-	-	0.6	8.8	10.2	**5.6**	10.0	0.4
mature *Pistia stratiotes*	-	-	-	-	-	-	1.2	5.0	8.8	**6.0**	7.0	1.6
Spirodela sp.	-	-	-	-	-	-	0.0	2.8	4.0	0.0	13.6	1.0
Normal range	1-5	0.2-20	1-400	0.02-5	0.03-14	0.1-2.4						
Critical range	5-30	30-300	100-400	10-100	5-30	5-30						
Threshold limit	35.7	18.0	123.0	35.0	37.3	0.596						

Table 3. Uptake of heavy metals by the wetland plants (sampling done on 6/6/2017)

Plant samples	***Inlet**						***Outlet**					
	Heavy metals (mg/kg)						Heavy metals (mg/kg)					
	Cu	Pb	Zn	Ni	Cr	Cd	Cu	Pb	Zn	Ni	Cr	Cd
medium *Typha* sp.	**14.4**	7.6	16.4	1.8	6.2	0.6	-	-	-	-	-	-
mature *Typha* sp.	2.0	4.6	5.2	4	6.0	0.8	1.0	13.6	10.4	0.0	2	0.2
mature *Alternanthera philoxeroides*	1.4	2.4	6.6	3.2	5.2	1.8	0.8	10.4	8.8	0.0	**19.6**	1.0
medium *Ludwigia* sp.	-	-	-	-	-	-	2.4	12.2	7.6	0.0	4.0	0.2
Normal range	1-5	0.2-20	1-400	0.02-5	0.03-14	0.1-2.4						
Critical range	5-30	30-300	100-400	10-100	5-30	5-30						
Threshold limit	35.7	18.0	123.0	35.0	37.3	0.596						

Metal uptake by wetland plants during the 1st week

The uptake of Cu by young *Typha* sp. from inlet exceeded the normal range and uptake of Ni by young *Alternanthera philoxeroides*, medium *Ludwigia* sp., mature *Pistia stratiotes* and *Spirodela* sp. from the outlet exceeded the normal range. (Table 1)

Metal uptake during the 2nd week

The uptake of Ni by young and mature *Typha* sp. shoot from inlet exceeded the normal range and uptake of Ni by young and mature *Typha* sp. shoot, mature *Alternanthera philoxeroides* and mature *Pistia stratiotes* from the outlet exceeded the normal range. (Table 2)

Table 4. Uptake of heavy metals by the wetland plants (sampling done on 13/6/2017)

Plant samples	*Inlet						*Outlet					
	Heavy metals (mg/kg)						Heavy metals (mg/kg)					
	Cu	Pb	Zn	Ni	Cr	Cd	Cu	Pb	Zn	Ni	Cr	Cd
medium *Typha* sp.	2.0	5.8	7.6	1.8	8.8	0.8	-	-	-	-	-	-
mature *Typha* sp.	1.6	5.2	6.8	2.2	9.0	0.6	1.8	12.2	23	3.0	5.4	1.4
young *Alternanthera philoxeroides*	2.2	9.8	8.6	1.4	10.2	0.2	-	-	-	-	-	-
medium *Alternanthera philoxeroides*	-	-	-	-	-	-	0.4	0.0	3.6	2.4	4.6	0.8
medium *Ludwigia* sp.	-	-	-	-	-	-	3.2	3.6	7.8	3.4	9.6	0.6
Normal range	1-5	0.2-20	1-400	0.02-5	0.03-14	0.1-2.4						
Critical range	5-30	30-300	100-400	10-100	5-30	5-30						
Threshold limit	35.7	18.0	123.0	35.0	37.3	0.596						

*Inlet and outlet are the sampling points at Jakkur Lake, Bengaluru.

C - Carbon; N - Nitrogen; P - Phosphorus; Cd - Cadmium; Cr - Chromium; Ni - Nickel; Zn - Zinc; Cu - Copper; Pb - Lead

Metal uptake during the 3rd week

The uptake of Cu by medium *Typha* sp. from inlet exceeded the normal range and uptake of Cr by mature *Alternanthera philoxeroides* from the outlet exceeded the normal range. (Table 3)

Metal uptake by wetland plants during the 4th week

The uptake of heavy metals by the macrophytes during the 4th week was within the normal range. (Table 4)

Wetland plants at storm water detention ponds have showed the ability for the uptake of nutrients and heavy metals[26]. *Eichhornia* sp. uptake heavy metals Pb, Cr, Zn, Mn, and Cu to a large extent from the wastewater[27]. *Ludwigia natans* is useful for remediation of Cd from contaminated water[28]. *Typha angustifolia* has been reported to assist remediation of various heavy metals (Cu, Pb, Ni, Fe, Mn, and Zn) and it has been found that the plant removes heavy metals from industrial wastewater containing metal, melanoidin, and phenol compounds[29]. *Spirodela* sp. has high uptake capacity of Cr, Zn and Pb[30]. The analyses highlights the use of plants for remediation as an environmental friendly approach because of their high efficiency in metal removal[31]. Removal of toxic metals by wetland plants viz., *Phragmites australis*, *Juncus effusus* and *Iris pseudacorus*[32]. *Carex pseudocyperus* and *C. riparia* have been reported to be effective for the removal of metals from water[33]. The heavy metal accumulation potential of wetland plants has been studied[34-38]. The spatial pattern of heavy metal accumulation in the sediments and macrophytes of Bellandur wetland, Bangalore, India show higher accumulation of all metals except for chromium in *Typha angustata*[35]. The bioremediation potential of aquatic macrophytes in Jakkur wetland, Bangalore, India is evident with the higher concentration of lead, zinc, nickel and chromium in *Typha angustatathan* at inlet. Nickel above normal range was observed in *Typha angustata* at inlet and in all plant species at outlet[36-38]. Similarly, there is a report on heavy metals in biotic and abiotic components of Varthur wetlands, Bangalore, India[37, 39]. The assessment of bioconcentration and translocation factors in macrophytes showed the select macrophytes are useful to remove heavy metals[37, 38].

Conclusions

The phytoremediation approach is an eco-friendly solution for the treatment of contaminated soils and water using plants. Common plants in wetlands such as *Typha* sp., *Cyperus* sp., *Ludwigia* sp., *Pistia stratiotes*, *Polygonum* sp., *Alternanthera philoxeroides* and *Spirodela* sp., have good remediation potential due to their metal uptake ability. Macrophytes have the ability for the uptake of heavy metals such as Cd, Pb, Zn, Ni, Cr and Cd, which will be the low cost option in the remediation of polluted soil and water. The uptake of heavy metals by the macrophytes during the fourth week was within the normal range suggesting that they can be used as fodder. Use of select local macrophyte species in the constructed wetland systems would aid in remediation through removal of contaminants (nutrients and heavy metals) from domestic and industrial wastewaters. Ensuring zero discharge from industries would be the best option toward ensuring heavy metals not getting into biotic food chain. Growing macrophytes in the flood plains of water bodies in rural area helps in treating agriculture run-off containing nutrients and heavy metals.

References

1. Vardhan K., Kumar P. and Panda R., 2019, *Journal of Molecular Liquids*, **290**.
2. Khulbe C. and Matsuura T., 2018, *Applied Water Science*, **8,** 19.
3. Azubuike C., Chikere B. and Okpokwasili C., 2016, *World Journal of Microbiology and Biotechnology*, **32,** 180.
4. Pande V., Pandey C., Sati D., Pande V. and Samant M., 2020, *Environmental Sustainability*, **3,** 91-103.
5. Etim E., 2012, *International Journal of Environment and Bioenergy*, **2,** 120-136.
6. Lee H., 2013, *Biotechnology and Bioprocess Engineering*, **18,** 431-439.
7. Wei Z., Van Le Q., Peng W., Yang Y., Yang H., Gu H., Lam S. and Sonne C., 2021, *Journal of Hazardous Materials*, **403**.
8. Gupta P., Roy S. and Mahindrakar A., 2012, *Resources and Environment*, **2,** 202-215.
9. Kochi L., Freitas P., Maranho L., Juneau P. and Gomes M., 2020, *Sustainability*, **12,** 9202.
10. Galal M. and Farahat A., 2015, *Environmental Monitoring and Assessment*, **187,** 701.
11. Guo-feng L., Zhang lu., Cheng-xin F., Shi-qun H., Jun H. and Paerl H., 2014, *Journal of Pollution Effects and Control*, **2,** 113.
12. Maddison M., Mauring T., Remm K., Lesta M. and Mander U., 2009, *Ecological Engineering*, **35,** 258-264.
13. Jha P., Samal A., Santra S. and Dewanji A., 2016, *American Journal of Plant Sciences*, **7,** 2112-2137.
14. Herath I. and Vithanage M., 2015, Phytoremediation in constructed wetlands. In: Phytoremediation: Management of environmental contaminants, Ansari A., Gill S., Gill R., Lanza R. and Newman L (Eds)., Vol 2, Springer International Publishing, Switzerland.
15. Gallon C., Munger C., Premont S. and Campbell P., 2004, *Water, Air, & Soil Pollution*, **153,** 135-155.
16. Kamal M., Ghaly A., Mahmoud N. and Cote R., 2004, *Environment International*, **29,** 1029-1039.
17. Hou W., Chen X., Song G., Wang Q. and Chang C., 2007, *Plant Physiology and Biochemistry*, **45,** 62-69.
18. Cook C., 1996, Aquatic and wetland plants of India, Oxford New York, Oxford University Press.

19. APHA, 1995, Standard Methods for the examination of water and wastewater, 19th ed., American Public Health Association, Washington, DC.
20. Arguello J., Raimunda D. and Padilla-Benavides T., 2013, *Frontiers in Cellular and Infection Microbiology*, **3,** 1-14.
21. Nas F. and Ali M., 2018, *MOJ Ecology and Environmental Sciences*, **3,** 265-268.
22. Gill M., 2014, *International Journal of Advanced Research*, **2,** 1043-1055.
23. Singh S., Parihar P., Singh R., Singh V. and Prasad S., 2016, *Frontiers in Plant Science,* **6,** 1-36.
24. Maiti S.K., 2003, Handbook of Methods in Environmental Studies: Air, noise, soil and overburden analysis, Vol 2, ABD Pub, Oxford Book Co, India.
25. MacDonald D., Ingersoll C. and Berger A., 2000, *Archives of Environmental Contamination Toxicology*, **39,** 20-31.
26. Istenic D., Arias C. and Brix H., 2011, The Proceedings Strepow Workshop, Andrevlje-Novi Sad, Serbia, p.111-117.
27. Tiwari S., Dixit S. and Verma N., 2007, *Environmental Monitoring Assessment*, **129,** 253-256.
28. Marbaniang D. and Chaturvedi S., 2014, *Keanean Journal of Science,* **2,** 29-34.
29. Chandra R. and Yadav S., 2010, *Ecological Engineering*, **36,** 1277-1284.
30. Ensley D., 2000, Rational for use of phytore-mediation. In: Phytoremediation of toxic metals: Using plants to clean- up the environment, Raskin I. and Ensley D (eds), New York, John Wiley & Sons, Inc, p.3-12.
31. Akbarzadeh A., Jamshidi S. and Vakhshouri M., 2015, *Pollution*, **1,** 1-8.
32. Perez-Sirvent C., Hernandez-Perez C., Martinez-Sánchez M., Lorenzo M. and Bech J., 2017, *Journal of Soils and Sediments*, **17,** 1384-1393.
33. Schuck M. and Greger M., 2020, *International Journal of Environment Research and Public Health,* ***17,*** 4623.
34. Wu H., Zhang J., Ngo H., Guo W., Hu Z., Liang S., Fan J. and Liu H., 2015, *Bioresource Technology*, **175,** 594-601.
35. Ramachandra T., Sudarshan B., Mahesh K. and Vinay S., 2018, *Journal of Environmental Management,* **206,** 1204-1210.
36. Sudarshan B., Mahesh M. and Ramachandra T., 2019, *Indian Journal of Environmental Protection*, **39,** 594-601.
37. Ramachandra T.V., Sudarshan B., Shivamurthy V., Asulabha S. and Varghese S., 2020, *SN Applied Sciences,* **2,** 3228-3238.
38. Bharath H. Aithal and Ramachandra T. V., 2016, *J. Indian Soc. Remote Sens.*, **44(4)**, 617-633.
39. Ahalya, N., Kanamadi R.D. and Ramachandra T.V., 2007, *Journal of Enviro. Bio.*, **28(4)**, 765-769.

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Synthesis and Biological Evaluation of New Mixed Ligand Complexes

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ABSTRACT
In the present study, we report the synthesis of Schiff base prepared by condensation between 4-Hydroxy-3-methoxybenzaldehyde and 4-Aminoantipyrine, as primary ligands and an amino acid L-Tryptophan as a co-ligand through refluxation to form mixed ligand metal complexes [M(VAAP)(Try)(H_2O)$_2$] where M= Cu,Ni,Mn,Zn metal ions. The prepared compounds were identified by C,H,N elemental analysis, Powder X-ray diffraction(PXRD), infrared spectrum (FTIR),UV-Vis spectral analysis,in addition to magnetic susceptibility using Vibrational magnetometer spectroscopic techniques. The magnetic properties as well as electronic spectra indicated that all the metal complexes exhibited octahedral geometry. The PXRD patterns suggested that all complexes were hexagonal systems with unit cell parameters.The cytotoxicity studies of mixed ligand complexes with VAAP and Tryptophan ligand against MCF-7 cell lines by using MTT assay method are presented. The results of the anti-cancer activity in terms of IC_{50}(μg/mL) were recorded and compared with standard drug doxorubicin. Thus, the mixed ligand metal complexes showed a high efficiency of anti-cancer property compared to ligand.

Keywords: *Mixed ligand complex, Doxorubicin, Tryptophan, PXRD, Anti-cancer Activity*

Introduction

Schiff bases and their metal complexes are compounds prepared by reaction of an primary amine and a carbonyl group (aldehyde or ketone). Literature survey shows that several Schiff base complexes with metal ions have been synthesized and characterized and their biological activities have been studied.[1-6] It is possible to synthesize a broad variety of mixed ligand complexes with one metal ion, or a variety of metal ions.

Mixed ligand Complexes derived from tryptophan display anti microbial assay[7,8]. Azo dye pigments of 4-Amino antipyrine are well-known multidentate ligands that coordinate in neutral and ionic form. 4-amino antipyrine is important in a variety of fields, including anti-cancer therapy. Anti-inflammatory, anti-oxidant, and anti-HIV properties have previously been reported-tryptophan is a rare amino acid due to the complexity of its catabolism[9,10]. Thus, the authors focused on the preparation of mixed ligand complexes [4N(4-Hydroxy-3-methoxybenzalideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one] (VAAP) with Cu(II), Ni(II), Mn(II), Zn(II) and an amino acid, L-Tryptophan as a co-ligand. The anti-cancer activities of the derived complexes were investigated in vitro.

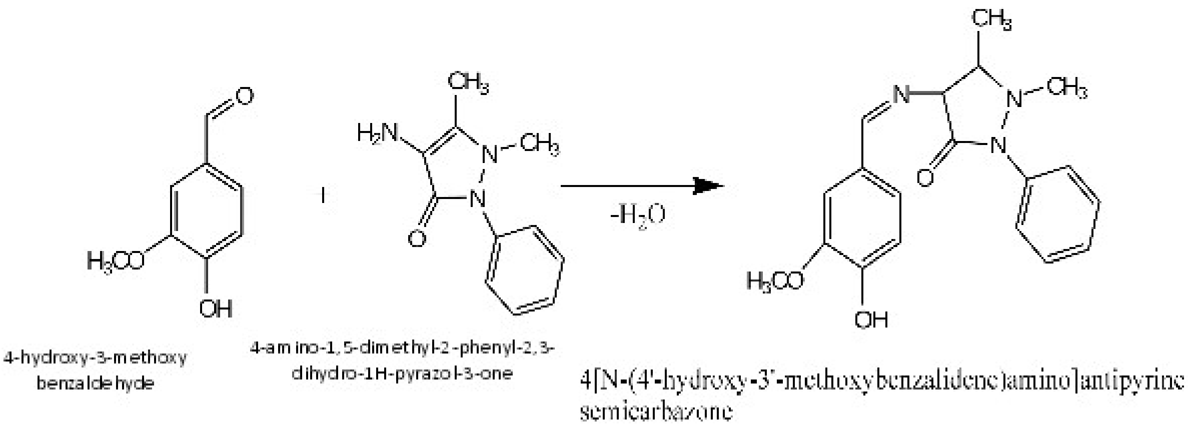
Materials and Methods

All the compounds were obtained from commercial sources and used as such. IR spectra were recorded using Potassium bromide (KBr) pellet using a Bruker Fourier Transform Infrared (FT-IR) spectrometer in the wavenumber range 4000-400 cm^{-1}. Ultra Violet (UV-Visible) 1800 series spectrophotometer was used in the wave length range 200-400 nm. Magnetic susceptibility was measured using Vibrational Magnetometer. Powdered XRD patterns were studied using Bruker-D8 Advance X-Ray Diffractometer.

Synthesis of Primary Ligand

The selected aldehyde for the preparation of primary ligand 4-Hydroxy-3-methoxy benzaldehyde (2g) (0.1M) was solvated in 50 mL of methanol. 4-Amino antipyrine (2.2g) (0.1M) was disintegrated in 50 mL of distilled water. The mixture was refluxed for 60 minutes. On cooling to room temperature, a yellow compound was obtained which was washed with hot water and dried in vacuum. The yield was 85%. Methanol was used to recrystallize the compound.

Scheme 1 Synthesis of primary ligand [VAAP]



Synthesis of Mixed Ligand metal complexes [M(VAAP)(Try)(H_2O)$_2$]

A warm methanolic solution of 10 mL 4[N-(4'-hydroxy-3-methoxybenzalideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one] (0.1M) was added as a primary ligand to a warm methanol suspension of Metal chloride (0.88g)(0.05M). After 40 minutes, a 5 mL of methanolic solution of L-Tryptophan (2.2g) (0.1M) amino acid was added as a secondary ligand in the amount. The mixture was vigorously agitated. The solution was then refluxed for about 2 hours. The crystals were strained, purified with warm water, and vacuum dried. The yield percentage was 72 percent. The formation of mixed ligand metal complexes is given in Scheme-2.

$$\mathbf{MX_2.2H_2O + VAAP \rightarrow [M(VAAP)(H_2O)_2] + Try \rightarrow [Co(VAAP)(Try)(H_2O)_2]}$$

Scheme 2 : Synthesis of mixed ligand metal complexes [M(VAAP)(Try)(H_2O)$_2$]

Results and Discussion

Infrared Spectral Studies

The FTIR spectrum of the primary ligand (VAAP) showed distinctive bands at 1648 cm^{-1} and 1598 cm^{-1}, which were attributed to (C=O) and (C=N) respectively. The band at 1648 cm^{-1} corresponds to (C=O) of the azomethine ligand and is transmitted to the substituted site at 1594cm^{-1} to 1579cm^{-1}, indicating coordination of oxygen from the carbonyl group towards the metal ion. The spectrum shows fine peaks at 3431-3080cm^{-1} that are attributed to the carboxyl component (O-H) and indole moiety (N-OH). There was a modification in the frequency with change in contour and intensity on co-ordination with metal ion at (1648-1899cm^{-1}) indicating that carbonyl oxygen of antipyrine was involved in chelation. There are two more frequencies observed in the IR spectra of Cu(II) complex at 549 cm^{-1} and 431 cm^{-1} which can be attributed to (M-N) and (M-O) stretching vibrations. The FTIR spectra of Schiff base and mixed ligand metal complexes are illustrated in Figures 1-3. The FTIR spectral data of Schiff base and metal complexes given in Table 1.

Table 1. IR absorption bands (cm^{-1}) of ligand and its metal complexes.

Ligand \ Metal complexes	C=O	C=N	C=C	C-N	C-O	M-N	M-O
(TMBAAP)	1648	1598	1496	1355	1295	–	–
[Cu(VAAP) (Try)(H$_2$O)$_2$]	1695	1578	1553	1355	1295	542	441
[Ni(VAAP) (Try)(H$_2$O)$_2$]	1694	1577	1562	1356	1295	541	446
[Mn(VAAP) (Try)(H$_2$O)$_2$]	1695	1596	1561	1356	1295	542	434
[Zn(VAAP) (Try)(H$_2$O)$_2$]	1696	1591	1545	1365	1275	581	440

Electronic Spectral studies

The electronic spectral bands of Schiff base and its mixed ligand metal complexes are given in Table 2. Two bands at 298 nm and 326 nm in the UV-visible spectrum of Schiff base may be attributed to $(n\rightarrow\pi^*)$ transitions within aromatic rings and azo-methine group. The electronic spectra of the mixed ligand complexes [M(VAAP)(Try)(H$_2$O)$_2$] shows bands near 23,529 cm^{-1} which can be assigned to d-d transition suggesting the octahedral geometry around the metal ions. The physico-chemical and Ultra violet spectral data, Magnetic susceptibility of mixed ligand complexes are presented in Table 2.

