Volume I, No. 01

Research Journal

SCIENCE VIEWPOINT 2021



Editor: Dr. Mithil S. Fal Desai

Dnyan Prabodhini Mandal's Shree Mallikarjun and Shri Chetan Manju Desai College, Canacona-Goa 403702

shreemallikarjuncollege@shreemallikarjuncollege.ac.in

Our Mission

To strive for community development through empowerment of individuals by providing opportunities for learning life's education and to lead by example.

Our Vision

To emerge as the torchbearer in the learner-centric Quality Higher Education and dedicated efforts continuously evolving to better the BEST.



- To provide the students with quality educational experiences.
 - To provide support services for successful completion of their Degree Education
- To provide support a learning environment that Fosters Critical thinking, effective communication, creativity and cultural awareness.
- Encourage positive attitude, support economically challenged students and empower girl students.
 - Mett the diverse needs of our demographically diverse student population.

• Embrace equity and accountability through measurable learning outcomes, ethical data driven decisions and student achievement.

From Principal's Desk

The year 2021 has been a blessed year, though it may be irony to say this is a blessed year given the difficult time in which we are living. But the good thing among all bad is we are happy alive and we are still safe. So those who believe in the almighty it is the blessing of the god and those who, don't believe in would say it is a gift of seignee. This is what seignee has given all of us 'A Hope'. Leaving this COVID year apart, this year is a blessed year



this COVID year apart, this year is a blessed year prof. Manoj Kamat for our college because this happens to be our first Principal and Chairman of T. Y. B. Sc. batch graduation year. Advisory Board

The seignee section started in the year 2018 and all these years our students and faculty members have striven their best to establish the department not only in teaching-learning but in terms of extra cocurricular activities. In these terrible times, the team has been successful in organizing special events on a grand scale like that on National Science Day. We could have interaction with esteemed world-famous researchers like Prof. R. Fernandes and Prof. Sheshnath Bhosle. All the activities organized by the science section of the college were intended to develop scientific temperament among the community. So I would say, the last three years were momentous for us.

This is an achievement of the science section and honors the dedication, we have decided to do something monumental. Therefore, our 'Viewpoint' which is a decade old in house research magazine, would be complemented with a new edition 'Science Viewpoint'. The college will continue with its decadal old magazine along with 'Science Viewpoint' to encourage the science section of the college.

Here we do not claim any originality in the work published and is of no competing interest, Science Viewpoint is a dedicated effort to promote science communication and research culture in our college. The efforts of the contributing authors may not be polished, however, this is a sincere effort to bring out good in us and let what is good, become better. in the hope to make it excellent in coming years. May readers excuse us, wish and bless us for this initiative. I wish the Science Viewpoint an excellent start and continuous success.

Editorial



Dr. Mithil Fal Desai. Editor The 'Science Viewpoint' is a decent new initiative of the college dedicated to the newly formed science section of the Shree Mallikarjun and Shri Chetan Manju Desai college that would develop skill research communication among the young staff.

It is my privilege to be appointed as the editor of this college research magazine. This would certainly help us to inculcate research aptitude which is a key feature in new education policy. I congratulate the editorial board members and all the contributing teachers for this publication.

Editorial Board Members



Prof. F. M. Nadaf **Vice-Principal**



Dr. Sucheta Naik IQAC-coordinator



Pr. Rupa Chari NAAC-coordinator



Ms. Divya R. Sawant Asst. Prof. Chemistry



Mr. Dipak Bowalkar Asst. Prof. Zoology



Dr. Nisha Kevat Asst. Prof. Botany



Ms. Diksha Naik Asst. Prof. Botany



Ms. Priyanka Kavalekar Ms. Shubhlaxmi Naik Asst. Prof. Chemistry



Asst. Prof. Chemistry





Mrs. Aswita Dessai

Ms. Aarti Tengse Asst. Prof. Chemistry Asst. Prof. Chemistry



Mrs. Sanchita Naik Gaunkar Asst. Prof. Zoology

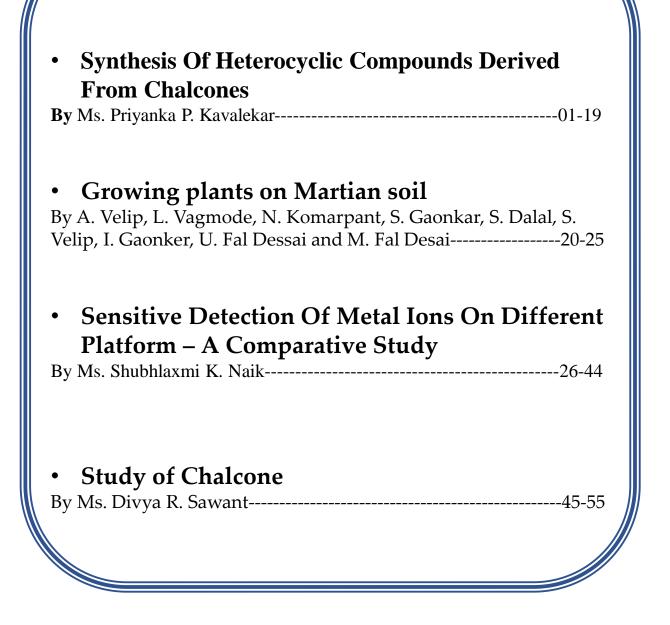


Ms. Sweta Moye Asst. Prof. Zoology



Ms. Sampada Naik Desai Asst. Prof. Chemistry

Table of Contents



Viewpoint

Research Article

Synthesis Of Heterocyclic Compounds Derived From Chalcones

Ms. Priyanka P. Kavalekar Assistant Professor in Chemistry Dnyan Prabodhini Mandal's Shree Mallikarjun and Shri Chetan Manju Desai College, Canacona-Goa priyanka.kavalekar@shreemallikarjuncollege.ac.in

Abstract: In this project study, we have tried to prepare heterocyclic compounds using Chalcone as a precursor and also we have tried determine the efficiency of different methods that have been employed to synthesise Chalcones and so here the synthesis of chalcones is carried out conventionally by Claisen-Schimdt condensation method using different alkaline bases like Sodium Hydroxide (NaOH), Barium Hydroxide (Ba(OH)₂) (solid base catalyst). Green chemistry plays a major role in today's world of chemistry. Therefore the two bases used here for preparation of chalcones are used in such a way, via which we can study the importance of employing green method for the synthesis of organic compounds. The Chalcone was prepared and it was first characterised on the basis of IR spectroscopy and Melting Point. And then it was used as a starting material for the next step that is for the preparation of heterocycles. Techniques such as thin layer chromatography, physical constant determination (melting point), IR spectroscopy have been used to check the purity and characterize chalcones and heterocycles prepared.

Keywords: Chalcone, Heterocycles, Anticancer, Antimalarial, Oxazine.

Introduction

The term 'Chalcone' was given by the two scientists namely, Kostecki and Tambor. Chalcone is a crystalline pale yellow solid compound. It is basic in nature having a physical constant (melting point) as 56°C. Chalcone structure contains two aromatic rings and one aliphatic three carbon chain. Other names of chalcones are benzalacetophenone and benzylideneacetophenone. Chalconare alpha, beta-unsaturated ketones consisting of two aromatic rings attached by an alpha beta-unsaturated carbonyl system with variety of substituents. Both the aromatic rings have conjugated double bonds and a completely delocalized pi-electron system. They are having keto ethylenic group (-CO-CH=CH-) and because of the presence of double bond they have a capability to exist in cis and trans form. Trans form is more stable as compared to cis form thermodynamically. Chalcones are open-chain flavonoids found and they are found in plants. They act as both intermediates and end products in synthesis of flavonoids. Chalcones have a broad spectrum of biological activities due to the presence of carbonyl group and on prohibiting of this carbonyl group,

chalcones are biologically inactive. Chalcones shows medicinal and pharmaceutical applications such as antiviral, antibacterial, anticancer, antioxidative, antiulcer, amoebicidal, insecticidal, and immunosuppressive. Along with this, they have also found application as light stabilizing agent, sweetening agent, analytical reagent for amperometric estimation of copper and spectrophotometric study of germanium. Chalcones and their derivatives can act as intermediates for the synthesis of heterocyclic compounds. Due to this reason chalcones are fascinated towards many researchers for their synthesis. Although chalcones occur naturally in plants, they can be synthesized in the laboratory using literature methods. There are various methods for the synthesis of chalcones including the most convenient method that is claisen-schmidt condensation which is an important C-C bond forming reaction. Generally, it is a simple condensation between acetophenone and substituted aryl aldehydes in presence of alkaline bases. The varied nature of catalysts, regents and reaction solvent universe requires simple, efficient and solvent-free synthetic protocols for biologically active compounds. Therefore green chemistry plays a vital role in development of synthetic strategies. The main criterion of green synthetic chemistry is to reduce minimum hazardous and make simple reaction procedure while design new synthetic molecules. Many researchers have reported grinding mode which is solvent-free synthetic route for some well-known reaction such as Reformatsky reactions, Grignard reactions, Aldol condensations, Dieckmann condensations, Reductions, and others. Most of these reactions require room temperature so these reactions are carried out at room temperature in absence of solvents that is they are carried out under solvent free environment, using only a mortar and pestle as a aparatus. Since Chalcone shows broad spectrum of biological activity including antimalarial, antibacterial, antifibrogenic, anticancer, antitrichomonal, antinflammatory, antileishmanial, cytotoxic and anti-Trypanosoma cruzi activities we have made an interest in synthesis if chalcone and study the factors while carrying out its reaction. In this research we reported solvent-free synthesis of some substituted 1,3-diaryl-2-propen-1-one chalcone with the reaction of Acetophenone with and substituted benzaldehyde under grinding technique in presence of solid base catalyst condition without using any organic solvent.[13] Essential and Important intermediates in the synthesis of many pharmaceuticals are mostly chalcones. Claisen-Schmidt Condensation is homogeneous reaction which is acid/base catalysed. Homogeneous reactions creates several extra steps, such as recovery of catalyst and then waste disposal problems, wastage of organic solvents. In this respect, heterogeneous catalysts are considered as an ecofriendly alternative.[19-25]

Lately, activated carbons have been used widely to catalyze fine chemicals reactions. They have extended surface area, microporous structure, and heavy degree of surface reactivity hence they carry out the reactions such as fine chemical reactions efficiently. Therefore, the catalyst of this type is viable with other typically employed catalysts for organic synthesis, such as zeolites, supported oxides and others.[12] A heterocyclic compound is a cyclic compound that has atoms of atlest two different elements in the ring.

Most common heterocycles are the ones having Five or Six membered ring which contains Oxygen, Sulphur, Nitrogen. Chemistry of Heterocycles is quiet inetersting and having a large variety of applications. They are predominantly used as pharmaceuticals, as agrochemicals and as veterinary products. They also find applications as sanitizers, developers, antioxidants, as corrosion inhibitors, as copolymers, dye stuff. They are used as vehicles in the synthesis of other organic compounds. Some of the natural products e.g. antibiotics such as penicillin, cephalosporin; alkaloids such as vinblastine, morphine, reserpine etc. have heterocyclic moiety. Some of the biological properties of some heterocyclic compounds, known in literature they act as anticancer, anti-HIV and Antimicrobials, they are Genotoxic active agents, Antiviral agents targetting virus proteins. COX-2 inhibitors and anti-inflammatory, they show antitumor activity, antioxient and cytotoxic activity etc. Thesse important bilogical applications gives rise to the wide research area under the heterocycles. Study of heterocyclic chemistry is one of the great importance to Organic chemists, polymer chemists, and many biological scientists. Some novel heterocyclic derivatives such as Thazines, Oxazines, Isoxazoles and Pyrazoles were synthesized from various Chalcones. These compounds cab be checked for their Anti inflammatory, Anti Bacterial and Anti fungal activities. Usually while using conventional methods there might be a production of waste, unwanted harmful byproducts, solvent waste etc. To avoid these major problems like production of unwanted or harmful by-products Green synthesis methods are required to build up a reliable, sustainable, and eco friendly synthesis procedures. The use of ideal solvent systems, reagents, solid catalysts etc is essential to achieve this goal. Green Chemistry works according to its 12 classic principles which contributes to less toxic reagents, less damage to humans and environment, saves energy, contributes to sustainability of the chemical processes all of which decreases the risk of global overheating and it makes a proper use of natural resources. The techniques like microwave heating, ultrasound-assisted and hydrothermal processes or ball milling in combination with the use of natural precursors, are of vital

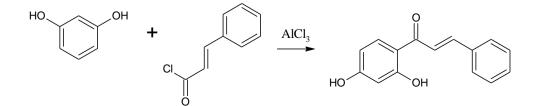
2. Materials and Methods

Literature Review:

Earlier chalcones were prepared via different methods like the ones listed below:

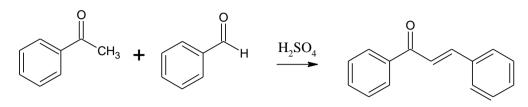
importance in the solventless synthesis, also in greener synthesis and biosynthesis techniques.

Shlnoda and Sato preferred to condense polyhydroxyphenols with cinnamoyl chloride in the presence of anhydrous aluminium chloride in nitro benzene when resorcinol was condensed, the main product formed was the chalcone derivative.

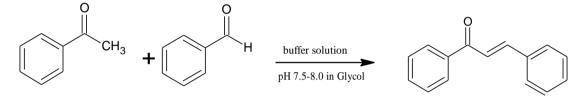


Claisen and Claparede were the first to use concentrated sulfuric acid for the condensation of acetophenone with benzaldehyde to

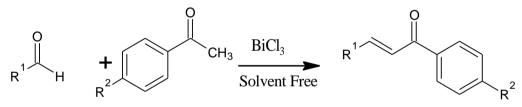
obtain simple chalcone.



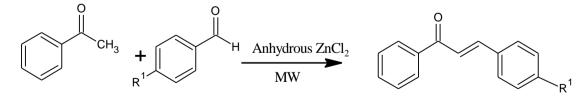
Reichel and Hampel employed very mild conditions for bringing about the condensation of acetophenone with benzaldehyde to chalcone using buffer solution of pH 7.5-8.0 in Glycol.



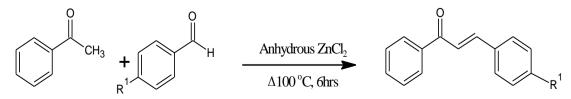
Dhruva Kumar, Suresh & Jagir S. Sandhu et al., in 2010 reported environmentally benign protocol for the synthesis of chalcones by the Claisen Schmidt condensation of aldehydes with ketones using eco-friendly non-toxic bismuth(III)chloride catalyst under solvent-free condition is reported. In this protocol, the reaction time is very short, yields are high, and there are no other pollutants formed.



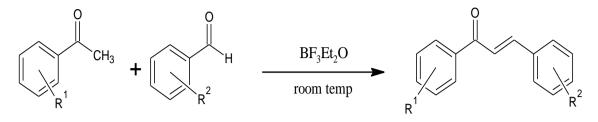
Microwave irradiation of aromatic aldehydes and acetophenones in the presence of anhydrous zinc chloride was resulted exclusively in chalcones in high yields. Easy isolation of products.



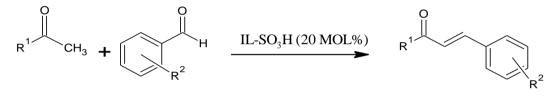
The reaction of aromatic aldehydes with various acetophenones thermally by using catalytic amount of zinc chloride was carried out.



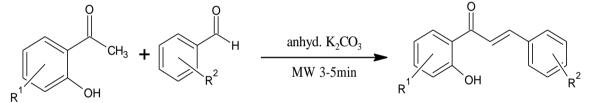
T. Narender and K. Papi Reddy et al., in 2007 reported the condensation of substituted acetophenones with various aromatic aldehydes in the presence of BF_3 -Et₂O at room temperature with dioxane gave chalcones with very good yields.



Hua Qian and Dabin Liu et al., in 2011 reported the procedure where in recyclable sulfonic acid-functional ionic liquids (ILs) were prepared and used as dual catalyst and solvent for the synthesis of chalcones via Claisen-Schmidt condensation between acetophenone and benzaldehyde. ILs proved to be very active, leading to an 85%-94% yield of chalcone in the presence of 20%

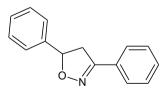


Ahmed Hassen Shntaif et al., in 2016 reported the reaction of O-Hydroxy acetophenone and substituted benzaldehydes in the presence of anhydrous K_2CO_3 under microwave conditions was carried out which gave good yields of the chalcone.

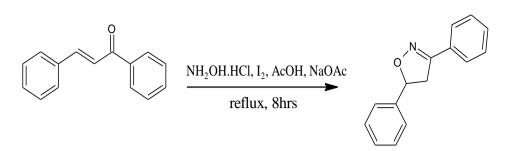


In this project we tried to prepare heterocycles simple methods but alternative methods used to prepare these heterocycles were:

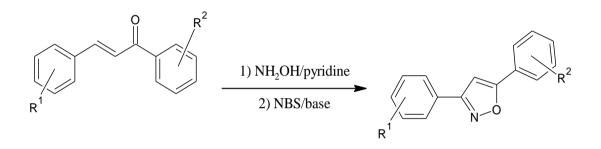
1) Isoxazole:



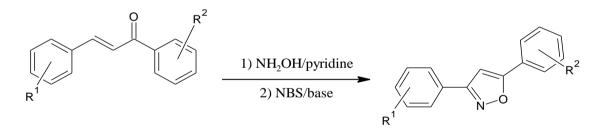
Sikkandarkani Akbar and Kannupal Srinivasan in the year 2013 proposed An iodine-mediated tandem reaction of 2alkynylchalcones with hydroxylamine hydrochoride gave naphtho[2,1-d]isoxazoles. This one-pot reaction involves an oxidative cyclocondensation followed by an electrophilic hydroarylation.



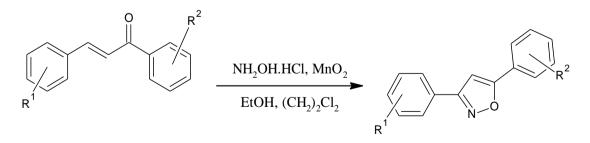
Elkasaby and Salem have reported the synthesis of 3,5-diaryl Isoxazoles from chalcone, By the action of NH₂OH/Pyridine on chalcone isoxazolines were obtained and then on further treatment with NBS/Base Isoxazoles were obtained.



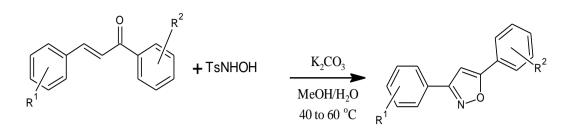
Solankee, Sejal et al., in 2008 reported the following reaction wherein potassium hydroxide is used.



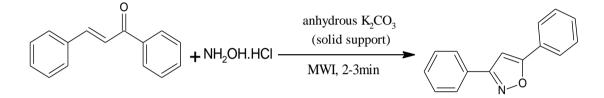
Tomishima masaki et al in 2008 tried the reaction with MnO₂ catalyst which gave good yields.



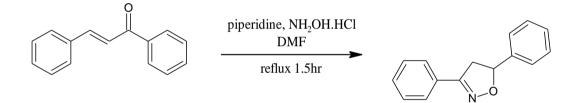
Tang, Shibing et al., in 2010 reported the synthesis of isoxazole using K_2CO_3 and N-hydroxy-4-toluenesulfonamide under heating conditions.



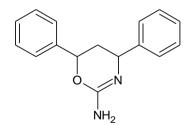
Mazaahir Kiswai, Shuchi Kukreja and Ruby Thakur, in 2006 reported the following reaction where in K_2CO_3 acts as a solid support under microwave conditions.



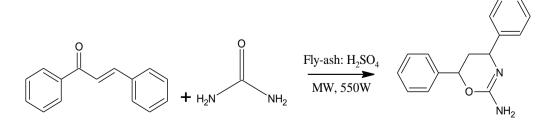
Bhoyar, A.D. et al., in 2012 carried out following synthesis of isooxazoline by using chalcone under refluxing conditions.



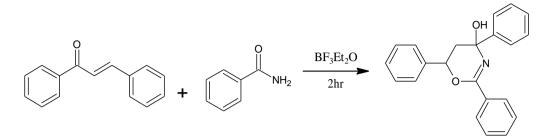
2) Oxazine:



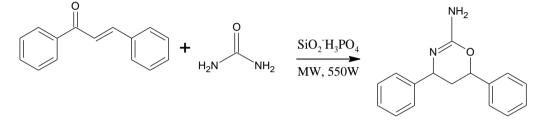
G. Thirunarayanan, R. Sundararajan, R. Arulkumaran carried out the green synthesis of oxazines under microwave conditions.



Girly Tony, Meena Chandran, A. R Bhat, K. Krishnakumar et al., reported the reaction of chalcones and banzamide to give oxazoles.

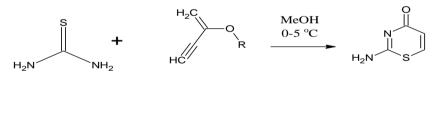


Ganesamoorthy Thirunarayan and velayutham Renuka, reported the following synthesis.



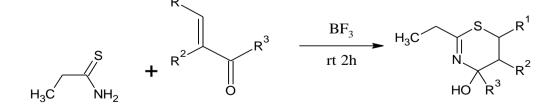
3) Thiazine:

Recently in 2016 a selective synthesis of 1,3-thiazinone in good yields was illustrated by Peddinti et al. by means of reaction of thiourea with acetylene monocarboxylates in methanol.

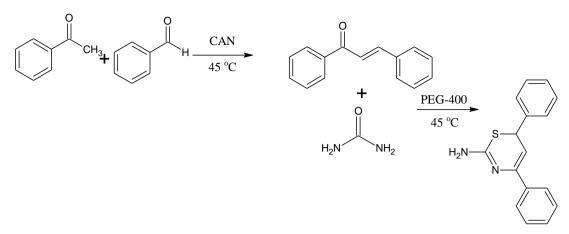


R= Me,Et

Mamoru Koketsu, Kohsuke Tanaka, Yuichi Takenaka, Cecil D. Kwong, Hideharu Ishihara et al reported the following synthesis.



Singh and colleagues developed an innovative, easy to use, inexpensive and highly efficient one pot, multi component ceric ammonium nitrate catalysed preparation of 1, 3-thiazine derivatives in PEG 400.



Present Work:

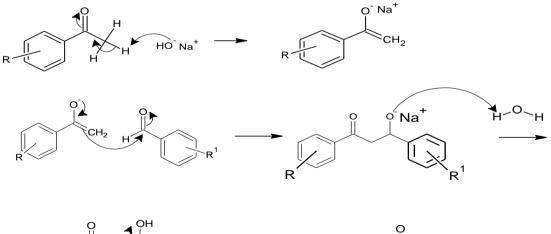
Step 1: Synthesis of chalcones:

Chalcones were synthesised by Claisen Schmidt Condensation between different acetophenones and different aldehyde derivatives in the presence of base.

General reaction:



Mechanism:





- 1. Claisen Schmidt condensation was carried out in two different ways:
- i) Using NaOH as a base
- ii) Using $Ba(OH)_2$ as a base.

Conventional method for the synthesis of chalcones:

i) Claisen Schmidt Condensation using Sodium Hydroxide:

A round bottom flask containing magnetic stirring bar kept in an ice bath was placed on a magnetic stirrer. Acetophenone (10mmol) was added, followed by 20ml ethanol. To this 10ml of 60% NaOH was added with controlled addition and then Aromatic aldehyde (10mmol) was added dropwise. After addition of all the reagents, the reaction mixture was stirred vigorously until it was a thick yellowish mass. The reaction mixture was monitored by TLC and it was neutralised with 2N HCl with continuous stirring. The product was filtered under suction and recrystallised using ethanol.

Green method for chalcone synthesis

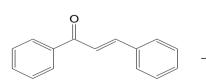
ii) Using $Ba(OH)_2$ as a base

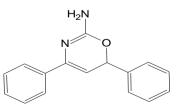
In a mortar and pestle, a mixture of aldehyde, ketone and anhydrous barium hydroxide (C-200) (2g) was taken and it was ground well at room temperature for 2-5 minutes. Then the reaction was allowed to stand for 10 minutes. Then 30ml of ice cold water was added to the reaction mixture and acidified it with concentrated HCl. The product then was collected by vacuum filtration and recrystallised from ethanol. . In the cases where an orange oil was formed, the mixture was extracted with CH_2Cl_2 , the extracts were dried (Na₂SO₄) and the solvent was evaporated to give the chalcone as a solid.