Table 2: Physico chemical and electronic spectral data of Schiff base and mixed ligand complexes

Compound	Molecular weight (g/mole)	Colour	(% Yield)	UV data (Assignments)	Magnetic Moments. M_{eff} (BM)
[VAAP]	355.39	Yellow	85	n-π^* (Transition)	-
[Cu(VAAP)(Try)(H$_2$O)$_2$]	825.28	Green	72	d-d (Transition)	1.79
[Ni(VAAP)(Try)(H$_2$O)$_2$]	826.74	Dark Green	76	d-d (Transition)	2.70
[Mn(VAAP)(Try)(H$_2$O)$_2$]	829.75	Pink	74	d-d (Transition)	5.73
[Zn(VAAP)(Try)(H$_2$O)$_2$]	874.75	white	75	C-T (Transition)	Diamagnetic

Powder X-RAY Diffractional studies

Powder X-ray diffraction data of three metal complexes [M(VAAPS)(Try)(H_2O)$_2$][where M = Cu, Ni, Zn metal ions is reported. The X-ray powder diffraction pattern for the Cu(II) complex shows 28 reflections ,Ni(II) complex shows 48 reflections and Zn(II) complex shows 38 reflections in the range of 11.44 – 43.69^0 (2θ), which arises due to the diffraction at the crystalline planes of the complex by the X-ray radiation. The distance between two planes which is called as an interplanar distance (d) was calculated by Bragg's equation (2dsinθ=nλ). The experimentally obtained and calculated values of d values along with the relative intensities for the selected peaks were tabulated. The unit cell parameters for the highest peaks and $h^2 + k^2 + l^2$ values were deduced from the spectra. The values for mixed ligand complexes were 1,3,5,6,7,9 and the presence of number 7 in these values indicated that the complexes may be tetragonal or hexagonal systems. The XRD patterns of mixed ligand metal complexes are represented in Figures 1-3.

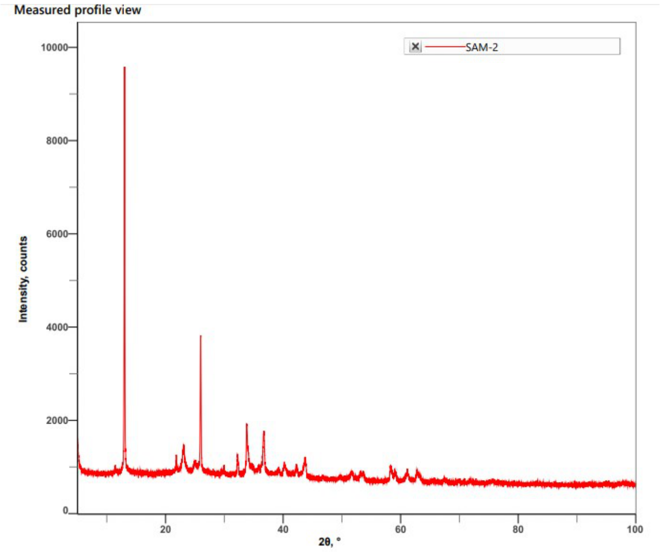


Fig. 1: Powder XRD pattern for Cu(II) mixed ligand complex

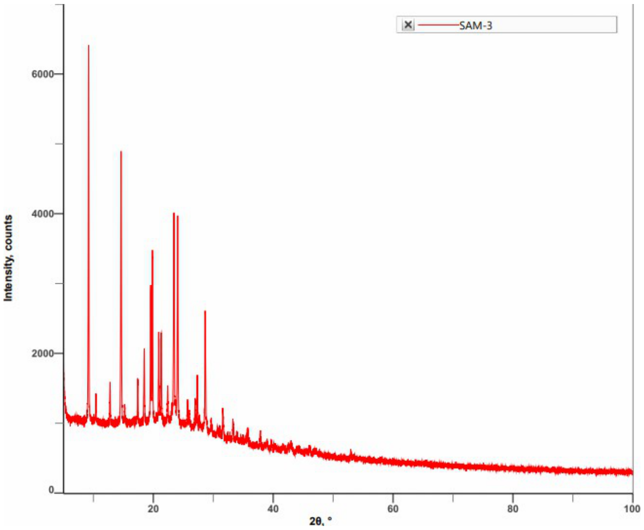


Fig. 2: Powder XRD pattern for Ni(II) mixed ligand complex

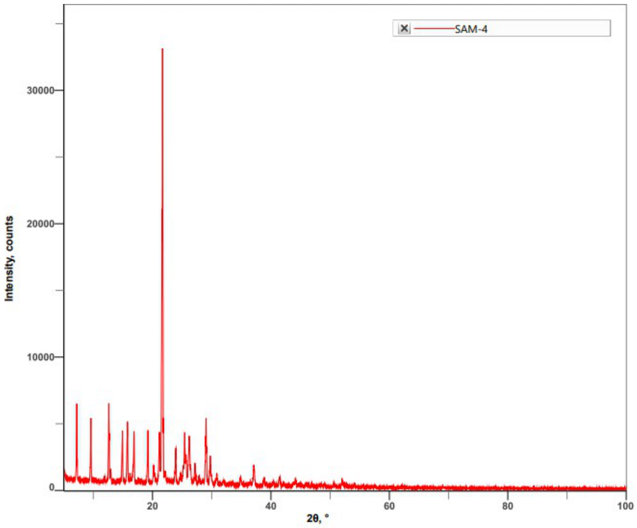


Fig. 3: Powder XRD pattern for Zn(II) mixed ligand complex

Anti-Cancer Activity

Method employed

The Schiff base (VAAP) and mixed ligand metal complexes were used to determine the cytotoxic effect against MCF-7 cell line by MTT assay. The results of the anticancer activity in terms of IC_{50} (μg/mL) were recorded (Table 3 and Figure 4) and compared with those of standard drug doxorubicin which has an IC_{50} value of 6.02 μg/mL. From the results, it is observed that compound **1a** exhibited highest anticancer activity with a IC_{50} value equal to 5.05 μg/mL which is very near to that of the standard drug. The rest of the complexes show good to moderate activity.

Table 1: Inhibition of cell viability of mixed ligand metal complexes [M(VAAP)(Try)(H_2O)$_2$] 1-1d against MCF-7 cell lines with standard drug doxorubicin

Compound	**IC_{50} (μg/mL)**
1. [VAAP]	**11.05**
1.a [Cu(VAAP)(Try)(H_2O)$_2$]	**5.05**
1.b [Ni(VAAP)(Try)(H_2O)$_2$]	**7.05**
1.c [Mn(VAAP)(Try)(H_2O)$_2$]	**6.01**
1.d [Zn(VAAP)(Try)(H_2O)$_2$]	**6.03**
Doxorubicin	**6.02**

Conclusions

The synthesis of a mixed ligand complex containing [M(VAAP)(Try)(H_2O)$_2$], where M represents Cu(II), Ni(II), Zn(II) metal ions has been reported. The Schiff base and its complexes are soluble in water and DMSO (Dimethyl sulfoxide), and have an octahedral geometry. The anti-cancer assignments were checked for MCF-7 Breast cancer cell lines against standard drug Doxorubicin. The results showed that the mixed ligand complexes had potential activity towards cancer cell lines, which we hope will aid in the development of new drugs to control cancer activity.

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References

1. Wail Al Zoubi, Abbas Ali Salih, Al-Hamdani, Susan Duraid Ahmed and Young Gun Ko, 2018, *Journal of Physical Organic Chemistry*, **31(2)**, DOI:org/ 10.1002/aoc.389.
2. Sudhakar Babu, K., Malleswar Reddy, P., Krishna Murthy Naik V. and Ramanjaneyulu, K., 2019, *International Journal of Pharmaceutical Sciences & Research*, **10(9)**, 4396-4403, DOI:10.13040/ IJPSR.0975-8232.
3. Ikechukwu P. Ejidike and Peter A.Ajibade, 2016, *Bio-inorganic Chemistry and Applications,* **1181,** 536-545. DOI:10.1016/.molstruc.2016.12.055.
4. Herandez-Molina, R., Abramov, P.A., Gonzalez-platas. J. and Oramas-Royo, S., Estevezraun, A., Guerra Rodriguez, M., Aranda Tavi. H., Guerra, B. and Fernandez, L., 2019, *Journal of Structural Chemistry*, **165,** 73-78, DOI:org/10.1016/ j.poly.2019.114280
5. Hao-yan Yin, Jing Lai,Juan Tang,Yanli Shang and JUN-Long Zhang, 2017, *Inorganics,* **6,** 20, DOI:10.3390/molecules6010020.
6. Sellamuthu Kathiresan,Subramaniam Mugesh and Jamespandi Annaraj, 2016, *New Journal of Chemistry*, 1-37 DOI.org/10.1039/C6NJ03501A.

7. Khawla M. Sultan Al-Juboori, 2017, *Oriental Journal of Chemistry*, **33(5)**, 2334-2340.

8. Le kaak.Abdul Kareem and Saba H. Mahdi, 2018, *Oriental Journal of Chemistry*, **34(3),** DOI:org/10.13005/ojc/340349

9. Subha, L., Balakrishnan, C., Thalamuthu, S. and Neelakantan, M.A., 2015, *Journal of Coordination Chemistry*, DOI:10.1080/00958972.2015.1008466.

10. Pallavi Jain, Dinesh Kumar, Sulekh Chandra and Namita Misra, 2019, *Advanced Science, Engineering and Medicine*, **11(1-2)**, 144-154.

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Synthesis of Selected Aza-Heterocyclic Compounds with a Green and Sustainable Approach and their Computational Assessment by Molecular Docking

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ABSTRACT

The aim of this study was to establish a green synthesis strategy for three aza heterocyclic compounds: 1, 4-dihydro-2, 3-quinoxalinedione; 2,3-diphenyl quinoxaline; and 4-dihydro-2, 3-quinoxalinedione (4-Hydroxyphenyl) ethyl ester of -6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid. Because of their enormous biological and pharmacological efficacy, heterocyclic compounds have risen in popularity in recent years. Heterocyclic compounds have a wide range of applications due to their synthetic versatility and biological actions such as antibacterial, antiviral, anti-inflammatory, antidepressant, antitubercular, anti-amoebic, and analgesic. The selected chemicals were produced using three distinct processes, with the most efficient and environmentally friendly method being chosen. Compounds were analyzed using Infrared Spectroscopy, Nuclear Magnetic Resonance Spectroscopy, and Mass Spectra. Computational modeling studies were conducted using MVD (Molegro Virtual Docker) 2013.6.0 software to estimate the biological activity of these medicinal compounds in order to determine the optimum green synthesis methodology. Our research showed that the most efficient method for producing 1,4-dihydro-2,3-quinoxalinedione was to use mechano-chemistry in a solvent-free environment with a simple pestle and mortar with 96 percent yield in 15 minutes, and in the case of 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl, the yield was 96 percent in reaction time of 3 minutes. In the case of 2,3 diphenyl quinoxaline, all three procedures, including the use of a sonicator and solvents such as ethanol and water, yielded nearly identical yields (97, 95, and 94 percent, respectively), and the reaction times were likewise similar, ranging from 8, 10 to 20 minutes. With the use of a molecular docking method on a particular protein, docking studies revealed that 1,4-dihyroxyquinoxaline-2,3-dione has anti-inflammatory action and 2,3-diphenylquinoxaline has anti-microbial activity. The target protein was successfully docked with the 1,4-dihyroxyquinoxaline-2,3-dione structure, as evidenced by the minimal binding energy. In-silico *drug development, which uses computational modeling to save time, effort, and expense, is a difficult, expensive, and time-consuming procedure.*

Keywords: *Green chemistry, Aza heterocycles,* In-silico *analysis, Computational modeling, Molegro Virtual Docker*

Introduction

Green chemistry and its twelve principles are a familiar term in the present era. It is a philosophical approach that contributes to sustainable development via application and extension of the principles of green chemistry. This approach is also known as "Environmentally benign" chemistry, "Clean chemistry" and "Benign-by-design chemistry". The term "Green chemistry" has been defined in numerous ways. P.T Anastas referred to this term for the first time in a special US Environmental Protection Agency (USEPA) program in 1991. The program was regarding implementation of sustainable development in chemistry and chemical technology by industries, academics and government. The objective of green or sustainable chemistry is enhancing the availability of useful compounds to human race at the same time preventing the harmful effects of the processes to the environment. This approach has become obligatory in present day chemistry [1].

Industrial production involves numerous chemical reactions that exploit enormous quantities and wide varieties of molecules, reagents, solvents, acidic substances, alkaline reagents etc. The side effect of these chemical processes is the generation of large quantities of undesired and harmful substances along with the generation of required products. The undesired substances may be in the form of solid, liquid or gas and pose the greatest challenge that chemistry has to deal with. So, it has become imperative for the chemists involved in synthesis to reduce chemical pollution. There has been significant enhancement of work in this direction in the last few decades. The intention is modification in chemical processes that will have less impact on environment and human health. By inventing new chemical processes that prevent pollution, green chemistry safeguards the environment. According to the principles of green chemistry, a threat can be eliminated in a simpler way, by using safe raw materials for production process[2].

Ionic liquids have extensively been used in recent years as alternative solvents in organic synthesis. No special apparatus and methodologies are required to carry out reactions in ionic liquids, along with the ability of them being recyclable[3]. These liquids are considered as good solvents for future improvements that can give "green" credentials to their use and applications[4]. In recent years, organic reactions in aqueous media have received considerable attention. The fact is that water is the inexpensive, most abundant, non-toxic, and environmentally friendly solvent. It exhibits unique reactivity and selectivity, which is different from that in conventional organic solvents[5]. Hence, water is proved to be an excellent solvent for many synthetic methods. One approach to making chemical synthesis greener is to use existing chemical synthesis processes but make the process itself safer and less polluting while also making the reagents required for it by greener processes. An example of the former might be to substitute a less volatile and less toxic solvent as an action medium for a chemical synthesis reaction. In some cases, a reagent may be made more safely by using biological processes for its preparation in place of chemical processes. A second general approach to making chemical preparations greener is to use different reagents for the synthesis that are safer and less likely to pollute[6]. The pharmaceutical industries are in need of new innovative alternate synthetic routes for synthesizing therapeutic and pharmacologically important compounds[7]. The development of economic and environmentally responsible methodologies for their inter-conversion remains a challenge, since conventional processes often lead to significant amounts of wastes and/or are performed under strong reaction conditions[8]. Facing with the ever-growing concern for environmental issues, methods with traditional organic synthesis has been greatly challenged. Thus, it places higher expectations on the future work for all the organic chemists[9].

Heterocyclic compounds are acquiring more importance in recent years because of their immense biological and pharmacological potency. Various biologically active synthetic compounds have five membered nitrogen containing heterocyclic rings in their structures. Many compounds bearing pyrazoles and their reduced forms, pyrazolines constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities such as antimicrobial, antiviral, anti-inflammatory, antidepressant, antitubercular, antiamoebic and analgesic. The presence of this core in any molecule has the ability to enhance its activity which has been verified by literature survey studies on synthetic protocols[10]. Pyrimidine and its derivatives have been recognized as important heterocyclic compounds due to their chemical and biological significance in medicinal chemistry. It is well known that the condensation of amino heterocycles and pyrimidine gives rise to the formation of bicyclic heterocycles known as triazolopyrimidines. The 1,2,4-triazolopyrimidines have attracted growing interest due to their important pharmaceutical properties and they appear in a variety of synthetic pharmacophores which possess anti- parasitic, antimicrobial, anticancer and antibiotic activities.

A pertinent category of nitrogen containing benzo heterocyclic compounds containing a ring complex made up of a benzene ring and a pyrazine ring are Quinoxaline derivatives[11]. These compounds have immense therapeutic values and potential activities; have ability to act as antimicrobial agents[12,13], cytotoxic agents, anti-tubercular, anxiolytic, anti-HIV, antioxidant[13], anti-inflammatory[14,15], antimalarial, anticancer, antidepressant[12], antibacterial and antifungal agents[14]. They are recognized to inhibit growth of gram positive bacteria and are active against various transplantable tumors and are thus used in antibiotics[15,16], and treatment of cancer, diabetes, diabetic retinopathy, rheumatoidarthritis, hemangioma and Kaposi's sarcoma[17,18]. Because of their diverse pharmacological and biological properties, they have emerged as privileged structures in combinatorial drug discovery libraries[19].

Most of the reported methods of traditional synthesis have the flaw of usage of expensive reagents/additives, metal catalysts, inflammable organic solvents or harsh reaction conditions, as well as difficult experimental/ work-up procedures. Hence, it is highly preferable to develop mild eco-friendly one-pot synthetic protocols for these highly significant classes of compounds. The goal of computational docking is to find the 3D configuration of the complex that minimizes the energy requirement. *In-silico* molecular docking has been widely used to determine the binding mode (pose) of a small molecule to a binding site. Computational and bioinformatics tools have become very important resources to identify the potential targets for various ligands in the present scenario[20].

Hence, the present work aimed at finding a green protocol for synthesis of selected aza heterocyclic compounds viz. 1, 4-dihydro-2, 3-quinoxalinedione; 2,3-diphenyl quinoxaline and 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5- carboxylic acid ethyl ester. After selecting the best green synthetic protocol, computational modeling studies were performed using MVD (Molegro Virtual Docker) 2013.6.0. software to predict the biological activity of these therapeutic compounds.

Materials and Methods

All the glassware used in the present study were of Borosil make. Prior to use, the glassware was soaked in Chromic acid solution for 24 hours. The glassware was then washed with tap water, further rinsed with distilled water and completely dried in the oven (at 110°C). All the chemicals and reagents used for the study were of analytical grade: o-phenylenediamine, Benzil, 4-Hydroxybenzaldehyde, Diethyl oxalate and Urea (extra pure) were procured from Lobachem, Mumbai; p-TSA was procured from Rankem and Ethanol, Diethyl acetoacetate was procured from MERCK, Germany. Acetic Acid Glacial, procured from SDFCL, Mumbai and Water (extra pure double distilled) were used as solvents

(Table 1). **Table 2** states the analytical instrumental details used in the study for synthesis and spectral analysis.

All the three selected compounds viz. **1,4-dihydro-2,3-quinoxalinedione**; **2,3-diphenyl quinoxaline** and **4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5- carboxylic acid ethyl ester** were synthesized by 3 different methods detailed in **Table 3**. Scheme 1-3 states the general reaction of the synthesis of compounds respectively. After synthesis, the compounds were confirmed by finding out melting point by Gallenkamp's Melting point apparatus. The spectral analysis of the synthesized aza-heterocycles was performed at IIT, Indore, India by FT-IR, NMR and Mass Spectroscopy. The yields of synthesized compounds were also calculated.

Table 1. Properties of Chemicals used in synthesis

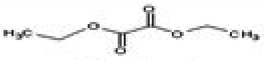
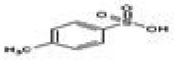
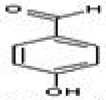
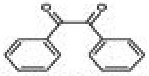
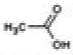
Compound Name	**Molecular formula**	**Molecular weight (gms)**	**Structure**
Diethyl oxalate	$C_6H_{10}O_4$	146.14	 Diethyl oxalate
p-TSA (monohydrate)	$C_7H_8O_3S.H_2O$ $C_7H_8O_3S$	190.22 172	 p-Toluene sulphonic acid
Ethyl acetoacetate	$C_6H_{10}O_3$	130.14 (g/mol)	 Ethyl acetoacetate
4-Hydroxy benzaldehyde	$C_7H_6O_2$	122.12	 4-hydroxybenzaldehyde
Benzil	$C_{14}H_{10}O_2$	210.23	 1,2-diphenylethane-1,2-dione
Ethanol	C_2H_5OH	46.07 (g/mol)	$H_3C—CH_2OH$ ethanol
O-phenylenediamine	$C_6H_4(NH_2)_2$	108.14	 o-phenylenediamine
Urea	CH_4N_2O	60.05	 urea
Acetic Acid Glacial	CH_3COOH	60.05	 Acetic acid glacial

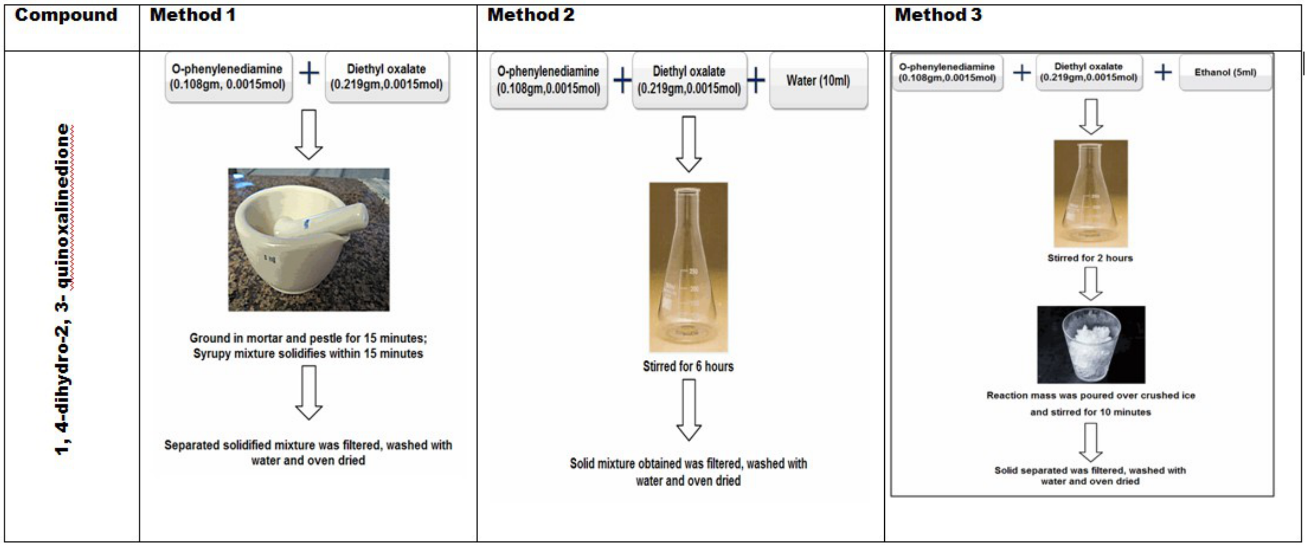
Table 2. Analytical Techniques and Instruments Used

Analytical Techniques and Instruments	**Model/Make/Maufacturer**	**Features**
Weighing balance Shimadzu Japan Fully Automatic Self-Calibration	One Touch	Weighing balance Shimadzu Japan Fully Automatic Self-Calibration
Microwave	Magic cook (Model no. 20S, Mech) Manufacturer: Whirlpool	600W, 50 Hz frequency, 230 Volt AC with timer.
Sonicator	Company: Rivotek	Ultrasonic probe sonicator with accessories, Input Power 230 V, 50 Hz, AC. Ultrasonic power 250 W. Horn diameter 10mm and 20mm with timer.
Orbital Shaker	Scientech (INSTAMES IN-1011)	Orbital incubator shaker
Gallenkamp's Melting point apparatus	Model No. IC0949	
Nuclear Magnetic Resonance spectrometer	BrukerPvt. Ltd. Switzerland, Bruker-Tensor 27	NMR spectrometer used for the analysis was Bruker-Avance-III, 400MHz
Fourier Transform-Infra Red spectrometer	Bruker Pvt. Ltd. Germany	
High Resolution Mass spectrometer	Bruker Pvt. Ltd. Germany	Bruker-MicrOTOFQ-II

The spectral analysis of the synthesized aza-heterocycles was performed in ambient conditions at IIT, Indore, India

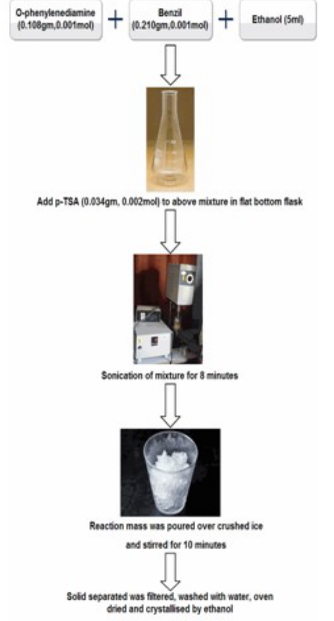
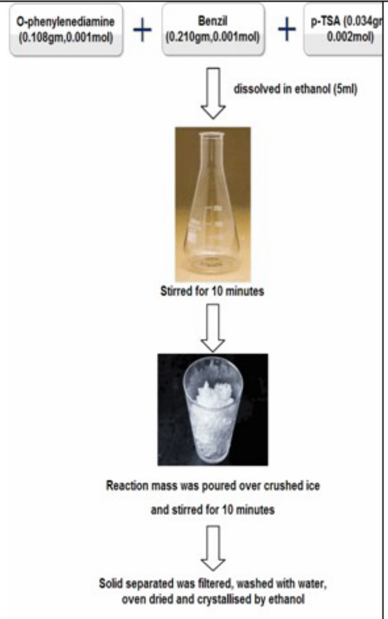
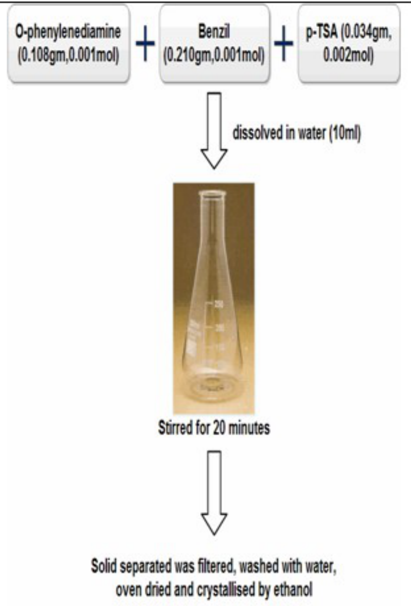
Table 3. Various experimental routes for synthesis of selected compounds

3a. Synthesis Procedures for 1, 4-dihydro-2, 3- quinoxalinedione

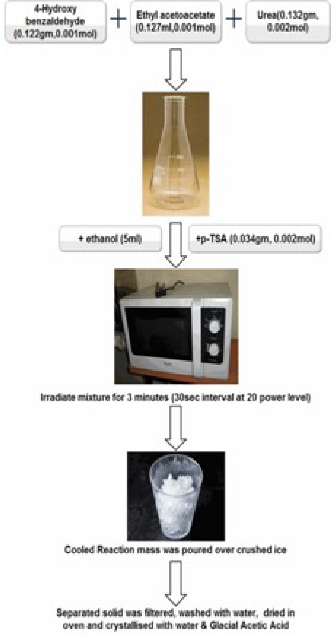
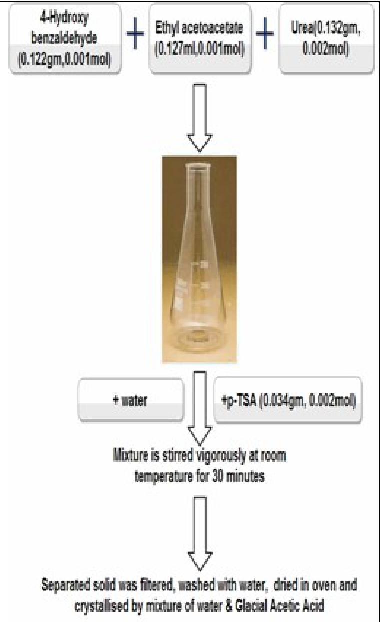
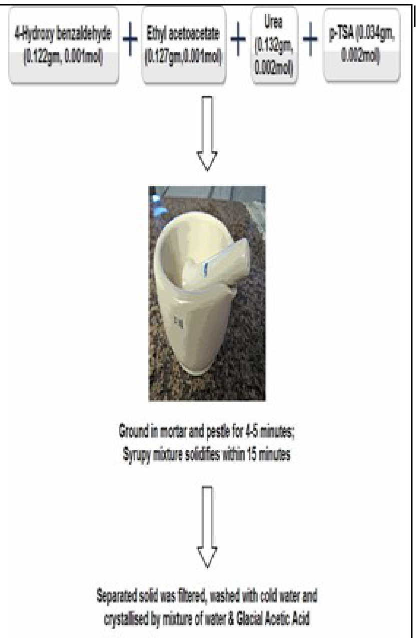


Compound	**Method 1**	**Method 2**	**Method 3**
1, 4-dihydro-2, 3- quinoxalinedione	O-phenylenediamine (0.108gm, 0.0015mol) + Diethyl oxalate (0.219gm,0.0015mol) ⇩ Ground in mortar and pestle for 15 minutes; Syrupy mixture solidifies within 15 minutes ⇩ Separated solidified mixture was filtered, washed with water and oven dried	O-phenylenediamine (0.108gm,0.0015mol) + Diethyl oxalate (0.219gm,0.0015mol) + Water (10ml) ⇩ Stirred for 6 hours ⇩ Solid mixture obtained was filtered, washed with water and oven dried	O-phenylenediamine (0.108gm,0.0015mol) + Diethyl oxalate (0.219gm,0.0015mol) + Ethanol (5ml) ⇩ Stirred for 2 hours ⇩ Reaction mass was poured over crushed ice and stirred for 10 minutes ⇩ Solid separated was filtered, washed with water and oven dried

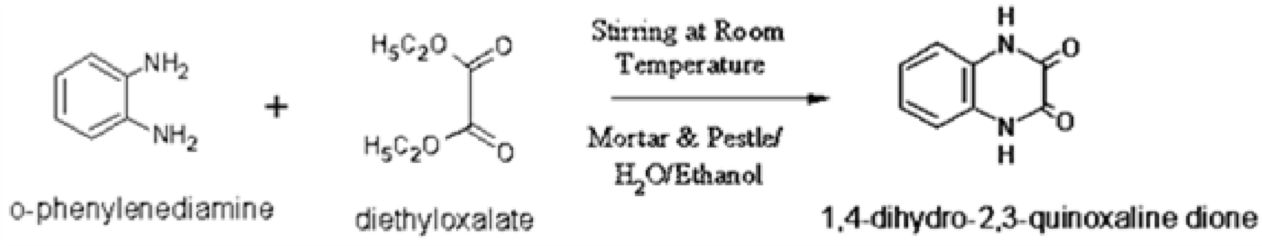
3b. Synthesis Procedures for 2,3-diphenyl quinoxaline

2, 3-diphenylquinoxaline			

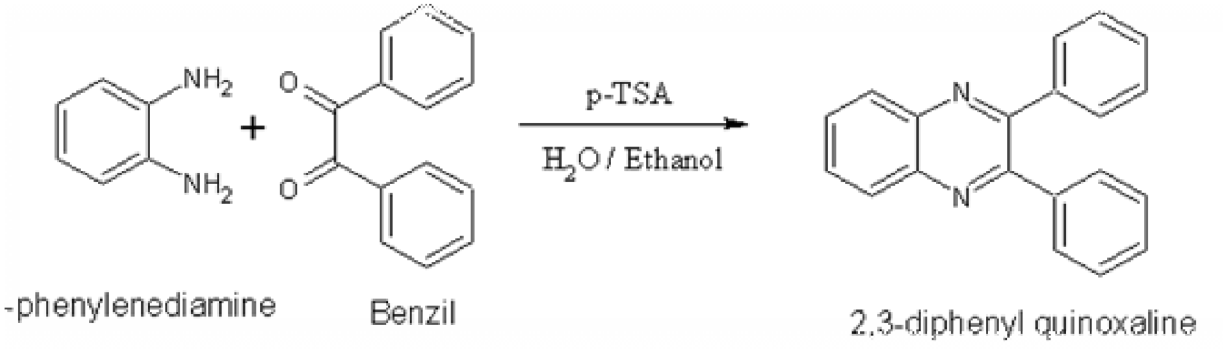
3c. Synthesis Procedures for 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylic acid ethyl ester

4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylic acid ethyl ester			

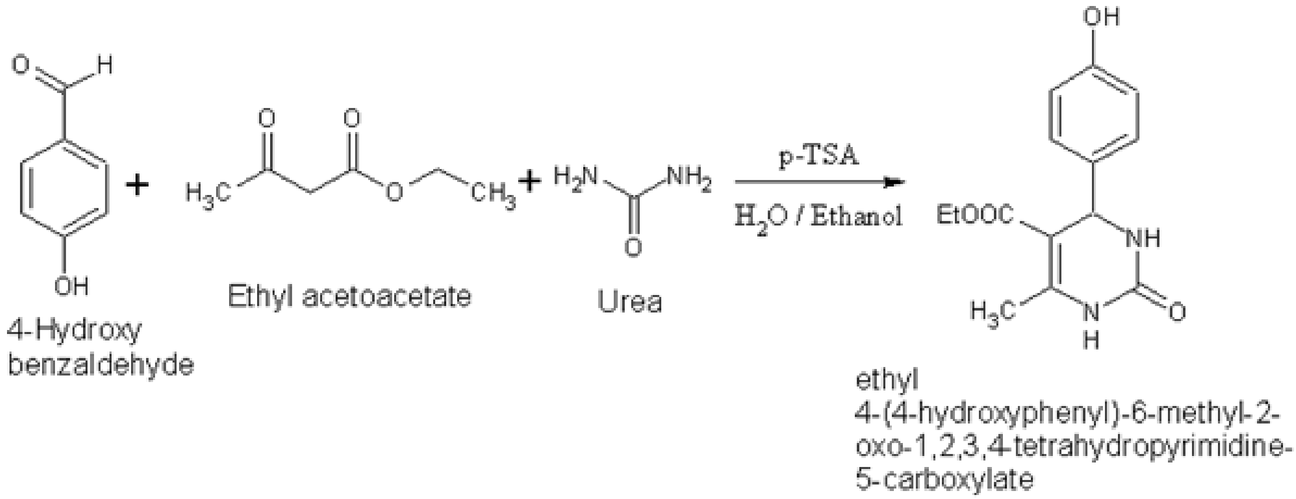
Synthesis Reaction Schemes of 1,4-dihydro-2,3-quinoxalinedione; 2,3-diphenyl quinoxaline and 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5- carboxylic acid ethyl ester



Scheme-1. Synthesis of 1, 4-dihydro-2, 3-quinoxalinedione



Scheme-2. Synthesis of 2, 3-diphenylquinoxaline



Scheme-3. Synthesis of 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylic acid ethyl ester

Molecular Docking Studies

The three dimension structure of receptor (PDB ID 3HSW) was downloaded from RCSB Protein data bank (Available from: http://www.rcsb.org/pdb/home/home.do). For docking analysis PDB coordinates of the target protein and 1,4-dihydroxy quinoxaline 2,3 dione molecule were optimize by MVD (Molegro Virtual Docker) 2013.6.0. In our study, molecular docking was performed on receptor (PDB ID 3HSW) for the above mentioned Quinoxaline derivatives. Molecular dynamics stimulation provides information about docking pose stability. For another derivative of quinoxaline used in our study, 2,3 diphenyl quinoxaline ,docking was performed on receptor (PDB ID 3ZV6).

Results and Discussion

The following section details the results of our experimental work. Table 4 states the percentage yield, reaction time and melting point of the synthesized aza- heterocycles. Table 5 delineates Energy and RMSD values obtained during docking analysis of 1,4-dihyroxyquinoxaline-2,3-dione as a ligand molecule. Table 6 gives the Energy and RMSD values obtained during docking analysis of 2,3- diphenylquinoxaline as a ligand molecule. Table 7 states the Energy and RMSD values obtained during docking analysis of 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester as a ligand molecule. Figure 1 specifies the 3D structures of synthesized Quinoxaline derivatives.

Table 4. Percentage yields, reaction times and melting points of the synthesized aza- heterocycles

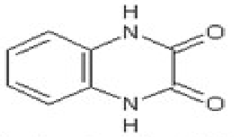
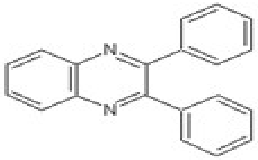
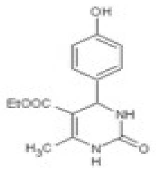
Sr. No.	Compound	Molecular wt. (gms)	Formula	Methods	Time	Yield (%)	Melting point (^oC)
1	 1,4-dihydroquinoxaline-2,3-dione	162	$C_8H_6N_2O_2$	A	15 minutes reaction	96	298
				B	6 hrs reaction	88	298
				C	2hr reaction	79	296
2	 2,3-diphenylquinoxaline	282	$C_{20}H_{14}N_2$	A	8 minutes reaction	97	126
				B	10 minutes reaction	95	125
				C	20 minutes reaction	94	127
3	 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetra hydropyrimidine-5-carboxylic acid ethyl ester	276	$C_{14}H_{16}N_2O_4$	A	3 minutes reaction	96	236
				B	30 minutes reaction	80	238
				C	4-5 minutes reaction	90	236

Table 5. Energy and RMSD values obtained during docking analysis of 1,4- dihyroxyquinoxaline-2,3-dione as a ligand molecule

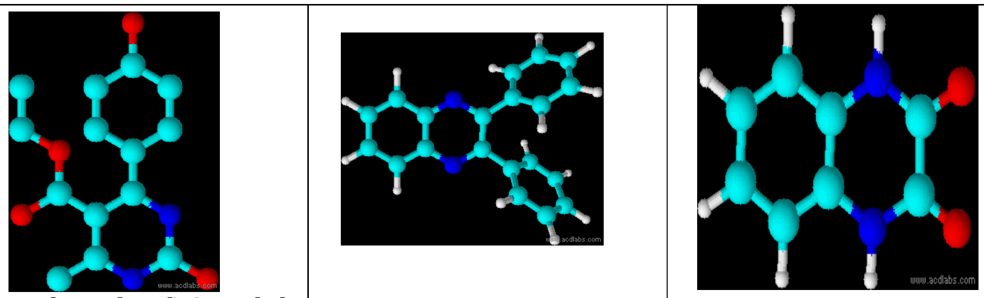
Docking Pose	PDB code	Moldock score	RMSD
1	3HSW _ligand	19.2112	11.1316
2	3HSW _ligand	19.2112	9.8562
3	3HSW _ligand	19.2112	8.8888
4	3HSW _ligand	19.2112	12.0354
5	3HSW _ligand	19.2112	9.4533

Table 6. Energy and RMSD values obtained during docking analysis of 2,3- diphenylquinoxaline as a ligand molecule

Docking Pose	PDB code	Moldock score	RMSD
1	3ZV6 _ligand	30.0426	5.4313
2	3ZV6 _ligand	30.0427	6.1986
3	3ZV6 _ligand	30.0427	3.6356
4	3ZV6 _ligand	30.0427	5.3784
5	3ZV6 _ligand	30.0427	6.2503

Table 7. Energy and RMSD values obtained during docking analysis of 4-(4- Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester as a ligand molecule

Docking Pose	PDB code	Moldock score	RMSD
1	3ZV6_ligand	-0.8593	5.6602
2	3ZV6_ligand	-0.8593	8.3054
3	3ZV6_ligand	-0.8592	3.4673
4	3ZV6_ligand	-0.8592	5.4109
5	3ZV6_ligand	-0.8592	3.7634



4-(4-Hydroxyphenyl)-6- methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester

2,3-diphenylquinoxaline

1,4-dihyroxyquinoxaline-2,3-dione

Figure 1. 3D structures of synthesized Quinoxaline derivatives

Discussion

The selected compounds were synthesized by Methods 1, 2 and 3 as stated in the Methodology of previous section. With **Method 1**; 96% yield of 1,4-dihyroxyquinoxaline- 2,3-dione was obtained by grounding reactants in mortar and pestle for 15 minutes. The melting point of the compound was observed to be 298°C **(Table 4).** In **Method 2**; there was 88% yield of the compound and melting point of 298°C was observed. **Method 3**; yielded 79% of the compound with melting point of 296°C.

On spectral analysis with FT-IR, NMR and Mass spectrometer the following peaks were observed: **IR (cm^{-1}):** 3372.94, 1631.13, 1590.07, 1498.21, 1458.78, 1267.91, 927.97, 809.52, 748.24. The absorption band at 1590 cm^{-1} is due to C— C stretching of aromatic ring system, that at 3372.94 cm^{-1} is due to -NH group, while that at 1631.13 cm^{-1} is due to –C=O group. **1H- NMR (400MHz, CDCl$_3$),** δ ppm 7.76, 7.75, 7.65, 7.64, 5.63, 5.50, 5.48; the peaks in the region ä 7.6-7.7 are due to 4 aromatic protons. **MS (m/z)** 162.14 (calc.), 163 (exp.) Similarly 2,3-diphenyl quinoxaline was synthesized by Methods 1, 2 and 3 [21] **. Method 1**; gave 97% yield of compound and melting point 126°C was observed. **Method 2**; gave 95% yield and melting point of compound was observed to be 125°C. **Method 3**; gave 94% yield and melting point of 127°C was observed **(Table 4**).

On spectral analysis with FT-IR, NMR and Mass spectrometer the following peaks were observed. **IR (cm^{-1}):** 1665.51, 1585.40, 1446.19, 1317.39, 1210.31, 1167.94, 1069.76, 995.32, 873.21, 786.54, 716.79, 639.85. The absorption bands at 1665 cm^{-1} and 1585.40 cm^{-1} are due to C-C stretching of aromatic ring (phenyl nucleus), that at 1210.31 cm^{-1} due to plain bending of C-H in aromatic phenyl ring, weak absorption band at 3061 cm^{-1} is due to aromatic C—H stretching, strong absorption bands at 716.79 cm^{-1} and 786.54 cm^{-1} indicate mono substituted benzene ring. The weak absorption band at 1446 cm^{-1} is due to –C=N stretching. **1H-NMR (400MHz, CDCl$_3$),** ä ppm 8.01, 7.99, 7.69, 7.67, 7.65, 7.54, 7.53, 7.51. **MS (m/z)** 282.33 (calc.), 283.13 (exp.) [21]

4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester was synthesized by Methods 1, 2 and 3 [22]. By applying **Method 1** 96% of compound was yielded in reaction time of 3 minutes which had a melting point of 236°C. **Method 2** gave 80% yield and melting point of 238°C was observed. **Method 3** gave 90% yield of compound and melting point of 236°C was observed **(Table 4)**.