2) Procedure for the synthesis of heterocyclic derivatives from chalcone:

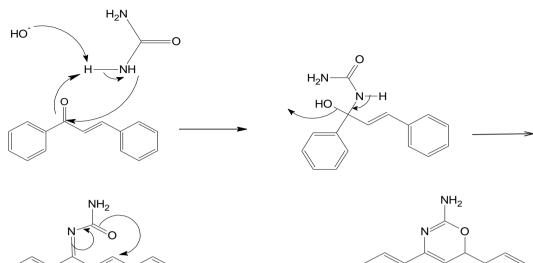
NaOH/C₂H₅OH

Synthesis of Oxazine:

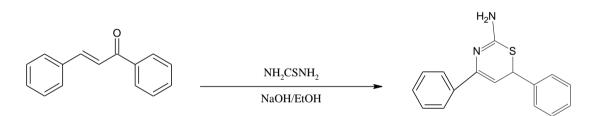




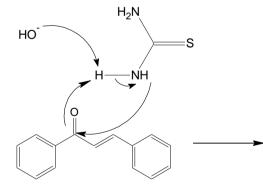
Mechanism:

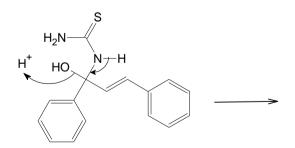


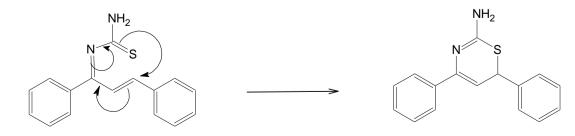
3) Synthesis of thiazine:



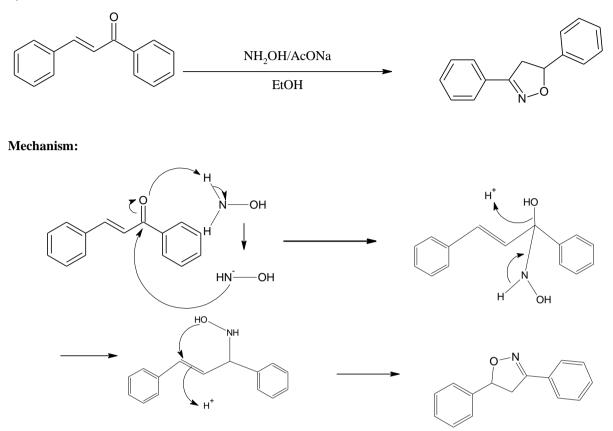
Mechanism:







Synthesis of isoxazole derivatives:



Procedures:

i) Synthesis of Thiazine derivatives:

Round bottom flask containing a magnetic stirring bar placed on a magnetic stirrer. To that flask 0.02moles of chalcone, 0.02moles of thiourea were added. And 10 ml of ethanolic sodium hydroxide was added in which the two substances were dissolved. And this reaction mixture was stirred for 3 continuous hours. Then it was poured in 400ml of ice cold water with continuous stirring for 1hour and the it was left overnight. The precipitate formed was filtered, washed and recrystallised from ethanol.

ii) Synthesis of Oxazine derivatives:

In a round bottom flask containing a magnetic stirrer bar, 0.02mol of chalcone, 0.02 mol of urea and 10ml of ethanolic sodium hydroxide were added. Contents were dissolved well and this reaction mixture was stirred for 3hours continuously and this was then poured in 400ml ice cold water and it was stirred for more 1 hour and then it was left overnight. The precipitate formed was filtered, washed well and recrystallised from ethanol.

iii) Synthesis of Isoxazole derivatives:

0.02 mol of chalcone and 0.02 mol of hydroxyl amine hydrochloride, sodium acetate in ethanol were taken in round bottom flask. This was refluxed for 6 hours, and then the reaction mixture was poured into ice water. The precipitate obtained was filtered washed and recrystallized.

3. Results

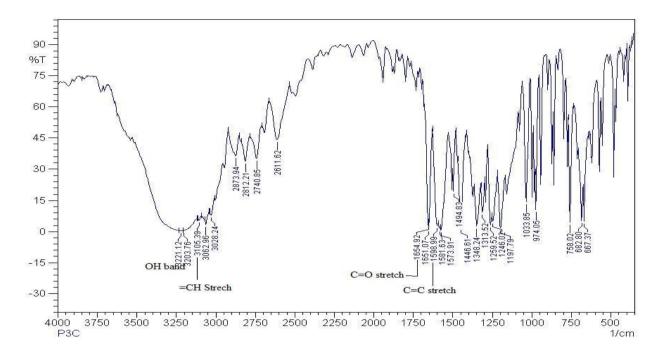
Different chalcone derivatives were synthesised via conventional and green method. Both the methods were employed to study the efficiency of these methods and also to understand the advantages of green method over conventional method. Here it was found that the yield of chalcones 1-6 were good in case of green method that was employed and the use of solved was minimized in case of this method. Poor yields were obtained when sodium hydroxide was used as a base also the reaction was not found to be completed after long period of time. The best results were obtained when 3-4 molar equivalents of C-200 were used. With the use of Ba(OH)₂ reaction time reduced from hours to minutes and the yields were also good.

The grinding technique was found to be the rapid procedure for the synthesis of chalcones which involved grinding of a mixture of aryl aldehyde, acetophenones and anhydrous barium hydroxide in a mortar and pestle for 2-5 minutes in the absence of any solvent. The product is also easily obtained by acidifying the mixture without extraction.

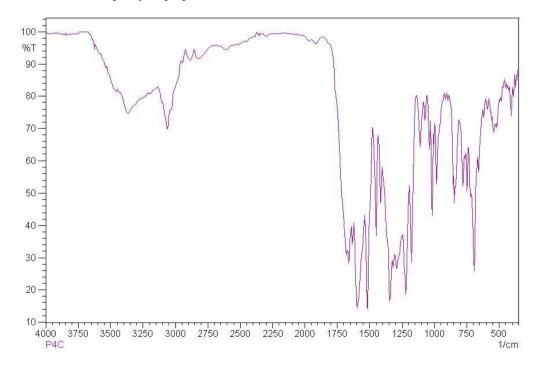
And no higher temperature and solvent was required. The formation of these Chalcones was confirmed by taking their IR spectras, where a prominent peak of carbonyl group was obtained at 1654.92 cm⁻¹ in case of 1-(2-hydroxy)-3-phenyl-2-propenone (2) which has shifted to lower value than the standard 1,3-diphenyl-2-propenone, aromatic C=C stretching vibration absorptions at 1598, 1581, 1573 cm⁻¹ were observed which shows the formation of this chalcone(2). Similarly IR spectras of formed compounds were recorded and they were characterised on its basis, melting points were determined too for the confirmation of the pure compounds.

Further reaction on the prepared chalcone was carried out to prepare heterocyclic compounds. Simple methods were employed to synthesise these heterocycles i.e. Oxazine, Thiazine, Isoxazole. Among which the successfully prepared heterocycle was Oxazine which was characterised by recording its IR spectra.

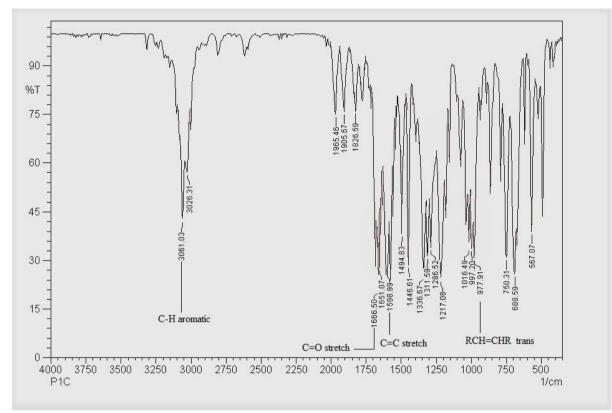
IR of 1-(2-hydroxy)-3-phenyl-2-propenone



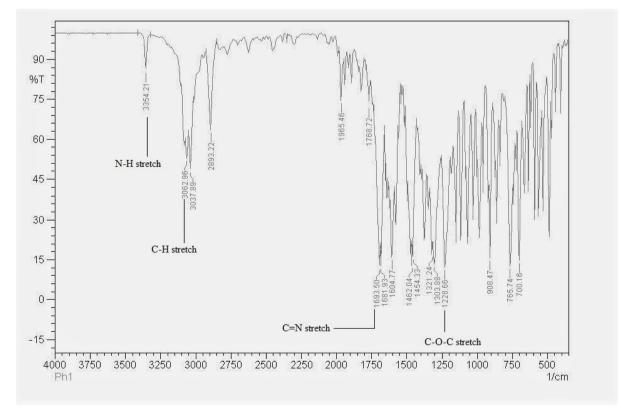
IR of 3-(4-nitro)-1-phenyl-2-propenone



IR of 1,3-diphenyl-2-propenone(step1)



IR spectra of oxazine (step2)



Following tables shows successfully synthesized chalcones, heterocycles.

1) Synthesised Chalcones:

Entry	Chalcones	Time [Ba(OH) ₂] Minutes	Time NaOH minutes	Yield [Ba(OH) ₂]	Yield NaOH	Melting points (°C)
1.		20	50	80%	65%	56
	1,3-diphenyl-2-propenone (C ₁₅ H ₁₂ O)					
2.	OH O 1-(2-hydroxy)-3-phenyl-2-propenone $(C_{15}H_{12}O_2)$	20	60	87%	70%	90
3.	HO 1-(3-hydroxy)-3-phenyl-2-propenone $(C_{15}H_{12}O_2)$	20	60	71%	63%	130
4.	0 NO ₂ 3-(4-nitro)-1-phenyl-2-propenone (C ₁₅ H ₁₁ NO ₃)	20	75	76%	50%	160
5.	O_{OH} $3-(4-Chloro)-1-(2-hydroxyphenyl)-2-propenone (C_{15}H_{11}O_2Cl)$	20	60	83%	70%	150
6.	Cl 3-(4-chloro)-1-phenyl-2-propenone	20	60	70%	53%	112
	$(C_{15}H_{12}O_2 Cl)$					

Table 1. Yield and melting points of Chalcone derivatives

2) Synthesized Heterocycle:

Entry	Heterocycle	Yield	Melting point
1.	H ₂ N NO Oxazine	40%	200 °C

3) <u>Heterocycles that we tried to synthesize but failed to obtain:</u>

Entry	Structure	Name
1.	H ₂ N N S	4,6- diphenyl-1,3-thiazine-2-amine
2.		4,5-diphenyl-1,2-isoxazole

Conclusion/Discussion

Our aim was to prepare heterocyclic compounds from different chalcone derivatives. So first of all we prepared different substituted chalcones by green method using a grinding technique and also by conventional method. Here it was observed that yields were good in case of grinding technique and it was an easy way to prepare chalcones where there was no use of any solvent. It was time efficient technique. It gave good yield. So it is atom economic. The compounds sythesised were identified via their IR spectra and melting points. Second step was to do further reaction on these chalcones to yield heterocycles Isoxazole, Oxazine, Thiazene. Attemp was to synthesise these heterocycles simple Stirring methods at room temperature. Out of three heterocycles, one was successful that is Oxazine was successfully prepared.