On spectral analysis with FT-IR, NMR and Mass spectrometer the following peaks were observed **IR (cm^{-1}):** 3510.63, 3150.20, 1674.20, 1596.10, 1452.33, 831.42, 776.55, 707.62, 646.66. The characteristic broad band in the region 3150.20 cm^{-1} is due to hydroxyl group (-OH).The absorption band at 3510 cm^{-1} is due to –NH group. The absorption band at 1596.10 cm^{-1} is due to C-C stretching. The absorption band at 831.42 cm^{-1} is due to paradisubstituted benzene. **1H-NMR (400MHz, CDCl$_3$)**, δ ppm 10.59, 9.78, 9.56, 9.32, 9.11, 7.64, 7.74, 7.62, 7.05, 7.03, 6.94, 6.92, 6.70, 6.69, 5.51, 5.06, 3.97, 3.95, 3.47, 2.50, 2.24, 2.07, 1.90, 1.10, 1.08. Anal. calculated for $C_{14}H_{16}N_2O_4$, MS (m/z) 276 (calc.), 277 (exp.) [22].

Chemistry plays a significant role in bridging physics, material sciences and life sciences. Our society has become dependent on chemical products in order to maintain our current standard of living and to improve quality of life. The last century has been highly productive in the developments in water treatment, pharmaceutical development, material science, polymers, agriculture pesticides and fungicides, detergents, petroleum additives and so forth. According to Paul Anastas, the goal of green chemistry is not just clean-up, it is about redesigning chemical processes from the ground level. It is about making industrial chemistry safer, cleaner and more energy efficient throughout the product's life cycle, from synthesis to clean up to disposal.

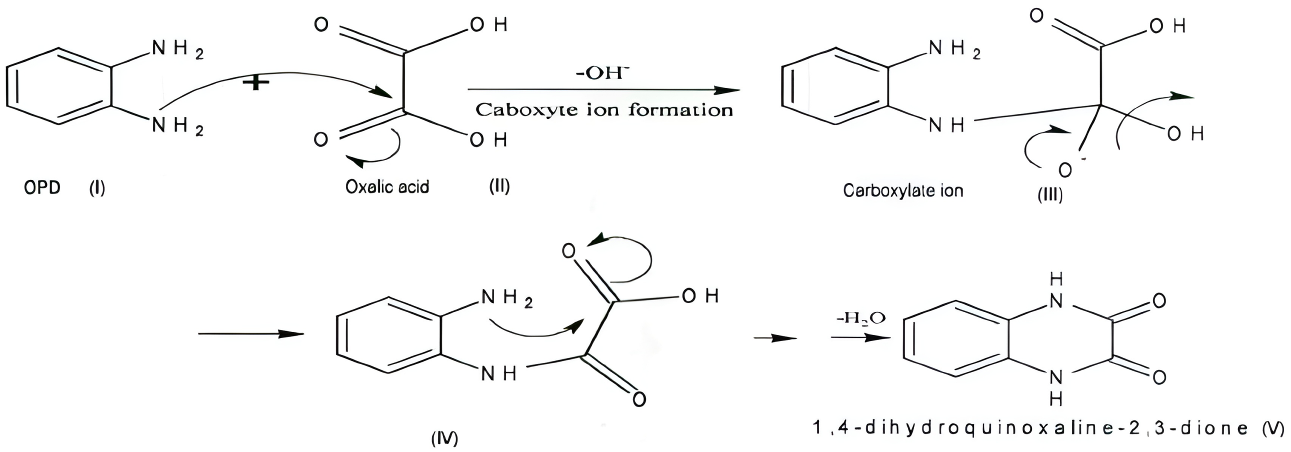
Quinoxaline-2,3-diones are mainly prepared by condensation of *o*-phenylenediamines with various ketoacid derivatives[23,24]. The last and crucial step is the ring closure of an *o*- phenylenediamine with oxalate derivatives forming a *para*diazine ring of quinoxaline-2,3- dione. Normally, this step is carried out using the method of Phillips[25] under the catalysis of strong acid[26,27,28]. Frequently a solvent is also employed. It is desirable to perform the ring closure in the synthesis of quinoxaline-2,3-dione under mild reaction conditions in order to avoid any side reactions. The general Phillips reaction carried out under harsh conditions, as commented by Piguet et al[29] requires modification for various reasons.

As far as possible, catalysts and solvents should be avoided, as these substances are potential impurities in the final product. In our modified procedure, green solvents and no catalysts have been employed. The reaction temperature is low, *i.e.* room temperature. Excess diethyl oxalate, a reactant, serves as a mild solvent and can be easily removed from the solid product by simply washing with ether. **(Table 4)**.

Organic synthesis in aqueous media is rapidly gaining popularity because of the fact that the use of many toxic and volatile organic solvents, particularly chlorinated hydrocarbons, contribute to pollution. Furthermore, using water as a solvent offers many advantages, such as simple operation and high efficiency in many organic reactions that involve water soluble substrates, reagents such as carbohydrates. Jaberi and Amiri[30] synthesized 2-substituted benzimidazoles by one pot reaction of o-phenylenediamine with aldehydes in presence of boric acid in water at room temperature in good yields.

Ratnadeep Ghadage and Pramodkumar[11] synthesised 1,4-dihydro-2,3-quinoxalinedione using oxalic acid dihydrate, conc. HCl and o-phenylenediamine. On stirring the mixture at 100 °C for 20 min, 77% of the compound was synthesized. They observed melting point of the compound at 300°C. While the melting point of our compound was (296-298°C) **(Table 4)**, which was in conformity to that observed by Ghadage et al.,[11]. They reported the spectra of the synthesized compound as; IR (cm^{-1}): 3404, 3176, 3113, 1682, 1618, 1522, 1499, 1426, 1383, 755, 744; 1H-NMR ($CDCl_3$), ä ppm 8.003 (s,2H, NH), 6.978 (t, 2H, CH), 6.715 (d, 2H,CH).

They have proposed a condensation reaction mechanism (Scheme 4) for the synthesis of 1,4- dihydro-2,3-quinoxalinedione under the same conditions[11.]



Scheme 4: Mechanism for synthesis of 1,4-dihydroquinoxaline-2,3-dione from oxalic acid and o-phenylene diamine [11]

Thakuria and Gopal Das[31] performed efficient synthesis of the potential pharmacophore 1,4- dihydro-quinoxaline-2,3-dione in a one-pot reaction at room temperature from substituted o- phenylene diamine and oxalic acid under solvent-free conditions by a simple grinding method with unsurpassed atom economy. They found the mass spectra of compound MS (m/z): 162.0 (M+), whereas we observed it at 163. The time required by Thakuria et al.,[31] was 0.5 hrs with 98 % yield, while we found the yield under solvent free conditions to be 96% at a reaction time of 15 minutes.

Grinding technique has been very successful in fulfilling the principles of green chemistry. Using this technique the reactions are carried out under solvent free conditions with maximum yield and minimum cost. Grinding of reactants can be carried out manually using a pestle and mortar or by using a high speed vibrating mill[32,33]. According to conventional approach, the collisions between reactants are necessary to carry out the reaction. Grinding forces the molecules of the reactants to undergo forced collisions and thus leads to the formation of products. As compared to conventional methods, the time required for completion of reaction using grinding is much less and this can be attributed to the large number of collisions at faster rate between the reacting molecules during the grinding process. In addition to this, the amount of heat produced because of friction during grinding process also helps in the completion of reaction. The general usefulness of solvent free conditions in chemical reaction has been well described by Toda[34] and Tanaka[35]in a review which covers a number of synthetically useful reactions.

Green chemistry is established as national responsibility in present time. It is highly amenable to have a convenient and rapid synthetic procedure that is energy efficient. Such a procedure should be suitable for large-scale operation also having a practically applicable orientation. Grindstone Chemistry is useful for desktop synthesis as well as kilogram scale operation.

The pioneering work of Toda et al[34] has shown that many exothermic reactions can be accomplished in high yield by just grinding solids together using mortar and pestle, a technique known as ‘Grindstone Chemistry’ which is one of the ‘Green Chemistry Techniques’. The energy generated through friction initiates the reactions. Grindstone Chemistry leads to reduction in wastes generation along with being highly reactive and energy efficient. It is simple to tackle such reactions as they reduce pollution, are more economical and ecologically favorable. Solid-state reactions are efficient and selective as compared to reactions in solution[36]. This method is superior to conventional methods as it is eco-friendly with high yield and does not require any special apparatus, It is also non-hazardous, operationally simple and convenient. A hand held electric food mixer with stainless steel rotor is a simple and inexpensive option for conducting Grindstone Chemistry on a large scale.

Conventional Method of synthesis of 2,3-Diphenyl quinoxaline: 1.26 gm of benzil was dissolved in 8 mL of warm rectified spirit and transferred into a 100 mL round bottom flask containing 1.08 gm of o-phenylene diamine dissolved in 8 mL of rectified spirit. Refluxing was done for 1 hour on a boiling water bath. Then, water was added until slight cloudiness persisted. The crude product was filtered and recrystallized from rectified spirit (75% yield, 1- 1.5hrs)[37,38]. Significant yield improvement in a shorter reaction time was observed when we synthesized the same compound with the help of ultrasound (97%, 8 minutes).

Joshi et al.[39], synthesized 2,3-Diphenyl quinoxaline by microwave irradiation of benzil (0.01M), o-phenylene diamine (0.01M) and ethanol (16ml) which gave 60% yield in a reaction time of 55 seconds, while we achieved 97% yield after 8 minutes of sonication. The yield of our compound was found to be significantly higher than that by the conventional method of synthesis which led to 51% yield in a reaction time of 0.5 hrs[304]. Although the reaction time by Joshi et al.[39] was less, the yield could be increased by modifying the technique and slightly varying the reaction time.

Jyotidas and Sarkar[40] synthesised quinoxalines in aqueous medium in the presence of tetraethylammonium bromate. They reported the spectral data of 2,3-Diphenylquinoxaline as ^{1}H NMR: (300MHz, CDCl3) δ 8.193(t, 2H, J1=2.7 Hz, J2=3.6 Hz, ArH),7.77-7.803(m, 2H, ArH), 7.519-7.544(m, 4H, ArH), 7.344-7.365(m, 6H, ArH).13C NMR: (75MHz, CDCl3) δ 153.43, 141.17, 138.99, 129.95, 129.79,129.15, 128.78, 128.25 and IR (cm^{-1}): 3057.17, 3028.24, 1548.84. The yield was found to be as high as 92% [41] ; they synthesized 2,3 diphenyl quinoxaline applying efficient practical techniques like sonication (sonochemistry sythesis), UV radiation and simple mechanochemistry using mortal-pastel method. They monitored the progress of the reaction by TLC and did the characterization by IR and NMR. Compared with traditional methods, these methods are more convenient and reactions lead to higher yield (95.8-98.3%), shorter reaction time (10, 15, 17 minutes) and milder conditions, without generation of pollution and are safer to the analyst. The melting point of the compound observed by Bendale et al., [41] was in the range 122-124°C, whereas we observed the melting point to be between 125-127°C. The spectroscopic studies revealed λmax: 292 nm IR: characteristic IR (KBr) bands found at: 3065, 1441, 1395, 768, cm^{-1}. Our observation of MS was (m/z): 283.13. As observed by Bendale et al.,[41] we also found that in comparison with traditional methods, our methods were more convenient and reactions can be carried out in higher yield (94-97%), shorter reaction time (8-20 minutes) and milder conditions (sonication and stirring at room temperature), without generation of pollution and are safer to the analyst.

To investigate the role of ultrasonic irradiation in our method, the reactions were carried out in the presence of p-TSA, dissolving o-phenylene diamine and benzil in EtOH and sonicating the above mixture at room temperature. From the results that are summarized in **Table 4**, it is clear that, under the same reaction conditions, reactions under ultrasonic irradiation led to relatively higher yields and shorter reaction times. It is presumed that the efficiency using ultrasound irradiation is due to the cavitation phenomena. An ultrasonic wave breaks intermolecular forces due to its pressure wave with alternate compressions and rarefactions. The chemical and physical effects of ultrasound derive primarily from acoustic cavitation which includes formation, growth and collapse of the cavity [42,43.] Bubble collapse in liquids results in an enormous concentration of energy from the conversion of kinetic energy of the liquid motion into heating of the contents of the bubble. The high local temperatures and pressures produced by cavitation lead to a diverse set of applications of ultrasound such as accelerating the rate of the reaction, changing the reaction pathway, enhancing chemical reactivity and synthesizing organic compounds[44.]

The two main sources of ultrasound in organic synthesis are ultrasonic cleaning baths and ultrasonic immersion probes, which typically operate at frequencies of 40 and 20 kHz, respectively[45]. The former are more commonly employed in organic synthesis simply because they are less expensive and hence more easily available to chemists, even though the amount of energy transferred to the reaction medium is lower than that of ultrasonic probe systems, which deposit the acoustic energy directly into the reaction medium.

Compared with traditional method, our method is more convenient and reactions can be carried out with higher yield, shorter reaction time and under milder conditions, without generation of pollution and safer to the analyst. This approach has several advantages, such as excellent yield, short reaction time, low cost, simple experimental as well as isolation procedures, and finally, it is in agreement with the green chemistry protocols.

Some organic reactions using water as the medium suffer from a serious disadvantage due to the non-homogeneity of the reaction mixture because most of the organic chemicals are almost insoluble in water and this results in a many fold decrease in reaction rates or sometimes reaction does not happen at all. This problem can be overcome by providing the activation energy to the reaction by using microwave irradiation. The microwave dielectric heating effect uses the ability of some liquids and solids to transform electromagnetic energy into heat and thereby drive chemical reactions. This *in situ* mode of energy conversion has many attractions for chemists, because its magnitude depends on the properties of the molecules. This allows some control over the properties of the material and may lead to reaction selectivity. There are a variety of methods for carrying out microwave-assisted organic reactions using domestic or commercial ovens; this is basically known as microwave-induced organic reaction enhancement (MORE) chemistry. Most of the published chemistry has been performed using domestic microwave ovens. The key reason for using devices intended for heating food items to perform synthesis is that they are readily available and inexpensive[46].

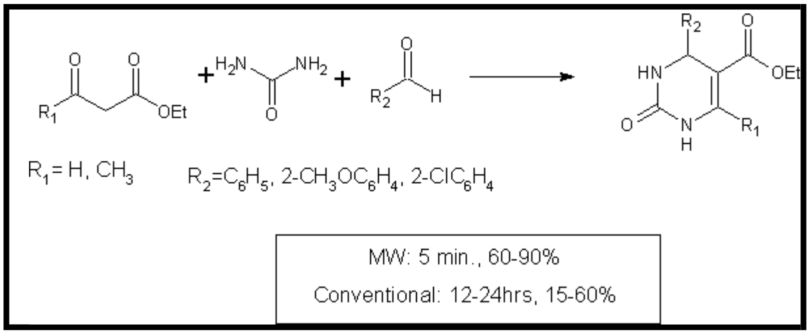
Anil Kumar Jogula et al.,[47] used Cyanuric chloride as a new catalyst for the one-pot Biginelli reaction coupling of β-ketoester, aldehydes and urea to give the corresponding tetrahydropyrimidinones. The reaction time required was 12 hrs, with 80% yield and the melting point was within 198-200°C. When we synthesized the same compound viz. 4-(4- Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester in the presence of p-TSA using 4-hydroxybezaldehye, ethyacetoacetate, ethanol and urea applying microwave irradiation, we observed a yield of 96% within 3 minutes reaction time and the melting point was 236-238°C[22]. Yadav et al.[48], performed microwave assisted synthesis of tetrahydropyrimidinones in the presence of TTSA. They irradiated an equimolar mixture (2 mmol each) of aldehyde, β-keto ester and urea (or thiourea) in acetonitrile, with catalytic amount of TTSA (3%) and the contents were irradiated by microwave radiation at interval of 10 sec. They found the yield of 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4- tetrahydropyrimidine-5-carboxylic acid ethyl ester to be 95% and melting point 201°C. M.V. Yadav[48] and Nayak et al.[49], synthesized Ethyl 4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4- tetrahydropyrimidine-5-carboxylate monohydrate using a mixture of ethylacetoacetate (0.1 mol), para hydroxy substituted benzaldehyde (0.1 mol) and urea which was refluxed in 50 mL of ethanol for 2.0 hrs in the presence of concentrated hydrochloric acid as catalyst. The quenching of reaction mixture was done in ice cold water. The precipitate obtained was filtered, dried and crystallized from methanol to obtain the title compound[49].

Heating under reflux for several hours is logical for endothermic reactions. For exothermic reactions, however, such energy input would be superfluous. The convenience and the time saving that results from the use of Grindstone Chemistry—for small scale as well as large scale reactions—is illustrated here in our work by describing the successful application of this technique to the multicomponent Biginelli reaction. We employed p-toluene sulfonic acid (p-TSA), an inexpensive and common organic chemical, which was an efficient catalyst for this reaction.

Bose et al.[50], synthesized tetrahydropyrimidinone using grindstone technique and found the yield to be 95%, with melting point 236–238°C. While Sun et al.[51] also reported the melting point of the compound to be in the range 236–238°C. Ushati Das et al.,[52] also synthesized Ethyl-4-(4-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate monohydrate using 4-Hydroxybenzaldehyde, ethyl acetoacetate, urea and p-TSA in mortar and pestel (yield 95%, m.p. 509–511 K). The observations by Ushati et al.[52], and Bose et al.[50], were almost similar to those noted by us under solvent free conditions of grindstone technique in terms of yield (our yield was 90%, with m.p. 236°C) and a reaction time of 4-5 minutes.

Mohideen et al.[53], synthesized Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine- 5-carboxylate by grounding a mixture of benzaldehyde (0.106 g, 1 mmol), ethyl acetoacetate (0.130 g, 1 mmol) and urea (0.070 g, 1.17 mmol) with four drops of *ortho* phosphoric acid for about 30 minutes. The reaction mixture was cooled for 15 minutes and poured into a beaker containing 50 mL of cold water. The precipitate obtained was filtered, washed with water and ethanol to get a white solid (0.26 g, 92% yield; mp 203–204). Physiologically active tetrapyrimidinones were successfully prepared by Biginelli reaction by grinding aryl aldehydes, ethylacetoacetate and urea/ thiourea in presence of p-toluenesulphonic acid at room temperature[54] Several mechanisms have been proposed for the synthesis along with that given by K. Folkers and T.B. Johnson[55] A proposal was also given by C.O. Kappe[55] in 1997 for the above synthesis.

The Biginelli reaction is important for the preparation of dihydropyrimidine derivatives and excellent results are found for reactions carried out with microwave enhancement as observed by Hayes[57]. He found that single-mode cavities offer more consistent and predictable energy distribution. Single-mode instruments produce one homogeneous, intense pocket of energy that is highly reproducible. Due to their uniform energy distribution and higher power density, these systems typically couple more efficiently with small samples[57].



Scheme 5 Biginelli reaction

The original Biginelli reaction was carried out by refluxing a mixture of the three components, ethyl acetoacetate, benzaldehyde and urea in presence of ethanol catalyzed by small amount of HCl[58] which often resulted in poor to variable yields of desired products (20-70%)[59.]

Multi-component systems being more efficient and economical have attracted the attention of organic chemists. Further, these reactions can be carried out without isolation of the intermediates (one pot and one step synthesis) and avoid the protection/deprotection strategies in the synthesis as well as time consuming purification processes. H.M. Hugel,[60] and Shete et al [61], checked the efficiency of various metal phosphates (NaH_2PO_4, KH_2PO_4 and K_2HPO_4) as promoter in the multicomponent dihydropyrimidine synthesis. They synthesized 5- acetyl / ethoxycarbonyl, 4-aryl / substituted aryl, 6-methyl, 3, 4- dihydropyrimidine-2-one / thione using a mixture of urea / thiourea, appropriate aldehyde, acetyl acetone / ethyl acetoacetate and metal phosphate in 20 mL of glacial acetic acid solvent which was heated at 45-50°C under magnetic stirring for 20-40 min. They found glacial acetic acid to perform well as a solvent as compared to ethanol. With glacial acetic acid, all products were obtained in satisfactory yields (moderate to excellent). Further, all metal phosphates they used were cheap and nontoxic. With NaH_2PO_4 the yield of

5- ethoxycarbonyl, 6-methyl, 4-phenyl, 3, 4-dihydro-pyrimidine-2(1H)-one was 66% and melting point was found to be between 192-195°C; with KH_2PO_4 yield was 89% and melting point was between 204-206°C; while with K_2HPO_4 yield was 50% and melting point was between 200-202°C. The melting point reported was 202-203°C [62]. The lower meting points of these products (as compared to the reported values) indicated low purity of the compounds. It can be seen that catalyzing the reaction by KH_2PO_4 gave superior results over the other two metal phosphates both in terms of yield and purity.

It is well known that Biginelli reaction is an acid catalyzed versatile one pot multi-component reaction for the synthesis of 3, 4-dihydropyrimidin-2(1*H*)-one / thione derivatives. The reaction occurs via formation of metal-enolate ion pairs and metal-*N*-acylimine intermediates which govern the overall progress of Biginelli reaction. The stabilization of *N*-acylium intermediate by the cation of the catalyst is the mechanism involved in this reaction[63].