References:

- Kumar,S.; Lamba, M.S.; & Makrandi, J.K., An efficient green procedure for the synthesis of chalcones using C-200 as solid support under grinding conditions, *Green Chemistry Letters and Reviews*, (2008) 1:2, 123-125. DOI: 10.1080/17518250802325993
- 2) Kumar, S., An improved one-pot and eco-friendly synthesis of aurones under solvent-free conditions, *Green Chemistry Letters and Reviews*, (2014) 7:1, 95-99. DOI: <u>10.1080/17518253.2014.895867</u>
- Detsia, A.; Majdalanib, M.; Kontogiorgisc, C.A.; Litinac, D. H.; Kefalasb, P., *Bioorganic & Medicinal Chemistry* 17 (2009) 8073–8085.
- Albuquerquea, M.T.H.; Clementina, M.M.; Santosa, b. J.; Cavaleiroa, A. S. and Silva, M. S. A., *Current Organic Chemistry*, 2014, 18, 000-000
- Kamal, R.; sharma, D.; Wadhwa, D.; Prakash, O. The chemistry of, ditosyloxy ketones: a new and convenient route to 4,5-diarylisoxazoles from , chalcone ditosylates. *Synlett.*, **2012**, 23, 93-96.
- 6. Solankee, A.; Patel, K.; Patel, R. Antimicrobial evaluation of some novel isoxazoles, cyanopyridines and pyrimidinthiones. *Indian J. Chem., Sect B.*, **2013**, 52, 671-676.
- Murthy, Y.L.N.; Nanda, R.; Kumar, K.R.; Swany, G. Synthesis and characterization of a new chromanoisoxazole. *Indian J. Chem., Sect B.*, 2006, 45, 532-534.
- 8. Voskiene1, A. and Mickevicius, V., Chemistry of Heterocyclic Compounds, 2009, Vol. 45, No. 12,.
- 9. Solankee, A. and Solankee, R.P. and Patel, *IJPSR*, 2013; Vol. 4(12): 4671-4675.
- 10. Solankee, A.; Patel, R. and Patel K., Der Pharma Chemica, 2011, 3(6), 317..
- Velikorodov, A. V.; Ionova, V.A.; Temirbulatova, S.I.; Titova, O.L.; and Stepkina, N.N., *Russian Journal of Organic Chemistry*, 2013, Vol. 49, No. 11, pp. 1610–1616
- 12. Upadhyay, A.; Gopal, M.; Srivastava, C.; and Pandey N.J., *j. pestic. sci.*, 35(4), 464–469 (2010)
- Mahgoub , H.H.; Bentwahab, H; Hussein, E. M. and Saeed Mahgoub, A.E.M. et al., *IJPSR*, 2014; Vol. 5(11): 5052-5058.
- Kalirajan, S.; Sivakumar, S. U.; Jubie, S.; Gowramma, G.; and Suresh, B.; *International Journal of ChemTech Research* Vol.1,No.1,pp 27-34, Jan – March 2009
- 15.] Kochetkov, N.K.; Sokolov, S. D., Adv. Heterocycl. Chem., 1963, 2, 365
- Kumara, K. S.; Reddya, K.T.; Vamsikantha, A.; Omprakasha, G.; Dubeyb, P. K., *Der Pharma Chemica*, 2011, 3 (5): 113-122
- 17. Raju, G. N.; Suresh, P.V.; Nadendla, R. R.; and Anusha, K., Der Pharma Chemica, 2015, 7(6):346-352)

- S. Shivshankar, S. Chavan, Y. Vibhute, Solvent-free synthesis of chalcones and antioxidant activity, J. Adv. Chem. Sci. 2(4) (2016) 373–375.
- 19. M.J. Climent, A. Corma, S. Iborra, J. Primo, J. Catal. 151 (1995) 60.
- 20. S. Saravanamurugan, M. Palanichamy, B. Arabindoo, V. Murugesan, J. Mol. Catal. A: Chem. 218 (2004) 101.
- 21. S. Sebti, A. Solhy, R. Tahir, A. Simahi, Appl. Catal. 235 (2002) 273.
- S. Sebti, A. Solhy, R. Tahir, S. Boulaajaj, J.A. Mayoral, J. Fraile, A. Kossir, H. Ouminoun, Tetrahedron Lett. 42 (2001) 7953.
- 23. R. Ballini, G. Bosica, M. Ricciutelli, R. Maggi, G. Sartori, R. Sartorio, P. Righi, Green Chem. 3 (2001) 178.
- 24. A. Fuentes, J.M. Marinas, J.V. Sinisterra, Tetrahedron Lett. 28 (1987) 4541.
- 25. M.J. Climent, A. Corma, S. Iborra, A. Velty, J. Catal. 221 (2004)474.

Research Article Growing plants on Martian soil

A. Velip¹, L. Vagmode¹, N. Komarpant¹, S. Gaonkar¹, S. Dalal¹, S. Velip¹, I. Gaonker¹, U. Fal Dessai¹ and M. Fal Desai^{1*}

¹ Shree Mallikarjun and Shri Chetan Manju Desai College, Canacona; mithil.faldesai@shreemallikarjuncollege.ac.in

* Correspondence:

Abstract: In the present work, we have attempted to study the growth of the plants on infertile replicate of martial soil which is rich in iron (III) oxide. A variety of edible crops like the black turtle, mung beans, radish, cluster beans and finger millet were attempted to grow. The nutrient supply is optimized to have a better growth of these plants on infertile soil. The growth parameters were monitored to understand the growth of these plants on the iron oxide substrate. The uptake of Fe was tried to analyze from the amount of ash content.

Keywords: Fe₂O₃; Martian soil; Fe

1. Introduction

After the success of Mangalyan the first Mars Orbiter mission, in coming years India is set to achieve some of the major space ambitions. The Indian Space Research Organisation (ISRO) got an 11% budget hike for 2019-20 and is all set to launch its first crewed mission, the Gaganyaan, before India's 75th anniversary of independence in 2022 [1]. America's National Aeronautics & Space Administration (NASA) has been campaigning for a manned mission to the planet in the 2030s, and the iconic entrepreneur Elon Musk has announced with his SpaceX company to do it by 2024 that would be a giant leap of mankind to colonize the Mars. Research on growing plants on the Martian soil would be an important step for self-sustainability before colonizing the planet. Apart from growing plants, scientists are trying to explore the resources that can be used from Mars [3]. The Martian soil is like dirt from the crushed laterite rock that is rich in iron oxide [4–6]. If we want to grow plants on such infertile Martian soil, we have to first identify the crops and make the soil fertile with nutrients and water to transform infertile substrate into something that grows in.

It is obvious due to Mar's extremely cold climate; scientists need to grow the crops inside a controlled environment. The soil on earth has moisture, minerals, microbes and other organic matter that could help the plants to grow. The Martian soil also has some of the nutrients that plants need for growth and survival. It is well-known fact that plant growth is affected by the availability of nutrients, water, substrate support material, as well as gravity and environmental conditions. The research on space station reveals that gravity affects the growth of the plant and the root structures are also significantly affected [7]. Martian soil consists mostly of volcanic basalt rock. It holds nutrients such as sodium, potassium, chloride and magnesium. It is made up of mineral matter.

Various Nasa rover mission like Viking 1, Viking 2 and Pathfinder, on Mars, has revealed the soil composition [8]. These missions revealed that the Martian surface has majorly mineral-like, silica (~45%) and haematite (~20%) alumina (~10%) with other minerals like lime and magnesia which are less than 10%. The presence of chloride is also observed in less than 1% of the Martian soil. The pathfinder revealed that there is a presence of P, Na, Mn and Mg in a small amount in Martian soil. As Martian soil has largely iron oxide and silica the study of the growth of plants on such infertile substrate is interesting to investigate. Si and Al are non-essential elements, while Fe is an essential micronutrient for plant growth.

Iron is a component of several vital enzymes and pigments [9]. It assists in nitrate and sulfate reduction and energy production within the plant [10]. Iron is required for electron chain reactions involved in photosynthesis and it is critical for its formation of

chlorophyll. The growth of the plants is affected in stressed conditions like that of high concentration of Fe. The atmosphere of Mars is made of 96 % CO_2 [11]. Iron is the third most limiting nutrient for plant growth and metabolism, due to the low solubility of the oxidized ferric form in an aerobic environment. Iron deficiency is a common nutritional disorder in many crop plants, resulting in poor yields and reduced nutritional quality. When the insoluble ferric form is reduced, it is converted to a ferrous form in the soil and is then absorbed by the plants. Even though iron is hardly present in living matter, it is still an essential element that is critical for plant life, as this element is involved in plant metabolism activities. As an important component of proteins and enzymes, iron plays a significant role in basic biological processes such as photosynthesis, chlorophyll synthesis, respiration, nitrogen fixation, uptake mechanisms through the action of the ribonucleotide reductase. It is also an active cofactor of many enzymes that are necessary for plant hormone synthesis, such as ethylene, lipoxygenase and abscisic acid. Iron deficiency severely affects plant development and growth and excess iron in the cell is toxic. Iron reactivity with reduced forms of oxygen produces radical elements that can cause a loss of integrity that can kill the cell. Therefore, there is a requirement for an optimal window for the iron concentration to ensure smooth plant development [10,12].

Iron limitation causes the decline of many photosynthesis components, including the Fe-S protein ferredoxin, which is involved in essential oxidoreductive pathways of chloroplasts. In this role, it is reversibly oxidized from Fe^{2+} to Fe^{3+} during electron transfer [10]. Iron oxide is a mineral compound that occurs abundantly in nature. It presents more than one crystal structure and also different structural and chemical properties. The main forms of these minerals are hematite, and magnetite [13].

In this brief study, we have attempted to investigate the growth of the plant on infertile replicate of Martian soil which is rich in iron (III) oxide. Different edible crops were tried to grow under different conditions. The growth parameters were monitored timely. The effect of iron oxide on plant growth is studied in this brief study.

2. Experimental

2.1 Preparation of Martian soil samples

The iron rust from the local workshop was collected and heated at 800 °C for 2 h. The rust was mixed with the washed sand in the ratio of 5:2 (w/w). This material is treated as Martian soil replicate.

Simultaneously, Fe_2O_3 was also prepared in the laboratory by using $FeCl_3$ as the source of Fe. About 100 g $FeCl_3$ (LR-grade) is dissolved in distilled water and filter through coarse filter paper. To this solution, 20 g ammonium chloride (LR-grade) was added. The 1:1 aqueous ammonia is added dropwise till complete precipitation of Fe^{3+} as $Fe(OH)_3$ is observed. The precipitate is filtered through a Buckner funnel and dried in the furnace at 800 °C.

2.2 Growing plants

Five different species of plants were identified and were cultivated in the control and experimental setup as shown in **Table 1** and **Table 2**. In the control setup backyard garden soil was used to grow the selected crop plants. About 50.0 g of Martian soil sample was taken in 5 plastic containers and seeds were sown in it and different nutrition treatments were given to it. The growth of the samples was monitored periodically.

Sample	Plant name (common name/botanical name)	No. of seeds in one treatment
А	Cluster beans/Cyamopsis tetragonolobus	02
В	Finger millet/Eleusine coracana	20
С	Mung bean /Vigna radiata	10
D	Radish, Mula/Raphanus sativus	05
Е	Black turtle bean/Phaseolus vulgaris	02

Table 1. Details of type of seeds and number of seeds

Table 2. Control and Treatments to grow the plant seeds

Sample	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Experimental	Normal soil	Martian soil	Martian soil	Martian soil 50g	Martian soil 50g	Martian soil
setup	50g + nor-	50g + sand 20	50g + sand 20	+ sand 20 g + 5%	+ sand 20 g $+$ 5%	50g + sand 20
	mal water	g+ normal wa-	g + 5% urea	NPK solution (5	urea solution	g + mineral
		ter	solution (5	mL)	(2.5 mL) + 5%	water
			mL)		NPK solution	
					(2.5 mL)	
А	+	+	-	-	-	-
В	+	+	-	-	-	+
С	+	+	-	-	-	+
D	+	+	-	-	-	+
F	+	+	-	-	-	-

(+) plant grew, (-) plant did not grow

2.3 Growth parameters of the plants

The height and weight were measured for all successfully grown plants. The height and weight are tabulated in **Table 3**. The day of the measurement is also tabulated.

2.4 Ash content

The plant samples were cut into fine pieces and dried in a silica crucible and ignited in the furnace at 800 °C and the weight of ash was measured for all controls and the T1. The data is represented in **Table 4**.

Sample	Со	ntrol	r	Γ1	Т	2	Т	3	Т	4	r.	Г5	No of days
Sat	Н	W	Н	W	Н	Н	W	W	Н	W	Н	W	No 0
А	7.2	0.286	12.0	0.517	-	-	-	-	-	-	-	-	09
В	9.0	0.045	8.4	0.030	-	-	-	-	-	-	6.0	0.020	12
C	6.2	0.652	1.5	0.163	-	-	-	-	-	-	0.6	0.082	10
D	12.0	0.217	10.0	0.145	-	-	-	-	-	-	12.0	0.160	11
Е	20.0	1.210	21.0	0.823	-	-	-	-	-	-	-	-	08

Table 3. Height and mass gained by the plant samples

H = height (cm), W = weight (g)

 Table 4. Ash content of the plants

	CONTROL			T1		
	Weight of	Weight of	Ratio of ash	Weight of	Weight of	Ratio of ash
Sample	plant sample	ash (g)	content to	plant sample	ash (g)	content to
	(g)		plant sample	taken (g)		plant sample
			weight			weight
А	0.286	0.005	0.017	0.517	0.010	0.019
В	0.210	0.004	0.019	0.208	0.003	0.014
С	0.652	0.015	0.023	0.163	0.004	0.025
D	0.217	0.005	0.023	0.145	0.007	0.048
Е	1.210	0.015	0.012	0.823	0.014	0.017

3. Results and discussion

Table 2 shows that all attempted plants grew in the control setup as expected. All selected species also grew in T1 where the tap water was used for hydrating the Martian soil substrate. However, the T2 to T4 probably could not have grown due to excess fertilizer in the early stages of the plants as the plants require optimized nutrient conditions to grow. These treatments were repeated several times and the data was found to be reproducible.

The physical characteristics of these plants were analyzed and compared with the control. The plants grown on Martian soil replicate were found to have a slightly yellow stem. This is probably due to the higher uptake of Fe. It was observed that all the plant samples grown in T1 were shorter than the control. An exception was seen in sample A. It was also observed that samples grown in treatments have poorly developed roots compared to the control.

Among the tested samples, plant height to weight ratio indicates that ratio more in treatment than the control for samples B and E. Although it is observed that the plants grown on Martian soil substrate are dwarf, the height to mass ratio indicated the plants were having more mass compared to their height in most of the cases. This could be because of a higher amount of Fe taken by these plants as seen from the ash content of plants in the control and treatment setup. Samples A, C and E show a higher amount of mass due to the presence of the inorganic content than that of control. The qualitative test with thiocyanate showed the presence of iron in all the ash samples of control and treatments (**Table 4**). However, the intensity of color was lower in control than that of the treatments. This reflects on the lesser uptake of Fe concentration in the control soil and higher uptake of Fe from Martian soil substrate.

The anatomical features of some of the plant species were studied using a compound microscope. The anatomical features were not thoroughly studied but among the tested samples, no evident changes could be identified in the sections of the treatment samples with that of control.

The prepare Martian soil substrate was tested for its iron content used in treatments. The Martian soil sample showed 0.042 g of Fe per 0.200 g of soil sample. The amount of Fe was found fivefold less than the theoretical value (0.139 g). This is observed as iron rust used as raw material was not pure and there was 30% silica added by mass. The planned studies were also to identify the Fe content in the control to have a comparative account of Fe uptake by control and treatment plants.

4. Conclusion

In this brief study, the growth of the plants on replicate of Martian soil which is rich in iron (III) oxide shows that plants can be grown successfully on such substrates. A variety of edible crops like the black turtle, mung beans, radish, cluster beans and finger millet were grown and their growth parameters were monitored. The plants grown on Martian soil replicate were dwarf and having yellow coloration on the tender stem and leaf petiole probably due to higher Fe uptake. The height to mass ratio of the treatments indicated the plants were having more mass compared to that of the control setup in most of the treatments. No significant difference in anatomical features was observed in these preliminary studies. The ash content of the treatment was also observed to be higher in many samples compared to that of the control. This is attributed to a higher amount of Fe uptake by the treatment plant samples as suggested by the qualitative test of Fe. The qualitative test showed the presence of Fe in control and treatment samples. The Fe in Martian soil was found to be less than that of pure iron oxide as rust was crude and there was a dilution of Fe concentration by the addition of silica.

References

- [1] Y. Wang, Y. L S. Priyadarshini. Nature Chemistry, (2019) 5–7.
- [2] M. Mele, C. Magazzino, Environ. Sci. Pollut. Res. 28 (2021) 2669–2677.
- [3] N. Savage, ACS Cent. Sci. 3 (2017) 1133–1136.
- [4] N. Latifi, A. Marto, A. Eisazadeh, Acta Geotech. 11 (2016) 433–443.
- [5] S. B. Ushijima, R. V. Gough, M.A. Tolbert, ACS Earth Sp. Chem. 4 (2020) 1947–1956.
- [6] Chem. Eng. News 54 (1976) 23–25.
- [7] F.J. Medina, R. Herranz, C. Arena, G. Aronne, V. De Micco, Gener. Appl. Extra-Terrestrial Environ. Earth (2015) 239– 254.
- [8] R. Rieder, T. Economou, H. Wänke, A. Turkevich, J. Crisp, J. Brückner, G. Dreibus, H.Y. McSween, Science 278 (1997) 1771–1774.
- [9] G. Hochmuth, SL353, One a Ser. Soil Water Sci. Dep. Florida Coop. Ext. Serv. Inst. Food Agric. Sci. Univ. Florida (2011)

1–8.

- [10] G.R. Rout, S. Sahoo, Rev. Agric. Sci. 3 (2015) 1–24.
- [11] S. Maurice, S.M. Clegg, R.C. Wiens, O. Gasnault, W. Rapin, O. Forni, A. Cousin, V. Sautter, N. Mangold, L. Le Deit, M. Nachon, R.B. Anderson, N.L. Lanza, C. Fabre, V. Payré, J. Lasue, P.Y. Meslin, R.J. Léveillé, B.L. Barraclough, P. Beck, S.C. Bender, G. Berger, J.C. Bridges, N.T. Bridges, G. Dromart, M.D. Dyar, R. Francis, J. Frydenvang, B. Gondet, B.L. Ehlmann, K.E. Herkenhoff, J.R. Johnson, Y. Langevin, M.B. Madsen, N. Melikechi, J.L. Lacour, S. Le Mouélic, E. Lewin, H.E. Newsom, A.M. Ollila, P. Pinet, S. Schröder, J.B. Sirven, R.L. Tokar, M.J. Toplis, C. D'Uston, D.T. Vaniman, A.R. Vasavada, J. Anal. At. Spectrom. 31 (2016) 863–889.
- [12] H. Eskandari, J. Appl. Environ. Biol. Sci. 1 (2011) 448-452.
- [13] E. A. Campos, D. V. B. S. Pinto, J. I. S. Oliveira, E. C. Mattos, R. C. Lazzarin, J. Aerosp. Technol. Manag. 7 (2015) 268-276.

Research Article Sensitive Detection Of Metal Ions On Different Platform – A Comparative Study

Ms. Shubhlaxmi K. Naik Assistant Professor in Chemistry DPM's Shree Mallikarjun & Shri Chetan Manju Desai College, Canacona – Goa

shubhlaxmi.naik@shreemallikarjuncollege.ac.in

Abstract: The major findings in the field of chemosensors for the detection of toxic pollutants in environment have been summarized and discussed in this research article. The major focus of current research efforts is on the development of chemosensors such as Isatin based organic compounds that can be employed for the detection of toxic pollutants such as Hg^{2+} , Cd^{2+} , Pb^{2+} ions using portable and inexpensive platforms.

Keywords: Chemosensor, Isatin, Mesoporous silica, LOD, Sensitivity, Range

INTRODUCTION

There is an increasing need for analysis of heavy metal contaminants in drinking water, ground water, industrial water etc. Toxic metals such as mercury, arsenic, lead and cadmium enter water supplies due to human activities such as landfills and plumbing which causes risks to human health even in trace amount. The detection and quantification of heavy metal ions are important in many applications, including environmental monitoring, waste management, developmental biology, and clinical toxicology. A number of techniques have been developed over the years for heavy metal ion analysis, including atomic absorption spectrometry, anodic stripping voltammetry, X-ray fluorescence spectrometry, and microprobes. These techniques in general require expensive equipment, sample pre-treatment, and/or analyte pre-concentration steps. Therefore, a simple, rapid, inexpensive, selective and sensitive method that permits real time detection of metal ions is still a challenging goal. In addition, due to the danger that the heavy metal ions pose to operators, minimal sample handling is desirable. [1]

Why There Is A Need For Field Portable Analytical Technique

Most modern heavy metal analysis systems for field analysis bulky and power- inefficient. The high cost and poor detection times are typically associated with the measurement of contaminants that clearly indicates there is a need for environmental screening and monitoring methods which are fast, east to use, portable and also cost-effective. To meet this need, a variety of field analytical methods are introduced which are commercially available or are under development.

Traditionally, water samples are collected and physically transported to central laboratories for analysis. The long time delays associated with this procedure are not acceptable, and hence on-line monitoring is required. In addition to this, the majority of samples which are negative are also subjected to costly laboratory analysis. A field-portable instrument for detection could save resources by employing costly analysis only on those samples which show positive results during the field analysis.

The need for analysis in the field is increasingly becoming more and more important. The ability to test a potentially contaminated drinking source at site is crucial. This was recognised in a 2007 report by the Royal Society of Chemistry in the UK regarding sustainable water - "National and International funding bodies should fund research programmes to develop portable field-testing kits for (heavy metals) that are quick, accurate, cheap and reliable to support remediation efforts."

Advantages Of Field Portable Analytical Technique

- The main advantage is that the results are available rapidly as the chemical analysis can be completed at the site of sampling rather than in laboratory.
- Other benefits include the elimination of sample storage with its associated problems and significant cost savings.
- The most useful applications of on-site analysis are in environmental monitoring and process analysis.

Criteria for selecting an Analytical technique: A method is the application of a technique to a specific analyte in a specific matrix. The requirements of the analysis determine the best method. In choosing a method, consideration is given to some or all the design criteria such as accuracy, precision, sensitivity, specificity and selectivity, robustness and ruggedness, scale of operation, sample throughput, equipment, time and cost.

Moving Your Laboratories To The Field - Advantages And Limitations Of The Use Of Field Portable Instruments In Sample Analysis

The recent rapid progress in technology of field portable instruments has increased their applications in environmental sample analysis. These instruments offer a possibility of cost-effective, non-destructive, real-time, direct, on-site measurements of a wide range of both inorganic and organic analytes in gaseous, liquid and solid samples. Some of them do not require the use of reagents and do not produce any analytical waste. All these features contribute to the greenness of field portable techniques. Several stationary analytical instruments have their portable versions. The most popular ones include: gas chromatographs with different detectors (mass spectrometer (MS), flame ionization detector, photo-ionization detector), ultraviolet–visible and near-infrared spectrophotometers, X-ray fluorescence spectrometers, ion mobility spectrometers, electronic noses and electronic tongues. The use of portable instruments in environmental sample analysis gives a possibility of on-site screening and a subsequent selection of samples for routine laboratory analyses. They are also very useful in situations that require an emergency response and for process monitoring applications.

Limitations:

- i. Quantification of results is problematic in many cases.
- ii. The other disadvantages include: higher detection limits and lower sensitivity than these obtained in laboratory conditions,
- iii. a strong influence of environmental factors on the instrument performance and a high possibility of sample contamination in the field. [2]

Field Analysis Kits

Field analysis kits are designed to get a fast, accurate, test result with a small kit. Some of the commercially available kits for field analysis are given below: [6,7]

Sr.	Name of the	Brand name	Metals	Number of	Detection	Results	Price
no.	kit		detected	samples	level	time	
				analysed			
	Water	Brand: ITS	Cd ²⁺ ,Co ²⁺ ,	50 strips	< 10,20, 50,		
1.	Metals Test	SKU: IT-TK-03	Cu ²⁺ , Fe ²⁺ ,		100, 200,	1 minute	\$ 19.99
	kit		Pb ²⁺ , Hg ²⁺ ,		400,		
			Zn^{2+}		1000ppb		
	Hg Drinking	Brand: ITS		50 test	0, 2, 5, 10,		
2.	Water Test	SKU: IT-TK-9	Hg^{2+}	strips	20, 40,	90 seconds	\$ 20.99
	Kit (Boris)				80ppb		
	Copper	Brand: ITS		Bottle of 50	0, 0.05, 0.1,		
3.	Water Test	SKU:IT-TK-29	Cu ²⁺	strips	0.2, 0.4,	Under 1	\$ 14.99
	Kits, John's				1.2ppm	minute	
	Arsenic	Brand: ITS		5 patented			
4.	Quick Test	SKU:IT-TK-04-	Arsenic	test kits	5ppb	12	\$ 27.49
	Mini-Kit	5				minutes	
	Mercury	Brand: ITS		50 test	< 50,100,	3 minutes	
5.	Check	SKU: IT-TK-05	Hg^{2+}	strips	200, 500,		\$ 20.99
	Water Test				1000pb		
	Kit						

 Table 1: Commercially available kits for field analysis

Heavy metals

Heavy metals are naturally occurring elements which have high density and atomic weight than that of water. Their multiple medical, agricultural, domestic, industrial and technological applications have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment. Their toxicity depends on several factors which include its dosage, route of exposure and the chemical species involved, as well as the age, gender, and nutritional status of exposed individuals.

Because of their high degree of toxicity mercury, lead, arsenic, and cadmium rank among the metals that are of public health significance. These metallic elements are considered systematic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Hence certain guidelines are given for the permissible limit of heavy metals in aqueous medium. [3]

METAL	WORLD HEALTH		
	ORGANIZATION (mg/L)		
Cu	2.000		
Hg	0.002		
Рb	0.010		
Cd	0.003		

Table 2: Standards and guidelines for heavy metals in drinking water

Chemosensors For Selective And Sensitive Determination Of Heavy Metals

- Chemosensors offers a low cost and accurate method with a high selectivity and method for detection of heavy metal ions. Due to which they play a very crucial role in analytical chemistry, environmental chemistry and bio-medicinal science.
- In the host guest chemistry the term chemosensor is more closely related with a molecular event. Sensor is a system that on stimulation by any form of energy undergoes change in its own state and thus one or more of its characteristics. This change is used to analyse the stimulants both qualitatively and quantitatively. These receptor molecules exihibit selective response to specific ions or neutral species to be used as chemosensors.
- Chemical sensors are miniaturized devices that can deliver real time and online information on the presence of specific compounds or ions in even complex samples. Chemical sensors employ specific transduction techniques to yield analyte information. The most widely used techniques employed in chemical sensors are optical absorption, luminescence, redox potential etc. but sensors based on other spectroscopies as well as on optical parameters, such as refractive index and reflectivity, have been also developed.
- Design of the chemosensors consists of three components such as a chemical receptor capable of recognizing the guest of interest usually with high selectivity; a transducer or signalling unit which converts that binding event into a measurable physical change and finally a method of measuring this change and converting it to useful information.