Computational modeling in drug discovery utilizes *in-silico* tools for drug design or for identifying ‘lead’ compounds from the existing databases that exhibit potential of inhibitory effects. For docking analysis PDB coordinates of the target protein and synthesized molecules were optimized by MVD Software as described in methodology section. **Table 5, 6 and 7** states the results of docking analysis of 1,4-dihyroxyquinoxaline-2,3-dione; 2,3- diphenylquinoxaline and 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4- tetrahydropyrimidine-5-carboxylic acid ethyl ester respectively. The 3D structures of selected compounds were optimized by ACD Lab software **(Figure 1)**. These coordinates were found to have minimum energy and stable conformation. 1,4-dihyroxyquinoxaline-2,3-dione shows anti inflammatory activity as shown by the molecular docking process. This can be used as lead compound for development of novel anti- inflammatory drugs. In our study, molecular docking was performed on receptor (PDB ID 3HSW) for the above mentioned quinoxaline derivatives. Molecular dynamics stimulation provided information about docking pose stability. Other derivatives of quinoxaline which were used in our study were 2,3-diphenylquinoxaline and 4-(4-Hydroxyphenyl)-6-methyl-2-oxo- 1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester for which docking was performed on receptor (PDB ID 3ZV6). 2,3-diphenylquinoxaline was found to exhibit anti-microbial activity. The minimum binding energy indicated that the target protein was successfully docked with 1,4-dihyroxyquinoxaline-2,3-dione structure. The top five poses are given in **Tables 5, 6 and 7**.

Conclusions

We have developed efficient method for the synthesis of 1,4- dihydro-2,3-quinoxalinedione; 2 ,3 diphenyl quinoxaline and 4-(4-Hydroxyphenyl)-6- methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester. The reaction of o-phenylene diamine and Benzil in ethanol and p-TSA by using sonicator, has several advantages, such as excellent yield, short reaction time, low cost, simple experimental as well as isolation procedures, and finally, it is in agreement with the green chemistry protocols. We have found that p-toluenesulfonic acid (p-TSA), an inexpensive and common organic chemical, is an efficient catalyst for this reaction. The catalyst used in an inexpensive chemical that is commonly found in most organic laboratories.

The synthesis of 1,4-dihydro-2,3-quinoxalinedione by the reaction of o-phenylene diamine and Diethyloxalate by Grindstone technique is a simplified and rapid synthetic procedure. This procedure is much simpler and faster. The methodology does not require the use of any organic solvent, catalyst and additional oxidant, thus meeting green chemistry objectives. Another useful aspect is that this procedure is energy efficient. This reaction can be easily adapted for use as an interesting experiment in an organic chemistry teaching laboratory.

Microwave radiation was used for the synthesis of 4-(4-Hydroxyphenyl)-6- methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylic acid ethyl ester. The microwave assisted method is an efficient rapid, simple, feasible and eco-friendly method for the synthesis of a large number of organic heterocyclic molecules.

Hence our study concludes that the best method for efficient synthesis of 1,4-dihydro-2,3- quinoxalinedione was by mechano-chemistry under solvent free condition by employing simple mortar pestel leading to a yield of 96% at a reaction time of 15 minutes. For the synthesis of 4-(4-Hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5- carboxylic acid ethyl ester microwave irradiation is the best method (yield 96%, reaction time of 3 minutes). Whereas, in case of 2,3 diphenyl quinoxaline all the three methods i.e., application of sonicator and solvents like ethanol, water gave almost comparable yields (97, 95 and 94 % respectively), the reaction times were also comparable viz., 8, 10 and 20 minutes.

Drug discovery is a complex, expensive and arduous process and *in-silico* drug discovery employing computational modeling reduces the time, efforts and cost significantly. The docking analysis demonstrates that 1,4-dihyroxyquinoxaline-2,3-dione shows anti inflammatory activity whereas 2,3-diphenylquinoxaline was found to exhibit anti- microbial activity. The minimum binding energy indicated that the target protein was successfully docked with 1,4- dihyroxyquinoxaline-2,3-dione structure.

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References

1. Dichiarante V., Ravelli D. and Albini A., 2010, *Green Chemistry Letters and Reviews*, **3(2)**, 105-113.
2. Wardencki W., Curylo J. and Namieoenik J., 2005, *Polish Journal of Environmental Studies,***14(4)**, 389-395.
3. Badami B.V., 2008, Resonance, 1041-1048.
4. Valavanidis A. and Vlachogianni T., 2012, Green Chemistry and Green Engineering, Synchronathemata Non-profit publishing house Athens Greece, ISBN 978-960-9695-00-8.
5. Madje B.R., Shelke K.F., Sapka S.B., Kakade G.K. and Shingare M.S., 2010, *Green Chemistry Letters and Reviews*, **3(4)**, 269-273.
6. Manahan S.E., 2005, *ChemChar Research*.
7. Kanagarajan V., Thanusu J. and Gopalakrishnan M., 2009, *Green Chemistry Letters and Reviews*, **2(3)**, 161-167.
8. Bamoharram F.F., Heravi M.M., Roushani M., Toosi M. R. and Jodeyre L., 2009, *Green Chemistry Letters and Reviews*, **2(1)**, 35-41.
9. Wang Suhui, Yin Shan, Xia Sheng , Shi Yanhui , TuShujiang and RongLiangce, 2012, *Green Chemistry Letters and Reviews*, **5(4),** 603-607.
10. Kotla V.V., Dalavai V.K. and Chunduria V.R., 2012, *Der PharmaChemica*, **4(5),** 2003-2008.
11. Ghadage R.V. and Shirote P.J., 2011, *Bangladesh J Pharmacol*; **6**, 92-99.
12. Radhakrishnan M., Dhar A. K., Tara S.T.V.N.V., Sappanimuthu T. and Thangaraj D., 2011, *Chinese Chemical Letters*, **22**, 389–392.

13. Ingle R.G. and Marathe R.P., 2012, *International Journal of Pharmaceutical Research & Allied Sciences*, **1(4)**, 46-51.
14. Das P.J. and Sarkar S., 2011, *International Journal of Chemical Research*, **3(2),** 56-60.
15. Geethavan M., Reddy J.R. and Sathyanarayana S.V., 2012, *International Journal of Pharmacy & Technology*, **4(3)**, 4700-4710.
16. Mohammad V.S. and Saeed B., 2013, *World Applied Sciences Journal*, **21(3)**, 394-401.
17. Jesús J.M.C., Ramírez-Hernández K., Gómez-Flores N.S., Rodas-Suárez O.R. and Peralta-Cruz J., 2012, *Molecules*, **17,** 5164-5176.
18. Mohammad R.I. and Hassani Z., 2008, *ARKIVOC* (xv) 280-287.
19. Ding Chang-jiang, Wang Yan, Zhang Wei-wei, Liu Li, Liang Yong-jiu and Dong De-wen, 2009, *Chem. Res. Chinese Universities*, **25(2)**, 174-177.
20. Syed Mohammad and Nighat Fatima., 2015, *Pharmacogn Mag*. **11(1)**, 123–126. DOI: 10.4103/0973-1296.157712
21. Mahadik P, Jagwani D. and Joshi R., 2014, *International Journal of Innovative Science, Engineering & Technology*, **1(6),** 482-490
22. Jagwani D. and Joshi P., 2014, *Int. J. Pharm. Sci. Res*., **5(12)**, 5548-55, DOI: 10.13040/IJPSR.0975-8232.5 (12).5548-55.
23. Cheeseman G.W.H. and Cookson R.F., 1979, Condensed Pyrazines. In The Chemistry of Heterocyclic Compounds; Weissberger, A.; Taylor, E. C. Series editors; John Wiley & Sons, Inc.: New York, 35, 78-111.
24. Kurasawa Y. and Takada A., 1985, *Heterocycles*, **23(8)**, 2083-2119.
25. Phillips M. A., 1928, *J. Chem. Soc*., 2393-2399.
26. Ohmori J., Sakamoto S., Kubota H., Shimizu-Sasamata M., Okada M., Kawasaki S., Hidaka K., Togami J., Furuya T. and Murase K., 1994, *J. Med. Chem*., **37,** 467-475.
27. Epperson J. R., Hewawasam P., Meanwell N.A., Boissard C.G. and Gribkoff V.K., 1993, *Bioorg. Med. Chem. Lett*., **3(12)**, 2801-2804 b) Obafemi C.A. and Pfleiderer W., 1994, *Helv. Chim. Acta*., **77**, 1549-1556.
28. Piguet C., Bocquet B. and Hopfgartner G., 1994, *Helv. Chim. Acta*., **77**, 931-942.
29. Jaberi Z.K. and Amiri M., 2012, *E.J. Chem*., **9(1)**, 167-170.
30. Thakuria Harjyoti and Das Gopal, 2006, *J. Chem. Sci*., **118(5)**, 425–428.
31. Wang G.W. and Liu L., 2004, *Chin. Chem. Lett*., **15**, 587-590.
32. Mashkouri S. and Reza Naimi-Jamal M., 2009, *Molecules*, **14(1)**, 474-479.
33. Toda F., 1995, *Acc. Chem. Res*., **28(12)**, 480-486.
34. Toda F. and Tanaka K., 2000, *Chem. Rev*., **100,** 1025.
35. Sachdeva H., Saroj R., Khaturia S. and Dwivedi D., 2012, *Green Processing and Synthesis*, **1(5)**, 469-477.
36. Rajasekhar K.K., Ananth V. Shankar, Nithiyananthan T.S. and Hareesh G., 2010, *Int. J. Chem. Tech. Res*., **2(1)**, (2010).
37. Vogel, 1989, Text book of Practical Organic Chemistry, p.1190.
38. Joshi Shrinivas D., Kulkarni Manoj and More Uttam., 2013, *Universal Journal of Pharmacy*, **2(3)**, 139-143.

39. Das P.J. and Sarkar S., 2011, *International Journal of Chemical Research*, **3(2)**, 56-60.

40. Bendale A.R., Kotak D., Damahe D.P., Sushil P. N., Jadhav A.G. and Vidyasaga G., 2011, *Pelagia Research Library*, *Der ChemicaSinica*, **2(2)**, 20-24.

41. Gogate P.R. and Pandit A.B., 2003, *Adv. Environ. Res.* **26**, 17.

42. Mason T.J., 2003, *Ultrason. Sonochem.*, **10**, 175-179.

43. Entezari M.H., Asghari A. and Hadizadeh F., 2008, *Ultrason. Sonochem*, **15**, 119**.**

44. Mason T. J., 1997, *Chem. Soc. Rev*. **26**, 443-451.

45. Lidstrom P., Tierney J., Wathey B. and Westman J., 2001, *Tetrahedron***,** **57**, 9225-9283.

46. Jogula A.K., Chellapalli S. and Pendem H.B., 2011, *Der Pharma Chemica*, **3(4),** 292- 297.

47. Yadav M.V., Kuberkar S.V., Khan F. G., Khapate S.R. and Sagar A.D., 2013, *Journal of Chemical and Pharmaceutical Research*, **5(1)**, 266-270.

48. Nayak S.K., Venugopala K.N., Chopra D., Govender T., Kruger H.G., Maguire G.E.M. and Guru Row T.N., 2009, *Acta Cryst.*, E**65**, 2502 [doi:10.1107/S1600536809037441].

49. Bose A.K., Pednekar S., Ganguly S.N., Chakraborty G. and Manhas M.S., 2004, *Tetrahedron Lett.*, **45**, 8351-8353**.**

50. Sun Q., Wang Y.Q., Ge Z.M., Cheng T.M. and Li R.T., 2004, *Synthesis*, 1047.

51. Das U., Chheda S. B., Pednekar S. R., Karambelkarc N. P. and Guru Row T. N., 2008, *Acta Cryst.*. **E64**, o2488-o2489.

52. Mohideen M. Nizam, Rasheeth A., Huq C.A.M.A. and Nizar S. Syed., 2008, *Acta Cryst.*, **E64**, 01752.

53. Bose A.K, Pednekar S., Ganguly S.N., Chakraborty G. and Manhas M.S., 2004, *Tetrahedron Lett.*, **45**, 8351-8353**.**

54. Folkers K. and Johnson T.B., 1933, *J. Am. Chem. Soc.*, **55,** 3784.

55. Kappe C.O., 1997, *J. Org. Chem.*, **62(21)**, 7201–7204.

56. Hayes, B.L., 2002, Microwave Synthesis: Chemistry at the Speed of Light, CEM, ISBN 0- 9722229-0-1, USA.

57. Biginelli P., 1893, *Gazz. Chim. Ital.* **23**, 360-416.

58. Falsone F.S. and Kappe C.O., 2001, *ARKIVOC*, 122-134.

59. Hugel H.M., 2009, *Molecules*,**14**, 4936–4972.

60. Shete D.K., Surve S.S., Patil S.B., Narade S.B., Patil K.S. and Pore Y.V., 2010, *Der Pharmacia Lettre*, **2(3)**, 59-65.

61. Ramatchandrian N., Sumathi S. and Buvaneswari G., 2009, *Indian J. Chem.*, **48B**, 865-867.

62. Reddy Ch. V., Mahesh M., Raju P.V.K., Babu T.R. and Reddy V.N., 2002, *Tetrahedron Lett.*, **43**, 2657.

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Reduction of Nitrobenzene in Aqueous Media by Fe–Al Bimetallic Material

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ABSTRACT
Nitrobenzene is a toxic agent which must be treated before discharging into the environment. One of the methods to treat nitrobenzene is to reduce it to amine compounds with zero-valent iron (ZVI). During the reduction, the iron surface can develop a passive film that decreases the reduction of nitrobenzene. To overcome this problem, many novel materials such as zero-valent bimetals like Pd-Fe, Cu-Fe, and Cu-Al have recently been shown to have enhanced potential to destroy passive layers as compared to ZVI. In this study, Fe-Al powder was used for nitrobenzene reduction. Optimal conditions for reduction of nitrobenzene to amine products, such as the ratio of Fe/Al, pH, and reaction time were studied.

Keywords: *Binary metal Fe-Al, Nitrobenzene, Reduction, Amine*

Introduction

Wastewater contamination by nitro aromatic organic compounds has gained great attention. Nitrobenzene (NB) has been considered a priority control contaminant by American Environment Protection Agency[1]. Wastewater containing NB must be treated before being discharged into the environment[2,3]. It is therefore necessary to find a novel and effective technology for the degradation of NB from wastewater. Nitrobenzene from wastewater has been reduced by zero-valent iron (ZVI) particles to triaminobenzene[4]. During oxidation, the iron surface is made passive by an iron oxide or hydroxide layer leading to a decrease in the rate of reaction with NB. To overcome this phenomenon, many novel materials such as zero-valent bimetals like Pd/Fe, Cu/Fe, and Cu/Al have recently been shown to have enhanced reactivity toward destroying passive layers compared to ZVI[6]. Bimetallic particles consist of two types of zero-valent Fe/Al metals.

Reduction by ZVI is an electrochemical corrosion process in which oxidation of Fe^0 to Fe^{2+} is an anodic half-reaction. The cathodic half-reaction involves electron acceptors like H^+, H_2O, O_2 or nitro group ($-NO_2$). The ZVI corrosion in the aqueous phase consists of the following reactions[7,8]:

$$Fe^0 + 2H^+ \leftrightarrow Fe^{2+} + H_2 \qquad (1)$$

$$Fe^0 + 2H_2O \leftrightarrow H_2 + 2\,OH^- \qquad (2)$$

The ZVI corrosion in aqueous phase as a system of Fe^0 - H2O - O_2consists of the following reaction:

$$2Fe + O_2 + 2H_2O \leftrightarrow 2Fe^{2+} + 4OH^- \qquad (3)$$

Bimetallic particles consisting of two types of zero-valent metals, aluminum – iron (ZVA and ZVI) are useful for the reduction of chloro and nitro aromatic compounds. Fe has electrode potential of Fe^{2+}/Fe^0, (–0.44 V) which is higher than that of Al^{3+}/Al^0, (–1.67 V). Fe and Al can form electrochemical cells in which iron plays the role of anode and aluminum that of cathode[6,9]. The metal iron can reduce organic compounds as follows:

$$Fe^0 + RX + H^+ \rightarrow Fe^{2+} + 2RH + X^- \qquad (4)$$

and $2Al^0 + 3\ Fe^{2+}$(on iron surface) $\rightarrow Al^{3+} + 3Fe^0$ (on iron surface) (5)

Thus, ZVI may be considered as a constant source of electrons. In this study, we apply the bimetallic zero-valent Fe/Al particles consisting of a core metal (aluminum) and a second metal (iron) for reduction of NB from water. The reaction between Al/Fe with NB (denoted as RNO_2) is as follows:

$$3Fe^0 + RNO_2 + 6H^+ \rightarrow 3Fe^{2+} + RNH_2 + 2H_2O \qquad (6)$$
$$3Fe^{2+} + Al^0 \rightarrow Fe^0 + Al^{3+} \qquad (7)$$

The bimetallic Fe/Al particles are used in which metal Al, as an electron source prevents the precipitation of the corroded layer of iron layer at the Fe^0 surface and thus enhances the surface reactivity of iron.

Materials and Methods

Chemicals

All chemicals used for this study were of analytical grade. Nitrobezene solution (ACS Sigma – Aldrich, reagent ≥ 99.0%, molecular weight, 123,11) was purchased in Vietnam. Commercially available aluminum particles (99.5% pure, 20 micrometers) were procured. Bimetallic Fe-Al particles used for this study were prepared according to the method published in literature[6,9], using the zero-valent aluminum powder (ZVA) and ferric chloride as precursors in a fume hood under ambient conditions, according to the reaction:

$$Fe^{3+} + Al^0 \rightarrow Fe^0 + Al^{3+}$$

Fe^0 was deposited on the Al^0 surface and then washed with deionized water, filtered and dried in vacuum. Fe and Al in the samples were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). The ratio of Fe/Al was calculated as follows:

Fe / Al = (Applied Fe content—Residual Fe content)/(Applied Al content—Residual Al content).

Throughout the experiments, the Fe^0/Al^0 particles used for the study involved 0.5, 1.2, 2.1, 2.5, and 2.8 g Fe / g of Al.

Analytical method

The pH of solution was monitored using a Toledo pH meter. The concentration of NB was determined by anodic stripping Voltammetry with a mercury hanging drop electrode (HMDE) using Metrohom 797 VA Computrace[4]. Nitrogen was passed for 60s through the solution consisting of NB in 0.1M acetic acid -sodium acetate buffer as electrolyte to remove dissolved oxygen. The voltagram was run in the potential range from - 40.90 to -0.10 V. The current peak height appeared at -0.442 V (Figure 1), being proportional to the NB concentration in the electrolyte.

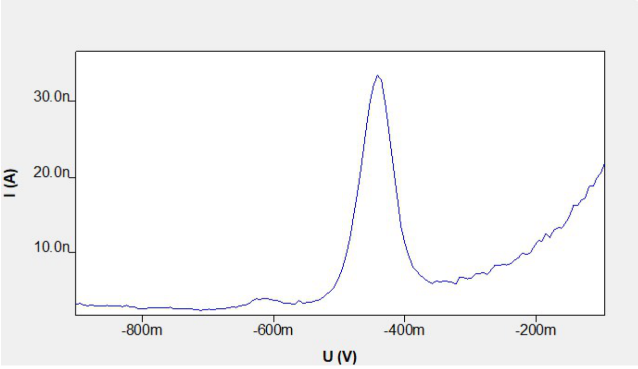

Fig. 1 Voltagram of NB on HMDE, NB conc. 10 mg/L, acetate buffer pH = 4.0, time for 60s.

The NB concentration of samples was determined using the following plot.

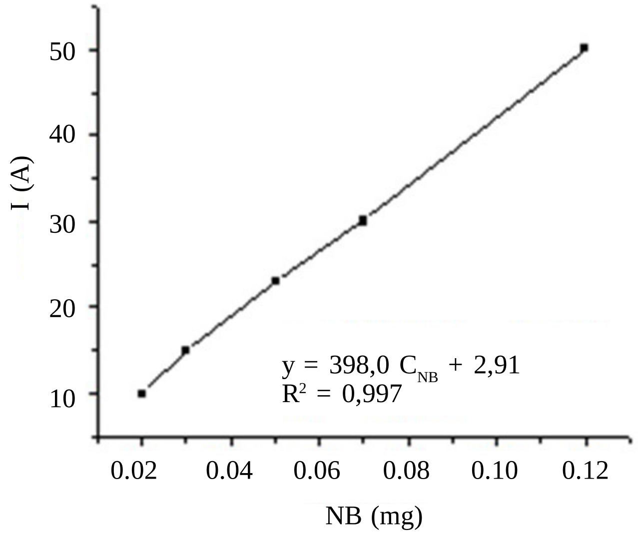

Fig.2 Calibration plot for Nitrobenzene

Reduction of NB by batch experiment

All experiments were conducted under N_2.The initial concentrations of NB and ZVI-Al particles were 80 mg/L and 1.0 g/L with different ratios of Fe^0/Al^0, at various initial pH values. The solutions were continuously stirred with a mechanical stirrer, in a batch of 200 mL. The pH of all solutions was adjusted with 0.1 M NaOH or H_2SO_4. Samples of solution collected from the reaction vessels at regular time intervals were filtered to determine the concentration of NB by Cyclic Voltammetry

Results and Discussion

The degradation of NB in ZVA - ZVI water system

Binary metallic ZVAl-ZVI consists of different ratios of Fe^0 and Al^0 that influence the kinetic curve of NB. The NB degradation rate increased with the increase of Fe^0 amount in ZVA-ZVI, and then reached a near-constant value. (Table 1, Figure 3).

Table 1. NB degradation dependence on the time and ratios of Fe^0 / 1g Al^0; M1, M2, M3, M4, M5 are samples involving Fe^0amounts of 0.5, 1.2, 2.1, 2.5, 2.8 g/1g Al^0: pH =3: NB with initial concentration of 80 mg/L in a 500 mL batch involving 200 mL sample.

Reaction time, min	0	5	10	15	20	25	30	35
$C_{NB/M1}$	80	74.5	70.1	66.6	62.5	61.4	58.3	55.2
$C_{NB/M2}$	80	65.3	40.7	23.7	14.1	7.8	4.2	1.8
$C_{NB/M3}$	80	50.3	30.2	15.1	6.5	4.5	2.1	0.3
$C_{NB/M4}$	80	50.0	29.5	15.7	6.6	4.5	2.2	0.3
$C_{NB/M5}$	80	50.1	29.7	15.8	6.7	4.5	2.2	0.3

The findings showed that the concentration of NB decreased when the reaction time increased from 0 to 35 min, but at a different rate, depending on the amount of Fe^0.

Table 1 indicates that when the Fe^0 in ZVAl powder increases from 0.5 to 2.1 g $Fe^0/1gAl^0$ corresponding to M_1, M_2, M_3 the NB concentration decreases from 80 to 6.5 mg/L and then reaches a near-constant value. This phenomenon can be explained by the Fe^0 saturated aluminum surface reducing the reaction between aluminum and Fe^{2+6}. Therefore the ratio ZVI/Al powder with value 2.1 was selected as the optimum value for the other experiments.

Influence of NB initial concentration on its degradation rate by ZVAl - ZVI powder

The experiments were carried out with NB concentrations 20, 40, and 80 mg/L, in a batch of 500 mL containing 200 mL of the sample with 2.1g Fe/1g Al. The average rate of NB degradation was measured for 5 minutes from the beginning. The results were presented in Table 2.

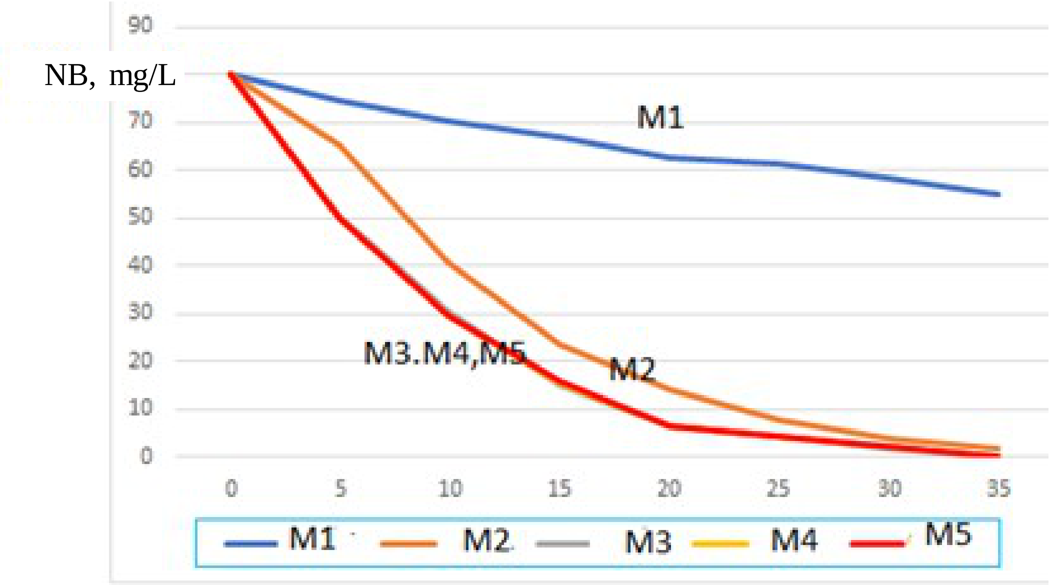

Fig. 3 Influence of Fe amount/1g Al on NB degradation, at pH =3, for the different samples M1, M2, M3, M4, M5.

Table 2. The average rate of NB degradation for 5 min from the beginning

$C_{\text{NB Initial, mg/L}}$	0	10	20	40	80
$R_{\text{Average rate,mg/L.min}}$	0	0.65	1.48	2.98	5.94

The results in Table 2 show that the initial average rate of NB degradation by ZVA-ZVI system, (at constant dosage of the powder), increases with the increased initial NB concentrations. The initial degradation rate of NB can be expressed by the following expression and Figure 4[10]:

$$dC^0_{NB} / dt = k_{obs}C^0_{NB}$$

where C^0_{NB} is considered as the initial concentration; k_{obs} is the rate constant in min^{-1}.

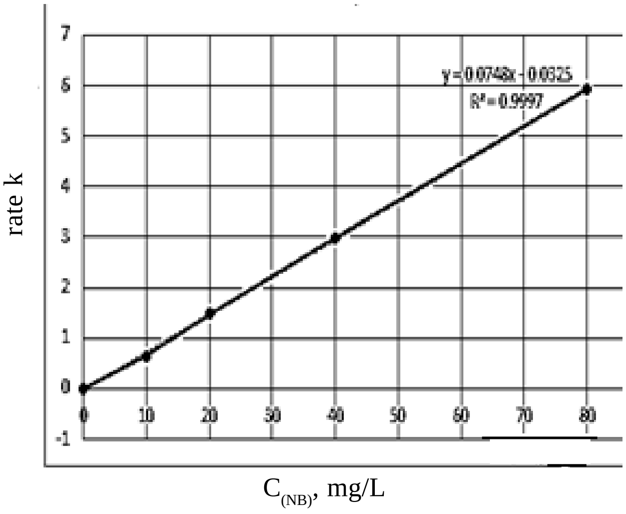

Fig. 4. The dependence of the reaction rate on the initial NB concentration

At the constant ZVA/ZVI dosage, the reaction rate of the NB degradation obeys first-order kinetics and is a case of Langmuir-Hinshelwood mechanism applied for surface adsorption reaction where the limiting step is adsorption/desorption [10]. k_{obs} observed is 0.0748 min^{-1}

Influence of pH on the NB reduction by ZVA/ZVI powders

The influence of pH on the reduction of NB by ZVI-ZVAl power is shown in Table 3.

Table 3. Influence of pH on NB reduction by ZVI-ZVA powder 80 mg/L NB, 2.1 g Fe/1g Al, reaction time 20 min, in a reaction batch involving 200 mL sample.

pH	2.0	3.0	4.0	5.0	6.0	7.0	8.0
NB, mg/L	5.2	6.5	7.4	10.5	25.7	39.2	56.8

The results indicated that the reduction reaction between NB and ZVI-ZVA was favoured in acid medium. It means that H^+ ions participate in the reduction reaction of NB.

Recycling and reuse of Fe/Al bimetallic particles

The effectiveness of the NB reduction reaction is strongly influenced by the activated iron surface and the formation of by-product on the iron surface. In this study, Fe(III) and Al(III) hydroxides may deposit on the surface of bimetal and form passive films, which could hinder the reduction reaction. These passive films can be removed in acid medium (pH = 2) after which the bimetal can be continuously effectively used for further experiments.

Conclusions

In this paper, the application of binary metal particles Fe – Al for the reduction of nitrobenzene from waste water was studied in detail. The reduction of nitrobenzene was evaluated by voltammetric method. The reduction of nitrobenzene depends on the presence of metallic iron deposited on the Al surface, pH, and reaction time. The optimal experimental conditions for the reduction of nitrobenzene were determined such as the ratio of Fe/Al (metal) i (2.1g Fe/1g Al), pH =3.0, and reaction time = 20 min.

References

1. Nitrobenzene Carcinogenicity, (CAS No. 98-95-3), EPA/600/R-95/100, April 1998.
2. Jingjuan Qiao, Weizhou Jiao and Youzhi Liu, 2021, *Green Energy & Environment*, **6(6)**, 910-919.
3. Jing Guo Le, Zhu Na Sun and Yeqing Lan, 2017, *Journal of the Taiwan Institute of Chemical Engineers*, **78,** 137-143.
4. Tran Duc Luong, Nguyen Hoai Nam, and Tran Van Chung, 2014, *Journal of Science of Hnue Chemical and Biological Sci.*, **59(9),** 59-65.
5. Jingjuan Qiao, Weizhou Jiao and Youzhi Liu, 2021, *Green Energy & Environment*, **6(6),** 910-919.
6. Li-Hua Chen, Chang-Chieh Huang and Hsing-Lung Lien, 2008, *Chemosphere*, **73**, 692–697.
7. Abinash Agrawal and Paul G. Tratnyek, 1996, *Environmental Science & Technology*, **30,** 153-160.
8. Michelle M. Scherer, Barbara A, Balko and Paul G. Tratnyek, 1999, American Chemical Society, 301-322.
9. Fenglian Fu, Zhang Cheng, Dionysios D. Dionysiou and Bing Tang, 2015, *Journal of Hazardous Materials*, **298**, 261–269.
10. Vasanth Kumara, K., Porkodib, K. and Rocha, F., 2008, *Catalysis Communications,* **9(1)**, 82-84.

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Synthesis of Chromone Mediated Aldimines and their Application for Cyanide and Cobalt Sensing

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ABSTRACT

Reactions of a series of o-substituted anilines and chromone-3-carboxaldehyde were reinvestigated which resulted in imines R1, R2 and R3. All the products were subjected to sensing of toxic analytes. Among all toxic anions, CN^- was selectively detected by R1 and R3 whereas R2 was not at all selective to any specific anions. R3 exclusively displayed sensing towards Co^{2+}cation. Cyanide sensing efficiency of R1-R3 receptors were characterized by various spectroscopic techniques namely IR, ^1H-NMR, ^{13}C-NMR, ESI, Mass and single crystal XRD. UV-Vis spectroscopic and fluorescence techniques were used for rest of the sensing studies.R1 showed sensitivity upto 0.321 ppb for cyanide and R3 showed selectivity towards cyanide and Co (II) upto 76 ppm for cyanide and 5.1 μM for cobalt respectively. The techniques were also applied for real time detection of CN^- and Co(II) from Ragi millet and commercially available Vitamin B_{12} tablets.

Keywords: *Chemosensor, Cyanide, Co(II), Supramolecule, Real time detection, Spectroscopic techniques*

Introduction

Schiff bases are known to be a prolific functionality in the field of synthesis. These bases find application as optical and fluorescence sensors for analytes, basic building blocks for total synthesis, as strong ligands in coordination chemistry and for binding with proteins and DNA. Schiff base reaction is named its discoverer, Hugo Schiff. In 1978, C.K. Ghosh and S. Khan[1] synthesized and characterized the Schiff base product of chromone-3-carboxaldehyde and o-phenylenediamine. They proposed a seven member diaza product which was stabilized by air oxidation. They proposed imine formation followed by cyclization resulting in the formation of a seven member ring which was red in colour .The reaction was carried out in reflux condition in ethanol. They reported two possible structures for o-phenylenediamine product with chromone-3-carboxaldehyde, among them one is 5-membered and another is 7-membered. For o-aminophenol and o-aminothiophenol, they proposed 5 membered and 7-membered ring structures. In 1982 Ivo Sigg et al.[2] re-optimized the reaction between chromone-3-carboxaldehyde and o-substituted aniline. They proposed a 14-member porphyrin like core with 4 nitrogen atoms in the middle for o-phenylenediamine which is of yellow colour, but in the case of o-aminophenol or o-aminothiophenol, only a seven member ring was proposed. Formation of a seven membered ring involving many hetero atoms is a bit distinct possibility, as it involves the aromaticity of chromone moiety. In this paper, we have reexamined the series of chromone-o-substituted aniline reactions to verify the products and elucidate the exact structures of the products. Chromone moiety is available in many natural products as well as in some medicines. Chromone is known to be a prominent fluorophore with strong binding sites and finds a prominent position in the sensor field.

In recent time, there are many reports on colorimetric and fluoremetric chemosensors for toxic and hazardous species based on the chromone molecule. Chromone based receptors for Al^{3+} have been reported based on the PET and FRET mechanisms[3-5]. Upon binding with Al^{3+}, PET is inhibited resulting in fluorescence. In recent time, chromone based organic nano particles are reported[6] based on AIE emission mechanism in water medium for sequential identification of Cu^{2+} (by quenching emission) and CN^- (by restoring emission).

Cyanide (CN^-) is one of the highly toxic anions that can affect many biological functions such as vascular[7], visual[8], central nervous[9], cardiac, endocrine[10] and metabolic systems[11]. About 0.5–3.5 mg/kg of cyanide is lethal and can lead to death[12]. Cyanide is of particular interest in various industrial applications like metallurgy, mining, electroplating and polymer synthesis, and also is involved in separating metal ions like, gold, silver and copper from platinum[13]. In recent times, chemosensor systems for CN^- detection based on the mechanism of coordination[14], hydrogen bonding interaction[15], supramolecular self-assembly[16], nucleophilic addition reaction and chemodosimetric detection[17] have been reported. K.S. Lee et al[8]. have reported a coumarin-based fluorescent chemodosimeter with a salicylaldehyde functionality as a binding site for selective detection of cyanide ions in water at biological pH[18]. A copper complex of receptor acts as a selective and specific colorimetric and fluorometric sensor for cyanide by displacing copper from the complex[19-21]. A dicyano-vinyl containing receptor has been reported as a colorimetric and fluorometric selective cyanide sensor[22-25]. Many receptors have been reported which are efficient in detecting cyanide based on chemodosimetric approach and ICT approach at ppm level[26-29].

As the imines mediated from chromone-3-carboxaldehyde and o-substituted anilines have some typical colorimetric and fluorogenic properties, we applied the examined compounds for sensing studies of various anions and cations which are a part of our daily needs.

Materials and Methods

Materials

Tetrabutylammonium salts of cyanide ($CN^–$), fluoride ($F^–$), chloride ($Cl^–$), bromide ($Br^–$), iodide ($I^–$), acetate ($AcO^–$), dihydrogenphosphate ($H_2PO_4^–$), hydrogensulphate ($HSO_4^–$), nitrate ($NO_3^–$) and hydroxide ($OH^–$) ions and chloride salts of metals (Zn^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, Mg^{2+}, Co^{2+}, Cr^{3+}, Fe^{3+}) were used as received for preparing stock solutions. Solvents were used in the pure and dry form. NMR grade DMSO-d_6 (SIGMA-ALDRICH) was used as received for titration.

Instruments used: SHIMADZU UV-2600 UV-Vis spectrometer and SHIMADZU RF-5301pc fluorescence spectrometer were used for photo-physical studies of all receptors. BRUKER-Ascend 500 NMR was used for receptor characterization and NMR-titration studies.

Synthesis

Chromone-3-carboxaldehyde (174mg, 1mM) and o-phenylenediamine (216mg, 2mM) (R1) were taken in 10 mL methanol in a round bottom flask. After 12 hrs of constant stirring at room temperature, a scarlet yellow precipitate appeared. The precipitate was collected by filtration and washed with methanol and dried in an oven. After drying, the solid product was dissolved in chloroform giving a red coloured solution. The solid product dissolved partially. The solution was then filtered. The filtrate part gave the imine product (R1A) which was red in colour, whereas 14-member ring product (R1) came out as yellow colour product.

For o-aminophenol (109mg, 1mM) (R2) and o-aminothiophenol (125mg, 1mM) (R3) with chromone-3-carboxaldehyde, the same synthetic procedure was followed, but neither of them gave the 14-member ring product.

Yield of R1 was found to be ≈ 81%. For R2 and R3 the yields were 75% and 78% respectively.

R1:- IR-1570cm^{-1}(-C=N-), 1637cm^{-1} (C=O), 3075cm^{-1}(-NH)). ^{1}H NMR-(ä) 14.3(t), 10.2(s), 8.6(s), 7.39(t), 7.33(d), 7.2(d) and 6.95(t).^{13}C NMR -(ppm) 192, 155, 153, 136, 132, 130, 127, 126, 119, 117, 116, 110 and 31. m/z- calculated-528.18, experimental-529.9 (M+1). (Fig. S1)

R1A:- IR-1548cm^{-1}(-C=N-), 1625cm^{-1} (C=O), 2918 and 2851(d)cm^{-1}(-NH$_2$)). ^{1}H NMR- (δ) 14.6(s), 11.4(s), 8.6(s), 7.6(s), 7.5(s), 7.2(d) and 7.1(t). ^{13}C NMR -(ppm) 207, 196, 161, 152, 136, 134, 131, 126, 119, 118, 115, 109 and 31. m/z- calculated-264.18, experimental-265.3 (M+1). (Fig. S2)

R2:- IR-1606cm^{-1}(-C=N-), 1643cm^{-1} (C=O), 3238cm^{-1}(-OH)). ^{1}H NMR- (δ) 12.01(d), 10.4(s), 8.2(d), 7.8(d), 7.5(t), 7.01(t), 6.98(2s) and 5.8(s). ^{13}C NMR -(ppm) 179, 155, 146, 144, 134, 128, 126, 124, 118, 116, 114, 101 and 55. m/z- calculated-265.06, experimental-266.9 (M+1).(Fig. S3)

R3:- IR-1608cm^{-1} (-C=N-), 1647cm^{-1} (C=O), 3445cm^{-1} (-SH)). ^{1}H NMR- (δ) 12(s), 8.3(s), 8.2(s), 7.9(s), 7.7(t), 7.3(s), 7.2(d) and 6.97(s).^{13}C NMR -(ppm) 180, 158, 144, 137, 135, 132, 127, 119, 118, 115, 101 and 55. m/z- calculated-281.05, experimental-280.04 (M-1). (Fig. S4)

Stock Solution Preparation

25 mL of 1.5×10^{-3} M solutions of the receptors R1, R2 and R3 were prepared in acetonitrile (ACN). All anion solutions ($1.5 \text{ x } 10^{-3}$M) were prepared in acetonitile from the corresponding Tetrabutyl ammonium salts. All cation solutions (1.5×10^{-3}M) were prepared from the respective metal chloride salts.

UV-Visible Spectroscopic Studies

100µL of receptor stock solution was taken in a 1mm cuvette and diluted to 3mL with solvent to get 5µM solutions. After dilution, the solution was subjected to irradiation with UV-light and the receptor UV-Vis spectrum was obtained. Analytes (e.g. CN$^-$, F$^-$, OH$^-$ etc.) were then introduced into the receptor and UV-Vis spectra were recorded. The same technique was followed for all other receptors studies.

NMR Studies

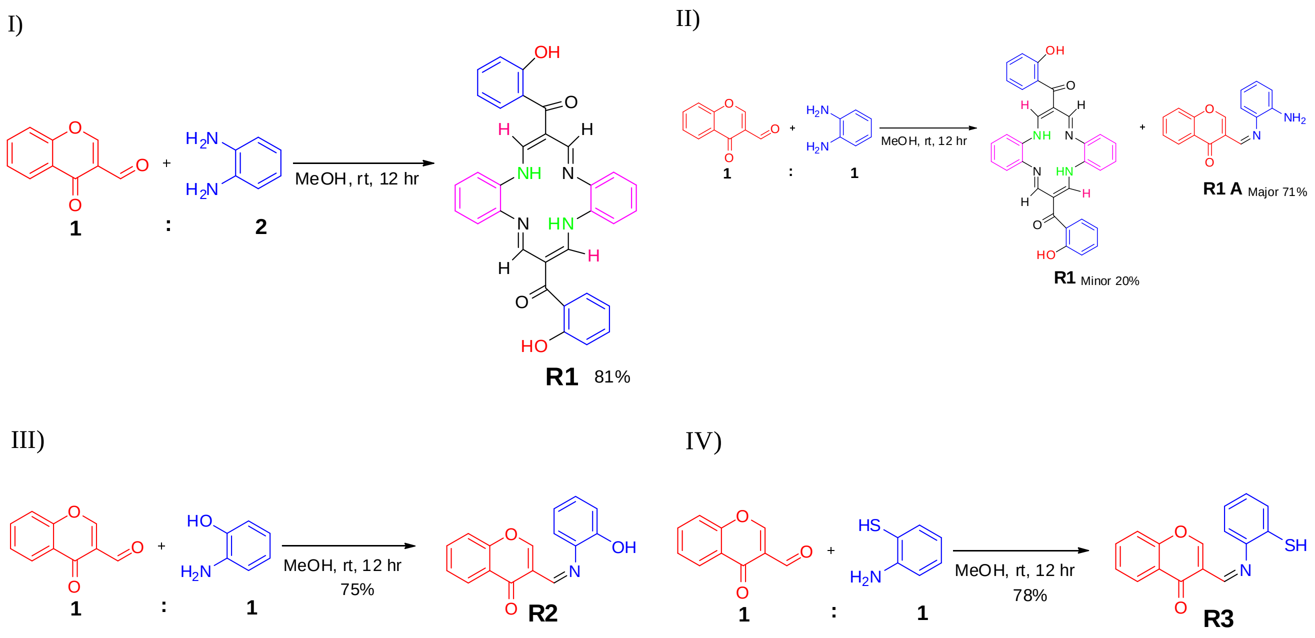
1.5mM solutions of receptors were prepared in DMSO-d$_6$. Solutions of Tetrabutylammonium cyanide and copper nitrate (1.5mM) were prepared in DMSO-d$_6$. Two equivalents of analytes were introduced in the receptor and the ^{1}H-NMR spectra of the resulted mixtures were recorded.