- Chromogenic reagent Isatin and its derivative attracted our attention due to their analytical significance. Isatin based colorimetric sensors have been known for visual detection of heavy metal ions in an aqueous medium. These colorimetric sensors are Schiff base derivative which involve more than one synthetic procedures. Schiff bases has following properties: are able to stabilize many different metal in various oxidative states, relatively easy to prepare, synthetic flexibility and special property of C=N group, Schiff bases are considered as an excellent chelating agents and are stable.
- Hence design and synthesis of an inexpensive and disposable sensor such as disposable sensor strips are highly desired. These chromogenic reagent display significant colour changes in the presence of particular ion solution. These show high selectivity for a particular ion in the presence of other ions of similar nature. The design and synthesis of sensors that can detect small concentrations of the ion of interest in the presence of other interfering ions is of considerable interest. [8]

Paper Based Sensor

Currently, there is a particular need for simple and fast field analytical tests. One of the most modern trends is the development of paper based analytical devices, such as the lateral flow strips made of cellulose. Paper is light, flexible, inexpensive, biodegradable, and is made from abundantly available materials such as plants and non-pathogenic bacteria found on fruits. As a result, it is a useful substrate for many applications. Paper offers several advantages, with its very low cost being the most highlighted benefit in literature. In fact, this naturally abundant and renewable material can be machined using similar methods to those traditionally employed. Another asset relates to the facility on discarding this eco-friendly material by incineration, thereby avoiding hazardous waste. Because fluids can move throughout the porous substrate by capillary action, there is no need for pumping systems and only small sample volumes are required. Moreover, patterning paper with hydrophilic channels delimited by hydrophobic wall allows samples to be dispensed into several spatially separated zones, which enables running multiple assays simultaneously, on a single device. All these characteristics are highly valuable for paper based sensor not only for resource-limited settings but also for applications in developing regions, opening up world of new applications.

Paper-sensing platforms have currently been utilised in other configurations, mainly dipstick tests and micro fluidic paperbased analytical devices (µPADs). [4,10]

Mesoporous Silica As A Supporting Medium For Metal Ion Sensor

Mesoporous material has pore size ranging between 2-50nm. Microporous material have lower pore size compared to mesoporous material i.e. < 2nm.

MCM-41:

- Mobil Composition Of Matter No. 41 is a mesoporous material with a hierarchical structure from a family of silicate and aluminasilicate that can be used as catalysts or catalyst supports.
- Synthesis: To achieve a defined pore diameter surfactants are used that form micelles in the synthesis solution. These micelles form templates that help build up the mesoporous framework. For MCM-41 mostly cetyltrimethylammonium bromide (CTAB) is used.

- The surfactant first forms rod-like micelles that subsequently align into hexagonal arrays. After adding silica species these cover the rods. Later, calcination leads to condensation of the silanol groups so that the silicon atoms are bridged by oxygen atoms. The organic template is oxidized and disappears.
- Surface modification: By modification the adsorption property can be significantly affected. This chemisorptions of chelating molecules (ligand) onto the modified mesoporous material provides immobility, mechanical stability, and water in solubility as a result increasing efficiency, sensitivity of analytical applications.

APTMS Functionalised Surface Modification

Among various organotrialkyloxysilane molecules for the modification of silica surface, (triethoxysilyl) propan-1-amine (APTMS) is widely explored by researchers. The amino functional groups are known to enhance the dispersibility and miscibility of silica fibres, and to be used as a linkage unit to attach other functional molecules.

A reaction was suggested as the interaction between amino group of APTMS and surface of Si-OH group in anhydrous condition, it was known that APTMS is readily soluble in water giving solution of unlimited stability at its natural pH in which the normal organotrialkyloxysilanes cause rapid condensation of Si-OH group to form insoluble gels, and internal hydrogen bonding suggested to explain its lack of activity. [5]

Objectives of the study

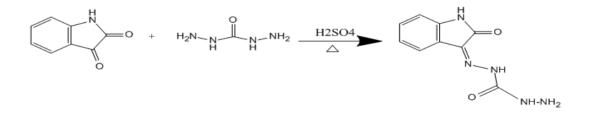
- To develop highly sensitive and selective (low ppb ranges) simple analytical detection kit for field analysis.
- The developed kit should be able to screen heavy metals in water with high sample throughput.
- Operations of kit should be user friendly.

METHODS

Synthesis Of Chemical Sensor For Quantitative Analysis Of Metal Ions In Aqueous Solution

I. Synthesis of Isatin-3-Carbohydrazone

Igm of Isatin (1) was dissolved in minimum quantity of ethanol and was kept on hot plate till clear solution was obtained. To this hot solution 0.6123gm of carbohyrazide (2) was added and stirred till it was completely dissolved. Further 10mL of 0.1N sulphuric acid was added to the above solution ad this solution was then kept on a hotplate for 30 minutes. The reaction mixture was then cooled, filtered and the product obtained was washed with water. The product Isatin-3-carbohydrazone (3) was recrystallized using ethanol with 76% of yield. [11]



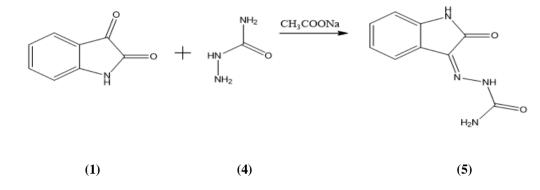
(1)

(2)

(3)

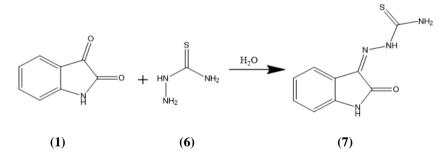
II. SYNTHESIS OF ISATIN-3-SEMICARBAZONE

A mixture of semicarbazide hydrochloride (0.1 mol, 1.0000g) (4) and sodium acetate (0.15mol, 1.830g) was dissolved in 100mL of water. To this isatin (0.1mol, 1.319g) (1) dissolved in 150mL of methanol was added and the whole mixture was stirred for 10-15minutes. Further the solution was warmed on a water bath to dissolve the contents and then cooled in ice, yellow precipitate of isatin-3-semicarbazone (5) was obtained. The precipitate was then filtered, dried and recrystallized with methanol with percentage yield of 60%.



III. SYNTHESIS OF ISATIN-3-THIOSEMICARBAZONE

1g of isatin (1) was dissolved in minimum amount of methanol and was kept on hotplate till a clear solution was obtained. To this hot isatin solution 0.6199g thiosemicarbazide (6) was added and stirred till it was completely dissolved. Further 100mL of distilled water was added to the above solution and it was kept on a hotplate for 30 minutes. The reaction mixture was then cooled, filtered and the product obtained was washed with water. The product Isatin-3-thiosemicarbazone (7) was recrystallized using methanol with percent yield of 78%. [11]



Binding Properties Of All Three Ligands With Mercuric Chloride Solution

Colorimetric sensing ability of the all receptors was investigated using 1mM solution of Isatin-3carbohydrazone in ethanol, Isatin-3-semicarbazone and Isatin-3-thiosemicarbazone in methanol with equivalent amount of mercuric chloride solution. Upon mixing receptor and metal ion in equivalent proportion, Isatin-3-carbohydrazone showed distinct colour change from yellow to red, whereas Isatin-3-semicarbazone did not show any colour change and little colour change was observed for Isatin-3-thiosemicarbazone.

Based on this observations Isatin-3-carbohydrazone was taken for further detection of mercury in water samples.

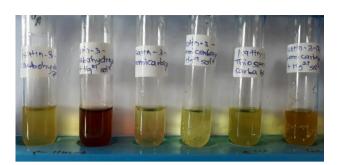


Figure 1: 1mM of each ligand with 1mM of Isatin-3-carbohydrazone, Isatin-3-semicarbazone and Isatin-3-thiosemicarbazone respectively.

Screening Of Various Receptors

Binding properties of all the receptors with mercury solution was investigated with UV-Visible absorption. Greater intensity of colour change was observed for Isatin-3carbohydrazone.

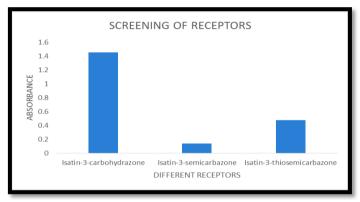


Figure 2: Showing absorption pattern for different receptors with 1mM of mercury solution.

Optimization Of Metal Ion And Ligand Concentration

Scope of visual detection of mercuric chloride solution with receptor i.e. Isatin-3-carbohydrazone was further screened for different concentration of metal ions. Corresponding to 0.25mM of receptor, metal ion concentrations such as 1mM to 0.0625mM were considered. When different concentrations of metal ion were added to 0.25mM of receptor, detectable colour change was observed up to 0.15mM of metal ion. Increase in absorbance was observed due to complete complexation of metal and ligand.

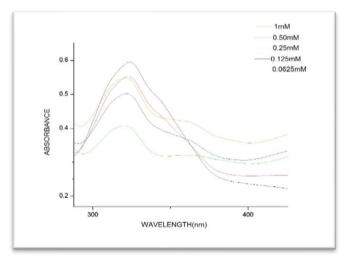


Figure 3: screening of different concentration of Hg (II) corresponding to 0.25mM of receptor

Further optical studies were performed by varying the

concentration of receptor (1mM to 0.0625mM) with 0.25mM of mercury solution. When different concentrations of receptor was added to 0.25mM of metal ion solution, detectable colour change was observed corresponding to 0.10mM of receptor, signifying the lower detection limit of receptor for detection of metal ion.

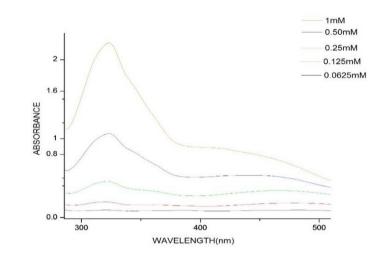


Figure 4: Screening of different concentration receptor corresponding to 0.25mM of Hg(II)

Jobs Plot For Studying The Binding Event Of Metal : Receptor

Spectroscopy is a valuable tool for determining the composition of complex ions in solution and for determining the composition of complex ions in solution and for determining their formation constants. There are various techniques for the complex-ion studies, one of them is the continuous variation method (Jobs Plot).

In the method of continuous variations, cation and ligand solutions with identical analytical concentrations are mixed in such a way that the total volume and the total moles of reactants in each mixture are constant but the mole ratio of reactants varies systematically (for example 9:1, 8:2, 7:3 and so forth). The absorbance of each solution is then measured at a suitable wavelength and corrected for any absorbance the mixture might exhibit if no reaction had occurred. The corrected absorbance is plotted against the volume fraction of one reactant, i.e., $V_M/(V_M + V_L)$, where V_M is the volume of cation solution and V_L volume of the ligand solution. A maximum occurs at a volume ratio V_M/V_L , corresponding to the combining ratio of metal ion and ligand in the complex.

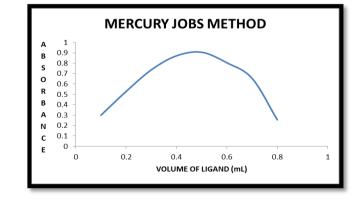


Figure 5: Jobs plot for mercury

The stoichiometric ratio of metal:ligand in mercury was found to be 1:1. For this stoichiometric ratio it may be proposed that for the binding ratio of 1:1, there are two binding sites for the receptor i.e. NH of isatin and the free NH_2 thus the metal binds to both NH of isatin and NH_2 of same ligand in the ratio 1:1.

Recovery Experiments

The recovery test was used to test the validation of the proposed method for mercury detection in real water samples. For this study different water samples like distilled water, tap water, purifier water, sea water and water from washroom tap was taken for spiking studies. In 10mL standard volumetric flask 1mL of ligand and mercury solution of 1mM was added and this solution was diluted with 8mL of distilled water. Similar procedure was followed for other water samples. Absorbance of all the solutions was measured and it's percent recovery was calculated by the above equation. As can be seen in the table, the recoveries of mercury in all real water samples ranged from %, indicating the reliability and practicality of method.