DFT Studies

All theoretical energy calculations were done using the DFT/TDDFT method with the hybrid exchange correlation functional B3LYP-6-31g by Gaussian 9.0 program.

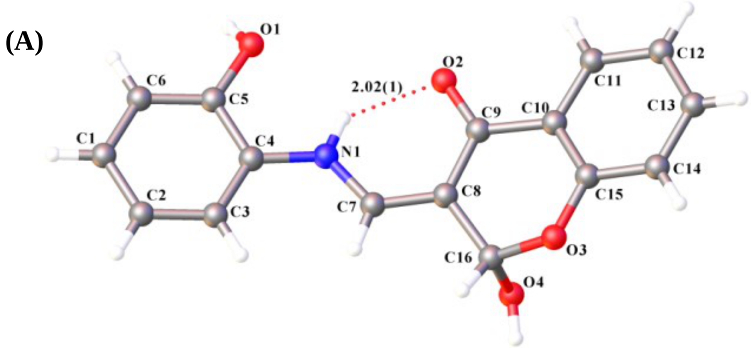
Result and Discussion

Structures of R1, R1A, R2 and R3 were well characterized by various spectroscopic techniques. Among the synthesized receptors, the ^{1}H NMR of R1 matches with the reported^{1}H NMR spectrum[2] but spectral data of R1(A), R2 and R3, is contatary to the structures proposed by C.K. Ghosh and S. Khan[1] and Ivo Sigg[2]. They have predicted the structures of R1A, R2 and R3 as seven membered, which is expected to follow an



Scheme 1- One step synthesis of receptors R1(I), R1A (II),R2(III) and R3(IV) by simple imine formation in methanol at room temperature (12 hrs).

intramolecular nucleophilic rearrangement to form a seven member cyclic ring. This was ascribed to the electrophilic nature of the C_{16} position and attack of OH and SH groups at C_{16} carbon. But we found it to be a simple imine for which ^{1}H-NMR spectra provide sufficient evidence. The single crystal structure studies further confirmed the structure of R2 (Fig. 1 (A, B, C)) [CCDC 1844119]. Ivo Sigg had proposed the nucleophilic attack at C_{16} carbon due to its electrophilicity. However crystal structure studies indicate the presence of methoxy group at C_{16}. From crystal structure studies it is evident that the molecule is a simple imine but C_{16} carbon in chromone framework is found to be chiral. It is due to the nucleophilic attack of methanol during the crystallization process. But upon moderate heating or melting the crystal, the methoxy group leaves the molecular framework hence the melting points of both crystalline and amorphous compound are same. And furthermore, formation of seven member ring will not take place by breaking a six membered aromatic ring, which is much more stable in comparison to a non-aromatic seven member ring.



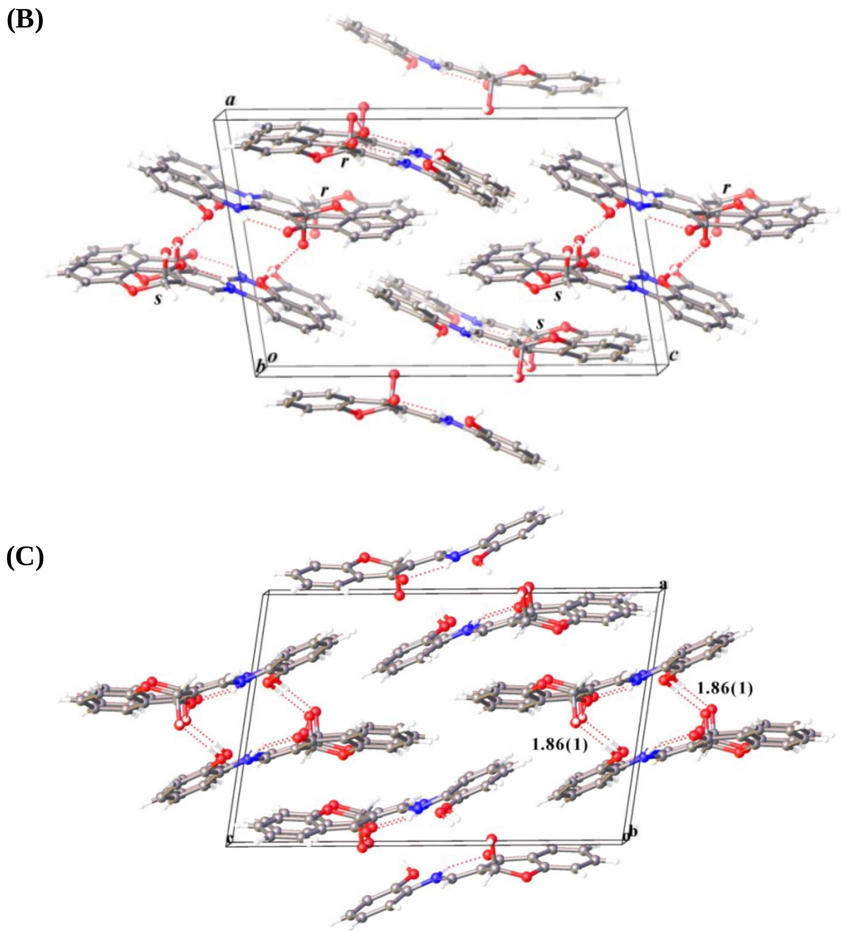


Fig. 1. (A) Solved crystal structure of R2, (B) Chirality of C_{16} position inside the unit cell and (C) Driving force and distance between two molecules inside the unit cell.

Disturbance in electronic framework is the key strategy in sensing mechanism. If any analyte is strong enough and very selective to disturb the electronic framework of the receptor, it results in colour change of the resultant solution (either blue shift or red shift) and which is observed in UV-Vis and luminescence spectroscopy. Perturbation in electronic flow changes the absorbance and emission properties of the receptor molecule. Hence, naked eye detection is easy, efficient and effective as compared to other techniques. The synthesized receptors R1, R2, and R3 were subjected to sensing for anions.

R1 shows exclusive selectivity and sensitivity for cyanide (CN^-) ion among all other anions in acetonitrile (ACN) medium. Upon introduction of cyanide in R1, immediately the colour of the receptor solution becomes yellow, which can be easily detected by the naked eye as depicted in **Fig 2. (A)**. The sensing of cyanide by R1 is evident in UV-Visible spectrum as decrease in absorbance intensity ($\lambda_{max(R1)}$= 354 nm) which is attributed to π-π^* transition of the R1 and near 450nm a new hump appears which is attributed to the n-π^* transition in the receptor upon addition of cyanide as it provides non-bonding orbitals. Cyanide is known to possess basic property and can abstract acidic protons from the receptor. R1 has -OH functional group which is a source of acidic protons and can be deprotonated easily by cyanide. The presence of non-bonding electrons on oxygen atom is responsible for the n-π* transition after addition of cyanide. Other anions like F$^-$, Cl$^-$, Br$^-$, I$^-$, AcO$^-$, HSO_4, $H_2PO_4^-$, NO_3^-, and OH$^-$ show hardly any influence on the photophysical property of the receptor as absorbance spectra appeared exactly similar to that of the receptor. The effect of cyanide on R1 is also confirmed by emission spectroscopy. Receptor R1 hardly shows any lumine-scence property upon excitation at 354nm. But after addition of cyanide, emission spectrum shows a maxima at 470nm with about a 100 fold increase in intensity, whereas other competitive anions do not show any influence on emission spectra. Apart from anion sen-sing, R1 can be used for sensing of cations.

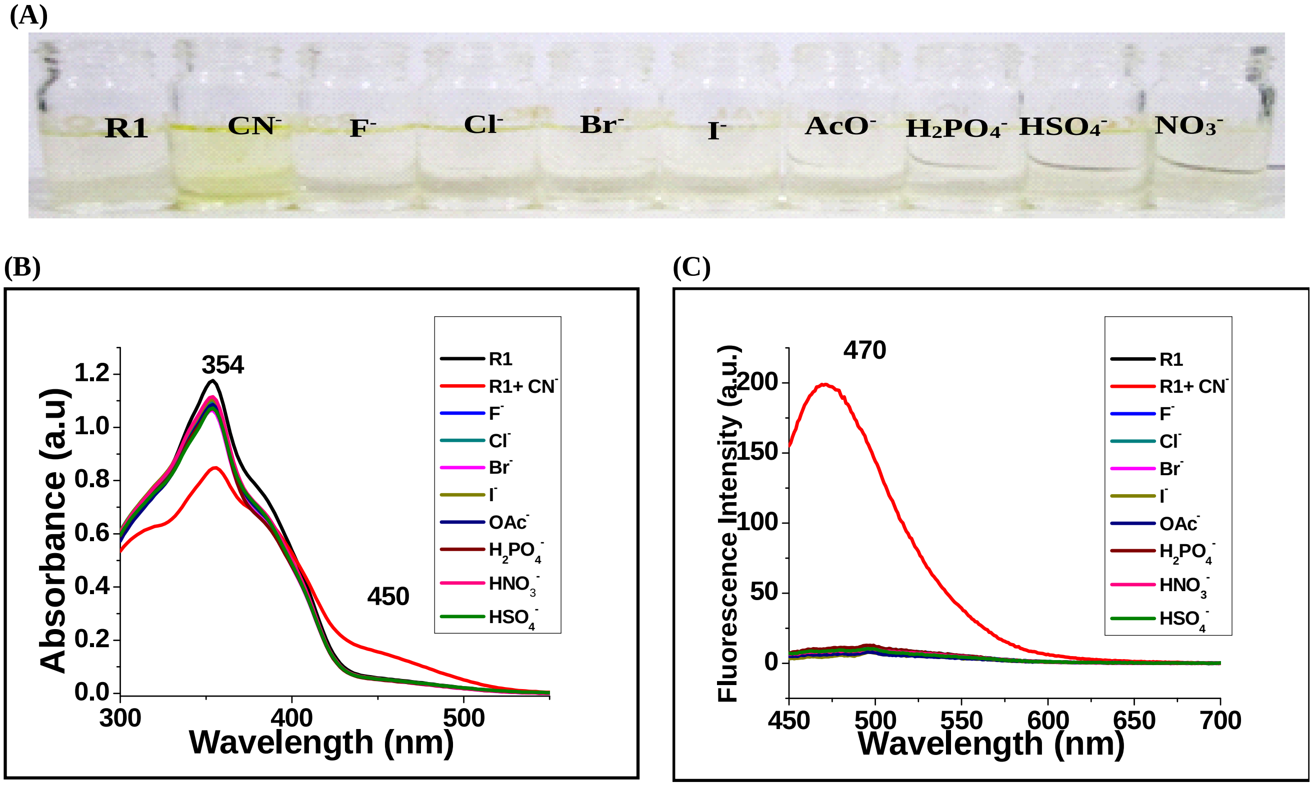


Fig. 2. A) Naked eye sensing of cyanide by R1. B) UV-Vis spectra of 5×10^{-5}M R1 against all anions of 5×10^{-5}M. C) Emission spectra of R1 against all anions.

Likewise R2 was also used for sensing studies but surprisingly, R2 does not show any specificity towards anions or cations. In case of anions, cyanide and hydroxide show colour change to yellow whereas hydrogen sulfate shows full discoloration of the R2 solution. Fluoride, chloride, bromide, iodide, acetate and dihydrogenphosphate show same intensity in colour of R2 solution as shown in **Fig. 3(A)**. It is observed in naked eye sensing that on addition of Co(II) and Cu(II), the colour of R2 solution immediately changes to intense yellow as shown in **Fig. 3(B)**. Upon addition of Pb(II), colour appears to be slightly bluish and on addition of Hg(II) the solution becomes colourless. Addition of Ni(II) results in a colour change to pale yellow. All the colour change are evident in the UV-Vis spectrum as all the cations show red shift in λ_{max}(R2) from 394nm to the range of 410nm to 440nm. Discoloration of R2 solution by hydrogen sulfate is also evident in UV-Vis spectrum as intensity at 354 nm decreases by about 5 times and also in emission spectrum, the intensity at emission maximum decreases about 5 times as compared to R2 emission intensity. All the anions show colour change due to their basic pro-perty. As R2 is formed from o-aminophenol and chromone-3-carboxaldehyde, the phenolic proton acts as an acidic proton with which all anions can show interaction. Cations also show the same phenomena.

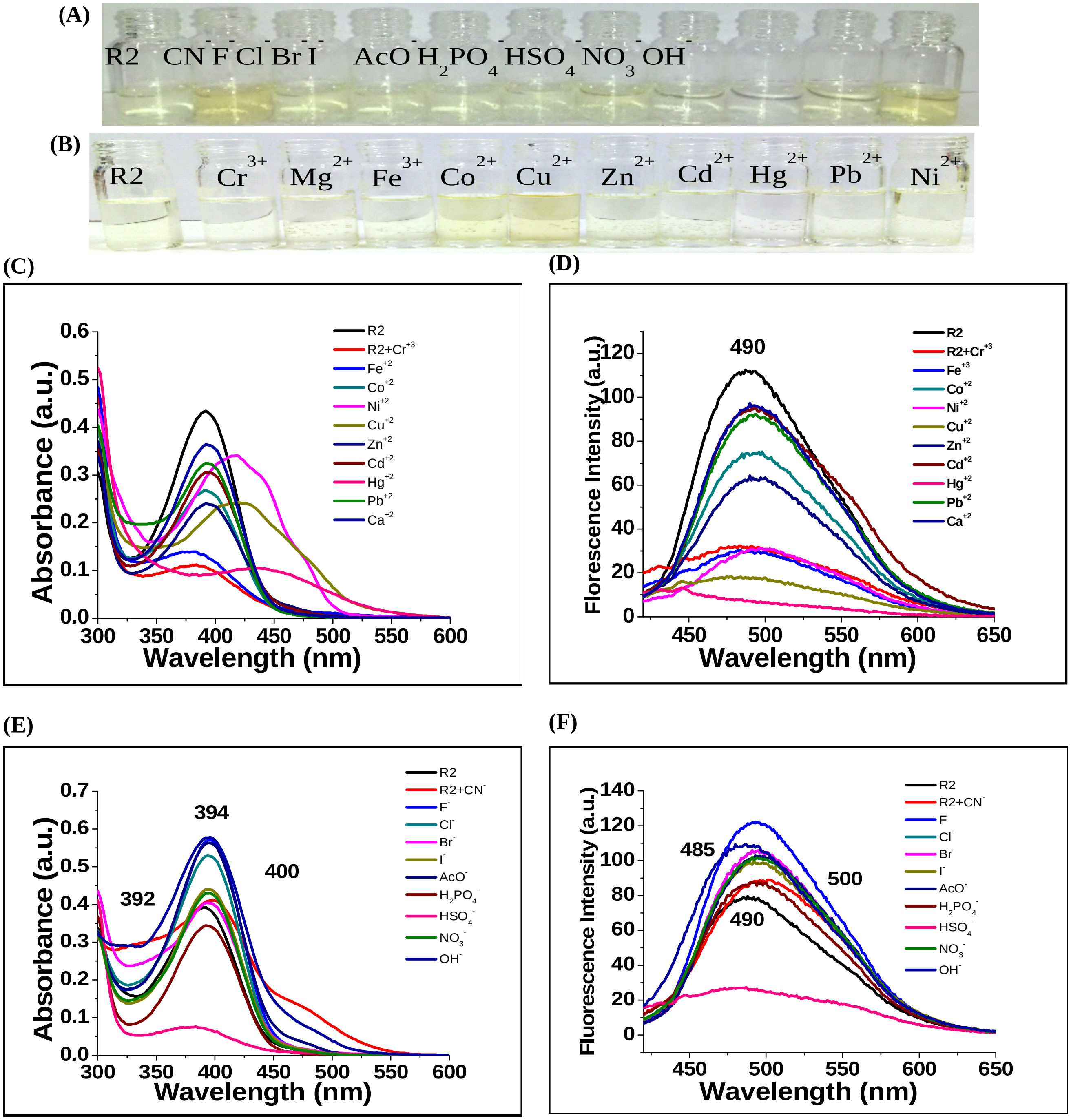
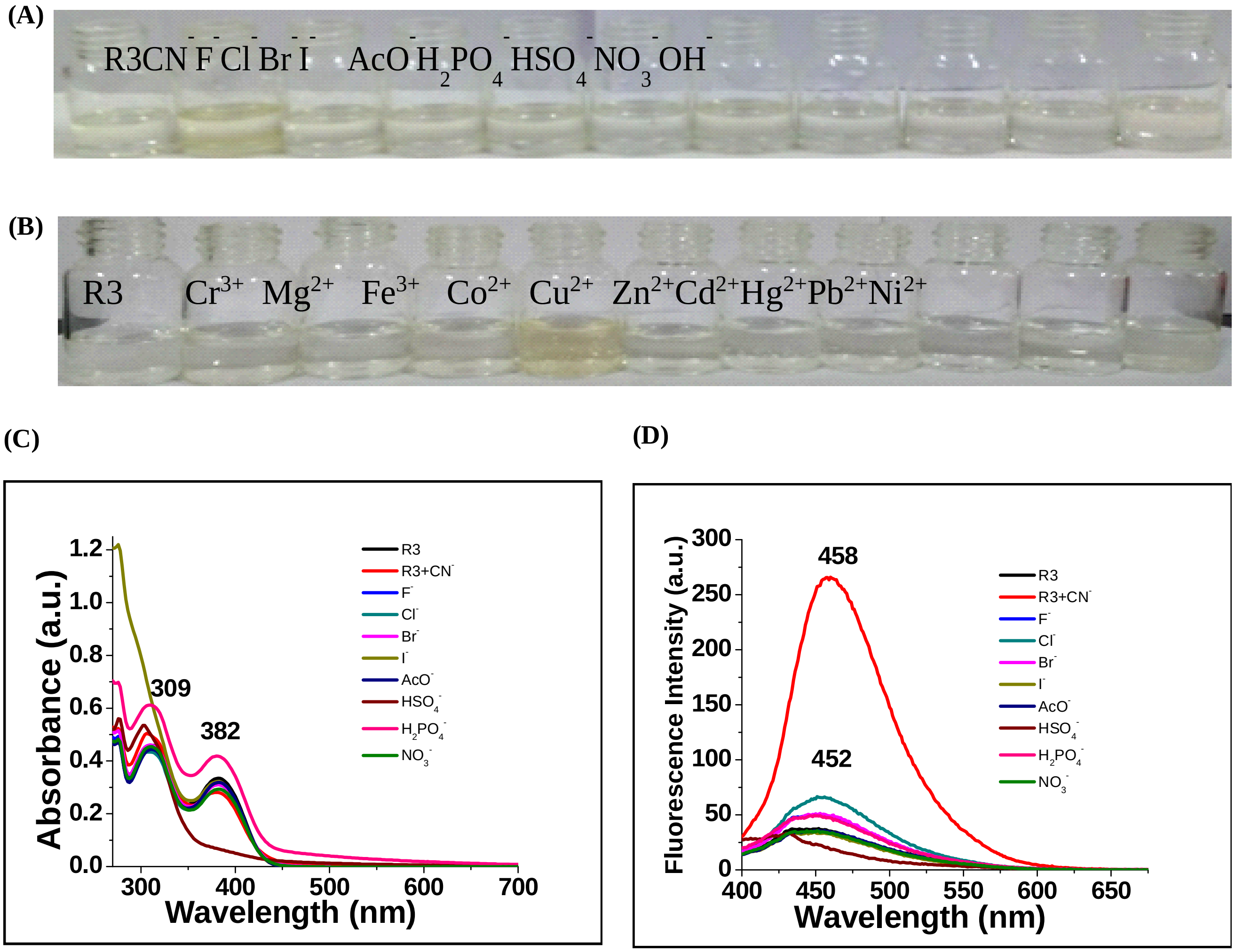


Fig. 3. A) Naked eye sensing of anions by R2. B) Naked eye sensing of cations by R2. C) UV-Vis spectra of 5×10^{-5}M R2 against all cations (5×10^{-5}M). D) Fluorescence spectra of 5×10^{-5}M R2 against all cations (5×10^{-}(M). E) UV-Vis spectra of 5×10^{-5}M R2 against all anions of (5×10^{-5}M). F) Fluorescence spectra of 5×10^{-5}M R2 against all anions of (5×10^{-5}M).

But in the case of R3, we again observed very specific interaction with cyanide which leads to the colour change from slightly yellowish to intense yellow (**Fig. 4A**). Upon exposure to all anions, R3 solution changes colour only in the case of cyanide which implies that only cyanide is capable of perturbing the electronic framework of R3. R3 shows λ_{max} at 382nm and a double hump at 310 nm. Absorbance at 382nm is attributed to n-π* transition and that at 310nm is due to for π-π* transition. UV-Vis spectra of R3 anion sensing revealed that cyanide addition make a significant difference in the electronic environment of R3 framework. After addition of cyanide, absorption maxima do not show any shift but intensity get altered. R3 spectrum shows two humps at 310nm and at 382nm. After introduction of cyanide the intensity at 382nm gets increased whereas that at 310nm gets decreased.