Sample	Spike	Found	Recovery (%)
	(mM)	(mM)	
Distilled water	1.0000	0.998	99.8%
Tap water	1.0000	0.90	90%
Sea water	1.0000	0.82	82%
Washroom tap	1.0000	0.89	89%
water			
Purifier water	1.0000	0.99	99%

Table 3: Results of Hg(II) Recovery Experiments Performed in real water samples.



Interference Studies For Developed Sensor

Figure 6: Screening of different metal ions for checking the response of sensor for other metal ions.

For checking interference of other metal ions during analysis with sensor response, series of solution of different metal ions of 1mM was taken and to it equivalent concentration i.e. 1mM of ligand solution was added. This test gave positive response only for lead along with mercury. From this we can infer that lead may interfere during analysis of mercury. Also it can be seen (from figure above) that this sensor can also be used for detection of lead as it shows different intensities of colour for both lead and mercury.

Analytical Features

SR.	PARAMETERS	EXPERIMENTAL
NO.		OBSERVATIONS
1.	Selectivity towards	Hg(II) >> Pb(II),
	metal ions	Co(II)
2.	Linear range	1mM to 0.0625mM
3.	LOD	0.10mM

Table 4: Showing features of the developed sensor

Preliminary studies for synthesizing novel platform for sensor attachment and optimizing sensor performance immobilized on novel platform

Mesoporous material as sensor platform: MCM-41

A] Synthesis of support material: MCM-41 and optimization of sensor performance on it.

Procedure: 1.922g of CTAB was dissolved in 89.2g (i.e. 89.2mL) of distilled water with 4.8g NH₃ (5.33mL NH₃) solution approximately 30%. This mixture was then stirred until clear solution was obtained and to this 6.9g i.e.7.39mL of tetraethylorthosilicate (TEOS) was added and stirred for 4hrs on magnetic stirrer. Once the 4hours stirring was over it was kept for aging for 24hours, the product was filtered and washed with 150mL of distilled water and kept in oven for 24hrs at 100^oC. The dried residue was then calcinated at 550^oC for 3 hours. This synthesized MCM-41 was characterized by IR spectroscopy. [9]

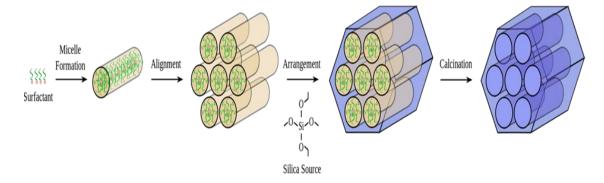


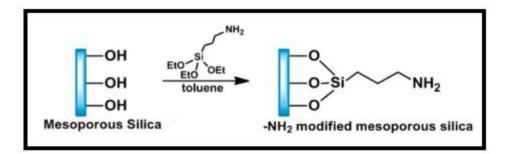
Figure 7: Diagrammatic representation of synthesis of MCM-41

FICTIONALISATION OF MCM-41:

APTMS Functionalisation

Procedure: 2g of mesoporous silica was weighed and taken in a 250mL round bottom flask. To this, 50mL of dry toluene along with 2mL of APTMS was added and was stirred and refluxed at 110°C for 12 hours. After 12 hours, it was filtered and washed with isopropyl alcohol. It was then characterized by IR.

Scheme:



HCI-APTMS-MCM-41 Functionalisation

Procedure: 1g of APTMS functionalised mesoporous silica was weighed and taken in a 100mL beaker. To it 50mL of 0.1N HCl was added and was stirred for 2 hours using a magnetic stirrer. After 12 hours, it was filtered and washed with distilled water and characterized by IR.

Attachment of ligand onto the acid functionalised mesoporous silica surface

Procedure: 0.5g of acid functionalised mesoporous silica was taken in a small beaker. To it, 15mL of 4mM Isatin-3-carbohydrazone was added. This mixture with the help of a magnetic stirrer was agitated for about 12 hours in order to immobilise the ligand onto the acid functionalised mesoporous silica supporting material. It was then filtered after 24 hours and was washed. After drying, it was characterized by IR spectroscopy.

Working with the sensor immobilised on silica

5mg of sensor immobilised acid functionalised mesoporous silica (sensor) was used each time for analysis in small vials. Here 5mg of sensor immobilised on silica was taken in a vial and to it 2mM of Hg(II) solution was added which gave following colour change.



(a)



(b)

Figure 8: representing the change in colour of the sensor (a) in absence of metal ion, (b) in presence of metal ion.

Determining Range and LOD of sensor immobilised on silica

Series of solution in the concentration range from 4mM to 0.25mM were tested for sensor response. It was observed that with naked eyes, sensor give distinct response up to 0.25mM concentration of solution. The experiment was repeated three times and based on the observations the range and LOD was optimized.

Optimized range: 4mM to 0.25mM

LOD: 0.25mM



Figure 9: representing detectable change up to 0.250mM of solution from right to left

Reusability of the sensor

Once the sensor immobilised on acid functionalized mesoporous silica support acted a good sensor for above metal ion used for sensing, then the next task was to check its efficiency for reusability i.e. up to how much cycles of Adsorption-Desorption with EDTA can it be successfully used.

Working: In a clean and dry vial, 5mg of the sensor material was taken. To this vial, 250µL of 2mM Hg(II) solution was added. It was observed that the colour of the silica particles changed from yellow to reddish orange in colour, the appearance of this colour is due to Adsorption of metal ion onto the silica surface.

Now so as to determine the reusability of this sensor, it was given three time washing with distilled water so as to check the disappearance of the red colour of the silica particle but it was observed that the colour did not change. To desorb the metal ion from the sensor, 250µL of EDTA of 10mM was added to the coloured silica particle, it was observed that on giving washings of EDTA three times, red coloured silica particle regained its initial colour i.e. yellow.



Figure 10: showing the adsorption (left vial) and desorption of metal ion (right vial) from the sensor.

The total number of Adsorption-Desorption cycle of sensor and metal ion was successful up to 5 times after which the sensor did not produce a change in colour of the silica particle.

Inference: Since the sensor can work for 5 Adsorption-Desorption cycle therefore the reusability of this sensor is 5 times usable.

SR.NO.	PARAMETERS	EXPERIMENTAL OBSERVATIONS	
1.	Range	0.25mM to 4mM	
2.	LOD	0.25Mm	
3.	Sample volume	250µL	
4.	Response time	In seconds	
5.	Cost per analysis	Considering the amount of chemicals and their cost and	
		reusability less than 10 rupees per sample.	

Analytical features:

Table 5: Analytical features of the developed sensor

PAPER SUPPORTED SENSOR AND OPTIMIZATION OF ITS PERFORMANCE

Protocol: Whatmann filter paper was used for sensor support which was cut into desired shape and size. On each paper strip, 50μ L of 0.1M sensor was spotted as a round spot using a micropipette. After complete drying, the strip was immersed in Hg(II) solution which showed a colour change.

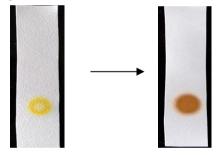


Figure 11: Representing the change in the colour of the sensor (a) in absence of metal ion, (b) in presence of metal ion.

Determing Range and LOD of paper strip

For determining the range and LOD of paper strip series of solution of Hg(II) solution was taken ranging from 1ppb to 5ppb which showed following observations.

Range: 1ppb to 5ppb

LOD: 1ppb

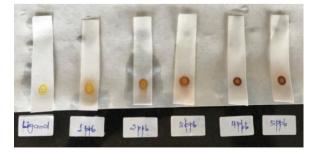


Figure 12: Representing ligand colour change before and after dipping ligand in Hg(II) solution ranging from 1ppb to 5ppb metal.

Quantitative estimation of paper strip using Imagej software

Paper strips were air dried and then was scanned on Epson scanner with 1200 resolution. For quantification Imagej software was used. First imagej.exe was runned and then in analyze option measurements were set by clicking mean gray value which gives the intensity of the colour. After that scanned image of 1ppb was opened, area of the spot was marked in rectangular shape and the intensity was measured. This gave the corresponding intensity of the colour. Same procedure was done for other strips. From the values calibration curve of intensity of each spot against concentration was plotted.

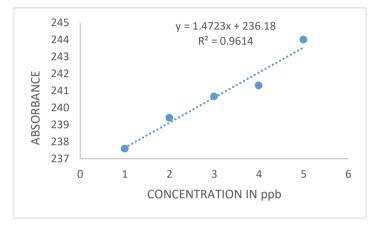
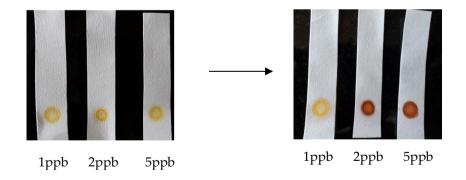
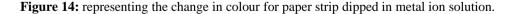


Figure 13: Graph of intensity v/s concentration in ppb

Checking the sensitivity of paper strip on water sample containing Hg(II) below its permissible limit

For this test paper strips were spotted with 0.1M sensor, dried and were kept in 20mL of 1ppb, 2ppb and 5ppb solution of Hg(II) solution. After some time colour change was observed from yellow to reddish orange colour.





Stability of sensor on paper strip

For determining the stability of sensor on paper strip, several strips of paper sensors were made. Some strips were stored in freeze and some at room temperature. This strips were then analysed for 15 days which showed same results in both cases which are seen in the following pictures. From this we can conclude that stability of sensor under room temperature and its storage is not an issue.

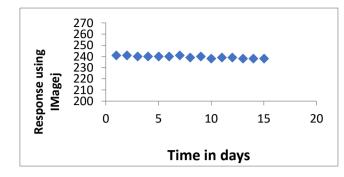
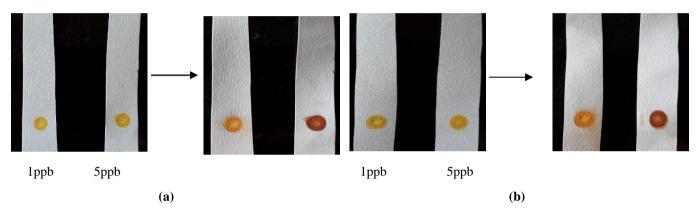
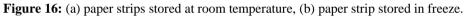


Figure 15: Calibration curve for response of paper strips for 15 days using imagej software

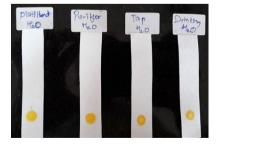


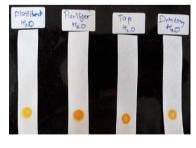


Inference: since paper strip stored at room temperature and freeze gave the same response we can conclude that stability and storage of paper strip is not an issue.

Testing strips on real samples

20mL of real water samples of distilled water, purifier water, tap water and drinking water was taken in a beaker and from each beaker $200\mu L$ of water was removed using micropipette. Then to the remaining samples $200\mu L$ of metal ion solution of mercury was added and paper strips were tested. It gave following response.





(b)

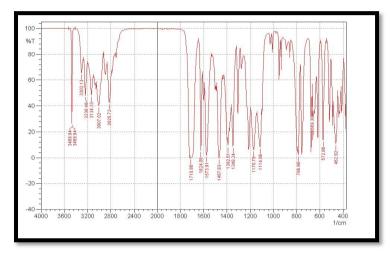
(a)

Figure 17: representing (a) paper strip before dipping in water samples (b) paper strip after dipping in water samples.

RESULTS

Characterization of Sensors

IR Spectra Of Isatin-3-carbohydrazone

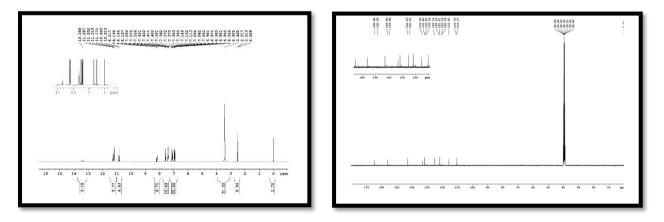


Interpretation:

FTIR (KBr) cm⁻¹: 3469.94 = 2 NH stretching, 3302.13 = Secondary amine, 3007.02 = C-H aromatic, 1710 = C=O stretching,

1624 = C=N, 750-1100 = C-C aromatic.