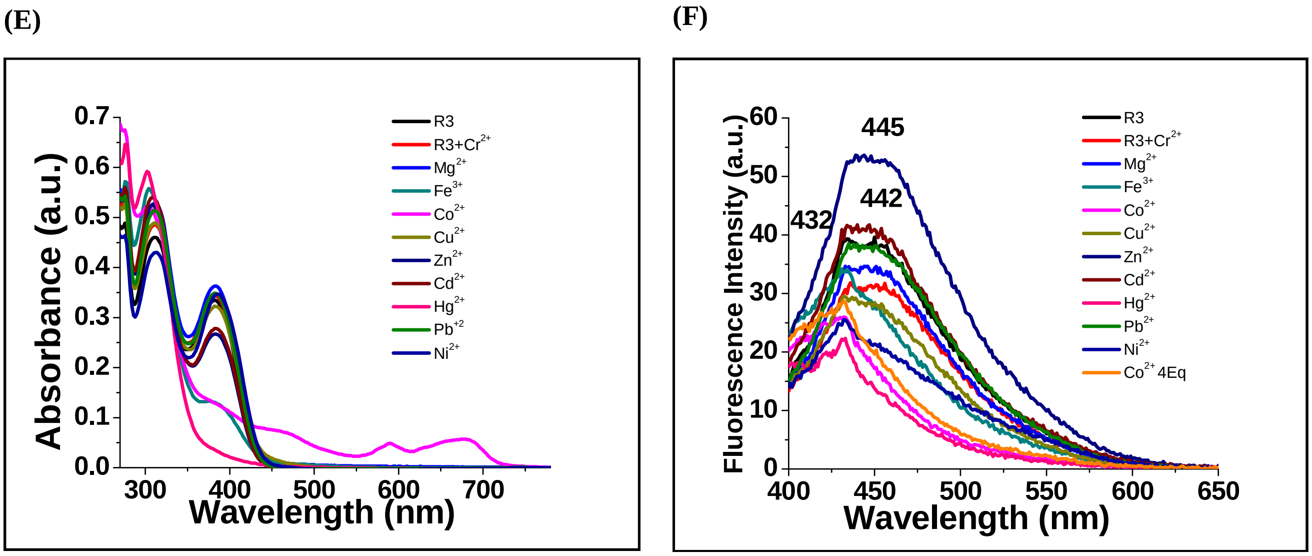


Fig. 4. A) Naked eye sensing of anions by R3. B) Naked eye sensing of cations by R3. C) UV-Vis spectra of R3 (5×10^{-5}M) against all anions (5×10^{-5}M). D) Fluorescence spectra of R3 (5×10^{-5}M) against all anions (5×10^{-5}M). E) UV-Vis spectra of R3 (5×10^{-5}M) against all cations of (5×10^{-5}M). F) Fluorescence spectra of R3(5×10^{-5}M) against all cations of (5×10^{-5}M).

Cross check of R3 sensing

Photophysical studies of **R3** show anion selectivity towards cyanide and cation selectivity for Co(II). Among CN^- and Co(II), permanent sensing has been observed for Co(II) even in the presence of cyanide (**Fig 5. (A)**). The addition of Co(II) followed by addition of cyanide does not have any impact on R3-Cobalt complex proving that Co(II) binds strongly with R3 and cannot be replaced by cyanide (**Fig 5. (B)**); but if the addition sequence is reversed, even in the presence of cyanide, Co(II) can bind with R3.

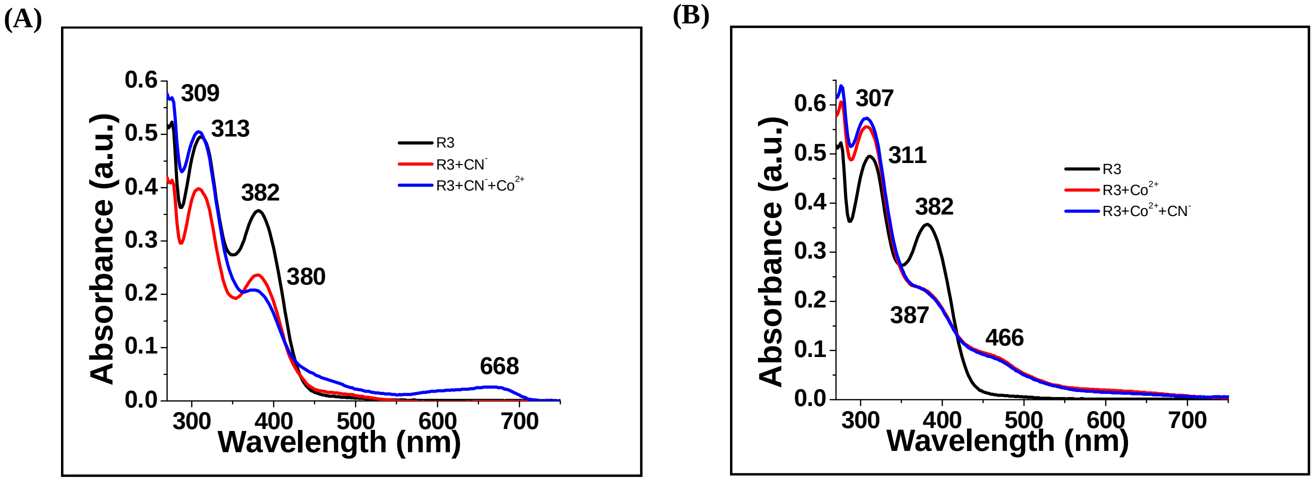


Figure 5. A) UV-Visible spectra of sequential addition of 5×10^{-5}M cyanide to 5×10^{-5}M R3 and followed by 5×10^{-5}M Co(II). B) UV-Visible spectra of sequential addition of 5×10^{-5}M Co(II) to 5×10^{-5}M R3 and followed by 5×10^{-5}M cyanide.

Theoretical studies

DFT studies of R1 and R1-CN were performed and the optimized electronic distribution of the possible orbital orientation was generated (**Fig. 6**). The energy of the generated HOMO and LUMO orbitals for R1 and R1-CN$^-$ were obtained from the program. The HOMO-LUMO energy difference for R1 was found to be 1.79 eV where the HOMO-LUMO energy difference for R1-CN$^-$ was found to be 0.814eV. Lower the energy difference between HOMO-LUMO, absorbance will appear on the red side of the absorption spectrum. So, from the calculations, HOMO-LUMO energy difference for R1 was higher than that of its deprotonated structure and can be correlated with the appearance of λ_{max}.

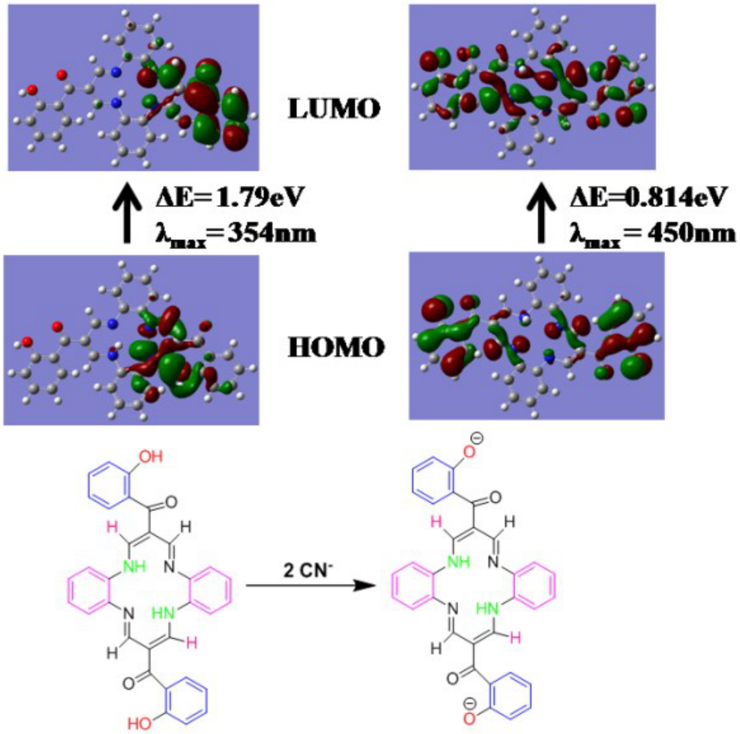


Fig. 6. Optimized electronic distribution of HOMO and LUMO orbitals of R1 and its deprotonated form.

Sensing Mechanism

Introduction of cyanide and Co(II) in R1 and R3 respectively showed colour change and the corresponding UV-Vis spectrum also supported the interaction of respective anion and cation. By ^{1}H-NMR titration, it is observed that cyanide shows colour change with R1 due to the deprotonation of the two acidic protons namely –OH protons, with a 2:1 ratio of saturation equivalence for cyanide with R1 (**Fig. 7**).

For R3 and Co(II), NMR titration shows that there is a covalent binding between metal and S, N and O present in the molecule. As saturation ratio for R3 and Co(II) is 2:1, it is expected that two molecules of receptor will covalently bind with one Co(II) atom leading to hexavalent complex. After saturation, we further examined the effect of cyanide on the Co(II) complex which not disturb the cobalt sensing.

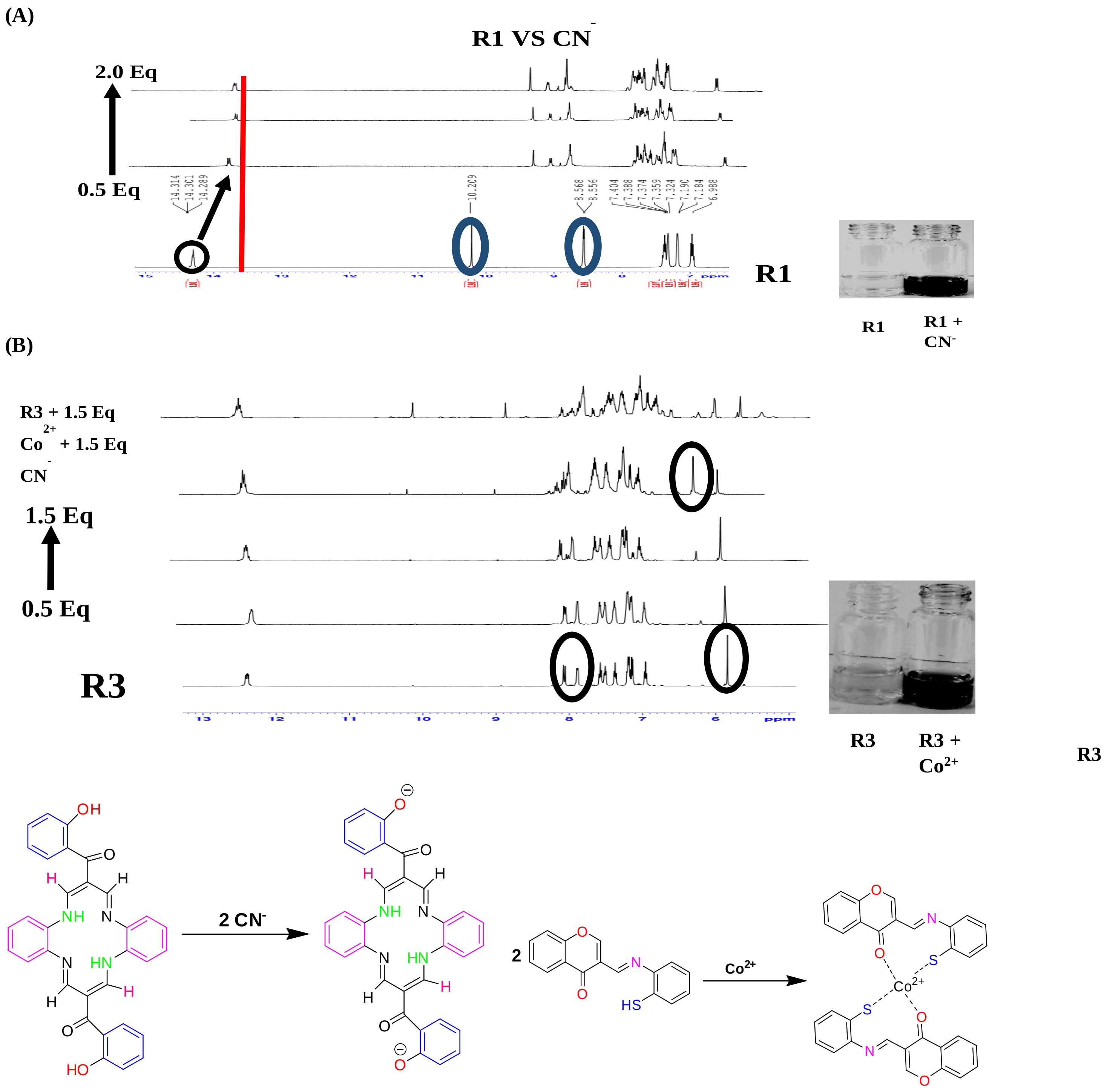
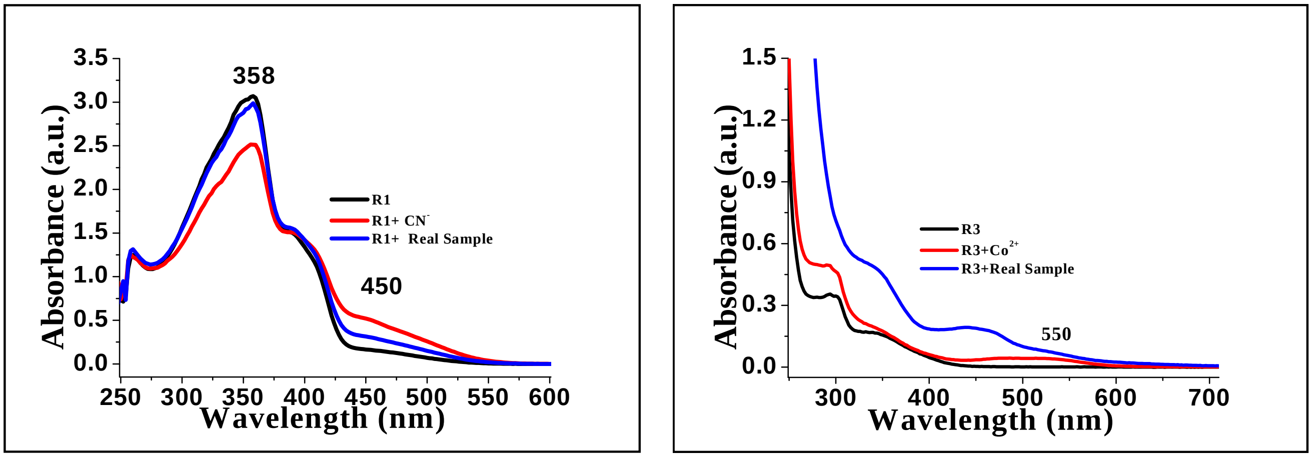


Fig. 7. ^{1}H-NMR titration of (A) R1 with cyanide and (B) R3 with Co (II). And probable sensing mechanism of cyanide by R1and of Co (II) by R3.

Real sample analysis

Since R1 and R3 are able to sense (CN^-) and Co (II) respectively, we have performed real sample analysis for R1 with Ragi as cyanide source and for R3 with vitamin B_{12} complex tablets available in local medical stores. Till date, this is the first report on Ragi used as real sample analysis. About 5-10 g of ragi was taken in 10-15mL of water and the resulting mixture was sonicated for around 30 minutes and allowed to stand for 1-2 hrs. The opaque solution was taken in vials and ultra centrifuged. From the resulting transparent liquid layer formed, about 200µL was taken out and added to the previously prepared R1 solution in DMSO. The colour change observed is similar to that observed in the case of cyanide addition. UV-Vis spectrum also reveals the same colour change **Fig. 8 (A)**.

Likewise vitamin B_{12}-complex tablets were brought from local medical shops. One tablet was dissolved in 5mL of double distilled water and sonicated for a period of 30 minutes. The resulting opaque solution was allowed to settle down for a day and then the solution was taken in vials for ultra centrifugation. The transparent solution was added in R3 solution and UV-Vis spectrum was recorded. The expected colour change was observed along with change in absorption spectrum **Fig.8 (B)**.



R3 + Co^{2+}

Figure 8.Naked eye colour and corresponding electronic spectral change upon addition of real sample and cyanide R1(A) and real sample and cobalt in R3 (B).

Conclusions

Reexamination of product formed by reaction of chromone-3-carboxaldehyde and o-substituted aniline (**R1**, **R2** and **R3**) was done successfully and structure confirmation of imines was done. The receptors were successfully used as colorimetric and fluorometric sensors for toxic anions and cations. **R1** shows selectivity towards cyanide with detection limit of 0.32 ppb and **R3** shows selectivity for Co(II) with detection limit of 5.1 µM. Though **R3** shows colour change for cyanide with detection limit of 76 ppm, in the presence of Co(II) its sensing ability for cyanide ceases. Cobalt shows 1:2 binding ratio with **R3** and cyanide shows 2:1 binding ratio with **R1**.

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References

1. Ghosh, C.K. and Khan, S., 1980, *Synthesis*, 701-702.
2. Sigg, I., Haas, G. and Winkler, T., 1982, *Helvetica Chimica Acta*, **65.1**, 275-279.
3. Liu, C., Yang, Z. and Yan, M., 2012, *J. Coord. Chem.*, **65(21)**, 3845–3850.
4. Fan, L., Li, T., Wang, B., Yang, Z. and Liu, C., 2014, *Spectrochim. Acta A.*, **118**, 760–764.
5. Fan, L., Qin, J., Li, T., Wang, B. and Yang, Z., 2014, *Sens. Actuators B*, **203**, 550–556.
6. Bhardwaj, S., Maurya, N. and Singh, A.K., 2018, *Sens. Actuators B.*, **260**, 753–762.
7. Kumar, V., Rana, H. and Kaushik, M.P., 2011, *Analyst*, **136(9)**, 1873-1880.
8. Wu, X., Xu, B., Tong, H. and Wang, L., 2011, *Macromolecules*, **44(11),** 4241-4248.
9. C.O. Ng, S.W. Lai, H. Feng, S.M. Yiu and C.C. Ko, 2011, *Dalton Trans.*, **40(39)**, 10020-10028.
10. Bhalla, V., Singh, H. and Kumar, M. 2012, *Dalton Trans.*, **41(37)**, 11413-11418.
11. Männel-Croisé, C. and Zelder, F., 2012, *Anal. Methods*, **4(9),** 2632-2634.
12. Isaad, J., Malek, F. and El Achari, A., 20132, *RSC Adv.*, **3(44)**, 22168-22175.
13. Holaday, M.D., Tarafdar, G., Adinarayana, B., Reddy, M.L.P. and Srinivasan, A., 2014, *Chem. Comm.*, **50(74)**, 10834-10836.
14. Aebersold, C., Amstutz, B., Steuer, A.E., Kraemer, T. and Zelder, F., 2015, *Anal. Methods,* **7(22)**, 9707-9712.
15. Yue, Y., Huo, F., Yin, C., Chao, J. and Zhang, Y., 2015, *Sens. Actuators B.*, **212**, 451-456.
16. Mardanya, S., Karmakar, S., Mondal, D. and Baitalik, S., 2015, *Dalton Trans.*, **44(36)**, 15994-16012.
17. Mardanya, S., Karmakar, S., Bar, M. and Baitalik, S., 2015, *Dalton Trans.*, **44(48)**, 21053-21072.
18. Lee, K.S., Kim, H.J., Kim, G.H., Shin, I. and Hong, J.I., 2008, *Org. Lett.*, **10(1)**, 49-51.
19. Chung, S.Y., Nam, S.W., Lim, J., Park, S. and Yoon, J., 2009, *Chem. Comm.*, **20**, 2866-2868.
20. Lou, X., Qin, J. and Li, Z., 2009, *Analyst*, **134(10)**, 2071-2075.
21. Shang, L., Zhang, L. and Dong, S., 2009, *Analyst*, **134(1)**, 107-113.
22. Wu, X., Xu, B., Tong, H. and Wang, L., 2011, *Macromolecules*, **44(11)**, 4241-4248.
23. Madhu, S., Basu, S.K., Jadhav, S. and Ravikanth, M., 2013, *Analyst*, **138(1)**, 299-306.
24. Zhao, H., Leamer, L.A. and Gabbaï, F.P., 2013, *Dalton Trans.*, **42(23)**, 8164-8178.
25. Ebaston, T.M., Balamurugan, G. and Velmathi, S., 2016, *Anal. Methods*, **8(38)**, 6909-6915.
26. Balamurugan, G. and Velmathi, S., 2016, *Anal. Methods*, **7,** 1705-1710.
27. Balamurugan, G., Venkatesan, P., Wu, S.P. and Velmathi, S., 2016, *RSC Adv.*, **29**, 24229-24235.
28. Qin, L., Hou, L., Feng, J., Chao, J., Wang, Y. and Jin, W.J., 2017, *Anal. Methods*, **9(2)**, 259-266.
29. Salomón-Flores, M.K., Bazany-Rodríguez, I.J., Martínez-Otero, D., García-Eleno, M.A., Guerra-García, J.J., Morales-Morales, D. and Dorazco-González, A., 2017, *Dalton Trans.*, **46(15)**, 4950-4959.

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