NMR Spectrum Of Isatin-3-carbohydrazone

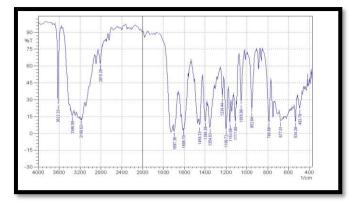


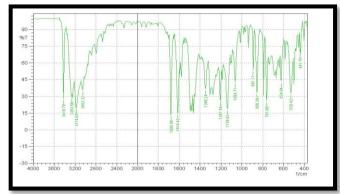
PMR spectrum

¹³C-NMR spectrum

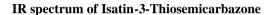
Interpretation of PMR Spectrum: chemical shift in ppm with respect to TMS: NH (hydride) = 7.082ppm, NH (urea) = 6.905ppm, NH₂ (amine) = 2.513ppm, NH (secondary amide) = 8.217ppm, CH (benzylidenimin) = 7.596ppm.

Interpretation of ¹³C-NMR spectrum: chemical shift in ppm with respect to TMS: 1-amide carbon = 179.84ppm, N-urea carbon =1.62.59ppm, 1-benzene carbon =142.30ppm, amine(C) carbon =132.00ppm, 1-benzene (CH) carbon -131.21ppm, 1-benzene (CH) carbon = 122.32, 120.91, 119.92ppm.





IR spectrum of Isatin-3-Semicarbazone



Interpretation of Isatin-3-semicarbazone: FTIR (KBr, cm⁻¹): N-H = 3550-3400cm⁻¹, CONH₂ = 1730cm⁻¹, C=O = 1710cm⁻¹, amide C=O = 1690cm⁻¹, C=N = 1680cm⁻¹.

Interpretation of Isatin-3-thiosemicarbazone: FTIR (KBr) cm⁻¹: 2 NH stretching = 3469.94cm⁻¹, sec. amine = 3302.13cm⁻¹, C-H aromatic = 3007.02cm⁻¹, C=O stretching = 1710cm⁻¹, C=N = 1624cm⁻¹, C-C aromatic = 750-1100cm⁻¹.

Comparative study of sensor used as Neat material (in aqueous medium), Immobilised on paper based and Immobilised on mesoporous material

PARAMETER	SENSOR IN	DISPOSABLE SENSOR	REVERSIBLE SENSOR
	AQUEOUS	ON PAPER STRIP	ON SILICA SUPPORT
	MATERIAL		
Range	1mM to 0.0625Mm	1ppb to 5ppb	4mM to 0.25mM
LOD	0.10Mm	1ppb	0.25mM
Sample volume	1mL	20mL	250µL
Sensor volume/ quantity	1mL	0.1M	5mg of silica
Need sophisticated	Not needed	Not needed	Not needed
instrument			
Cost involved	Less than 10 rupees	Less than 5 rupees	Less than 10 rupees
Reusability	Cannot be used	Cannot be used	Can be used five times

Table 6: Showing comparison between three platforms used for sensors

CONCLUSION

In this work three ligands have been compared for detection of mercury metal ions which were characterized by IR. Out of these three Isatin-3-carbohydrazone gave greater response in terms of colour intensity for mercury so it was chosen for further studies. Further three different platforms for sensing metal ion using the said ligand was compared. Also all the three different platforms for sensing metal ion using the said ligand was characterised by ¹H NMR and ¹³C NMR.

When the sensor was used in liquid phase it gave response to mercury and lead. Among this it showed greater response to mercury. When sensing ligand was immobilised on cellulose paper as disposable sensor, it showed more response to mercury i.e. distinct colour change w.r.t. ligand colour. Their quantification was done by usinf imagej software. This strips were checked for their stability and storage which showed that it is stable at both room temperature and freeze temperature. This run was carried outt for 15 days. Also they were used to detect mercury below and above its permissible limit in drinking water which gave good response for it showing the sensitivity of sensor.

When mesoporous material with surface modification was used for immobilization gave good response. The main advantage of this mesoporous material with surface modified sensor is that it can be used on a reversible basis up to 5 times adsorption-desorption cycles using EDTA as a desorbing agent for metal ions.

REFERENCES

- E R I C A S . F O R Z A N I, H. A. (2016). Detection of Heavy Metal Ions in Drinking Water Using a High-Resolution Differential Surface Plasmon Resonance Sensor, pg:593, DOI: <u>https://doi.org/10.1021/es049234z</u>
- 2. Agnieszka Gałuszkaa, Moving your laboratories to the filed- Advantages and limitations of the use of filed portable instruments in environmental sample analysis.
- 3. V. Vongelegt et al. / optical sensors for Determination Of Heavy Metal Ions. (2012).
- 4. LF. Captain-Valley et al. / Irreversible test strip for mercury determination based on neutral ionophore, AnalyticachimicaActa 524 (2004), DOI: <u>https://doi.org/10.1155/2018/3594020</u>
- J. Hak- sung et al./ Quantitative Analysis and Efficient Surface Modification of Silica Nanoparticles, Journal of Nanoparticle's, article ID 59347, (2012), DOI: <u>https://doi.org/10.1155/2012/593471</u>
- JOYTIDAS, P. J. Low-cost field test kits for arsenic detection in water, DOI: https://doi.org/10.1080/10934529.2013.824764
- 7. Richard J. Reay, A. F. (Accepted 13 May 1996). Microfabricated electrochemical analysis system for heavy metal detection, pg:450.
- 8. Rogers, K. R. (n.d.). Biosesnors for environmental applications, pg;533.
- 9. P.K. jalet al. / Chemical modification of silica surface by immobilization of functional groups for extractive concentration of metal ion, / Talanta (2004), pg:1004-1028
- L. Liu et al. / Paper based colorimetric array test strip for selective and semiquantitative multi-ion analysis, (2014), pg: 8829-8843, DOI: <u>https://doi.org/10.1021/ac5021886</u>
- 11. Kiran Gangarapu, A.J. (n.d.). Synthesis of thiocarbohydrazide and carbohydrazide derivatives as possible biologically active agents.

Research Article

Study of Chalcone

Ms. Divya R. Sawant Assistant Professor in Chemistry Dnyan Prabhodhini Mandal's Shri Mallikarjun and Shri. Chetan Manju Desai College, Canacona-Goa divya.sawantr@shreemallikarjuncollege.ac.in

Abstract: Chalcones which are also known as α,β -unsaturated ketones is an important class of organic compounds and reported to possess a wide spectrum of biological activities such as antibacterial, antifungal, anticancer, anti-inflammatory etc. Chalcones are present abundantly in nature, from ferns to higher plants. Many chalcones were been reported during 1960's and 70's which were isolated from various parts of plants, buds, leaves, blossoms, heart wood, roots, seeds, flowers, and inflorescence. It can exist in free and combined states either in the form of Chalcone or glycosides. Chalcone being unsaturated ketone it contains the ketoethyenic group -C0- CH=CH. Due to the presence of chromophore -C0-CH=CH , these compounds are coloured. Oxazine, Thiazines and Isoxazole derivatives can be prepared from Chalcone as starting materials.

Keywords: Chalcone, Heterocycles,

Introduction

The history of chemotherapeutic agents has been dynamic characterised by emergence of constant new challenges followed by investigations, production of new drug, discovery, though most of these are already known. Most of the scientific chemotherapeutic agents knows that the heterocyclic compounds were used as clinical agents. Chalcone can exist in free and combined states either in the form of Chalcone or glycosides. These compounds carry many different substituents like isopentyl, methyl, methoxy and hydroxyl, which is present on either ring A or ring B of Chalcone molecule. Many of the higher plants contains dihydrochalcones. Chemically Chalcone consists of open chain flavonoids in which a three carbon α - β - unsaturated carbonyl system joins the two aromatic rings. Antimicrobial activity in the Chalcone is due to the presence of reactive unsaturated keto function.

Activities such as anti-bacterial, antinuclear, antifungal, vasodilatory, antioxidant, antimalarial, antimitotic inhibition of tyrosinase and many more are found to be possessed in a number of chalcones having hydroxy, alkoxy groups in different positions.

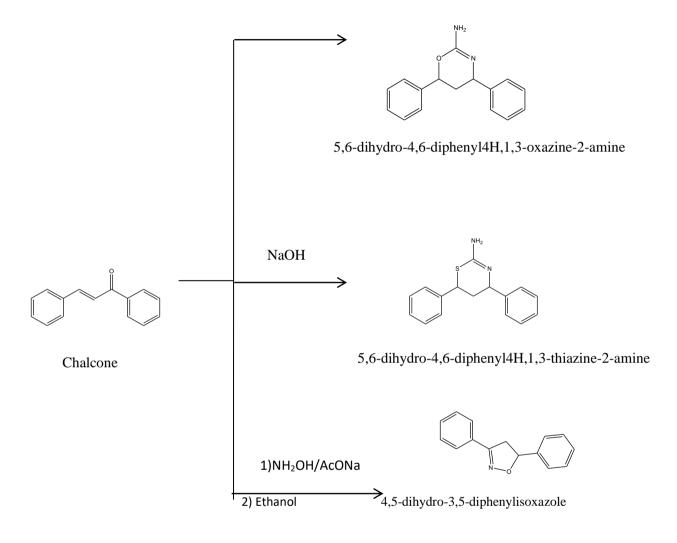
Chalcone being unsaturated ketone it contains the ketoethyenic group -C0- CH=CH. Due to the presence of chromophore -C0-CH=CH, these compounds are coloured. Derivative like cyanopyridines, pyrazolines isoxazoles and pyrimidines which have different heterocyclic ring system are synthesized using chalcones.

OBJECTIVES

- 1) To prepare Chalcone by Claisen-schmidt condensation reaction.
- 2) To prepare further derivatives like Isoxazole, Thiazine and Oxazine from Chalcone.

SCHEMATIC STRATEGY

Chalcone was synthesised by two methods in the first method it was synthesised by green methods using Acetophenone, Ba(OH)₂ ,Concentrated HCl, Ethanol and CH_2Cl_2 while second method is conventional method wherein The Claisen-schmidt condensation reaction is used to prepare Chalcone, it is a coupling reaction between aldehyde and ketone that allows the preparation of chalcone in presence of aqueous alcoholic alkali like NaOH. Further the chalcone prepared was used as a starting materials to prepare is derivatives like Isoxazole, Thiazine and Oxazine, following systematic strategy is used in preparing further derivatives.



Experimental procedure

1.Synthesis of Chalcone

1.1.GREEN METHOD :

Chemical Required :

- Acetophenone (4mmol) (0.48gm)
- Benzaldehyde (4.1mmol) (0.4350gm)
- $Ba(OH)_2$ (2g)
- Concentrated HCl
- Ethanol
- CH₂Cl₂

In a mortar and pestle, mixture of acetophenone (4mmol) (0.48g) and benzaldehyde (4.1mmol) (0.4350g) and Ba(OH)₂ (2g) was taken. It was grinded well at room temperature for 5 minutes. After allowing the reaction to stand for 10 minutes 30ml ice cold water was added to it and was acidified with concentrated HCl. It was then filtered and the product was collected from ethanol. The mixture was extracted with CH_2Cl_2 when orange oil was formed. When the extract was dried (Na2SO4) and solvent was evaporated, to gave chalcone as solid. The % yield was calculated and the melting point was reported.

1.2. CONVENTIONAL METHOD :

Chemical Required :

- Acetophenone (0.033mmol) (3.96g)
- Benzaldehyde (0.033mmol) (3.493g)
- Ethanol (25mL)
- 10% NaOH (25mL)

(3.493g) (0.033mmol) of Benzaldehyde and (3.96g) (0.033mmol) of acetophenone was dissolved in 25mL ethanol. 25 mL of 10 % NaOH was added to it. The mixture was stirred for 4 hours, and then approximately 400mL of distilled water was poured with constant stirring. It was than kept in refrigerator an overnight. The precipitate obtained was filtered, washed and recrystallised from ethanol. The % yield was calculated and melting point was reported.

2. Synthesis of 5,6-dihydro-4,6-diphenyl-4H-1,3-oxazine-2-amine

A clean healer was taken and exactly 2.08g of chalcone and 0.76g of urea was weighed. After weighing, NaOH of 0.4g was added in 5mL ethanol. It was stirred continuously for 3 hours. After 3 hours, 200mL of ice cold distilled water was poured. it was again stirred for an hour. It was than kept in refrigerator for an overnight. The compound was dried and filtered and its quantity was weighed. It was recrystalized with alcohol and its melting point was found.

3.Synthesis of 5,6-dihydro-4,6-diphenyl-4H-1,3-thiazine-2-amine

A clean beaker was taken and exactly 2.08g of chalcone and 0.76g of thiourea was weighed. After weighing NaOH of 0.4g was added in 5mL ethanol. It was than stirred continuously for 3 hours After 3 hours pour in 200mL of ice cold distilled water. It was again stirred for a hour. Then it was kept in refrigerator for an overnight. The compound was filtered and dried and its quantity was weighed. It was recrystalized with alcohol and its melting point was found.

4.Synthesis of 4,5-dihydro-3,5-diphenylisoxazole

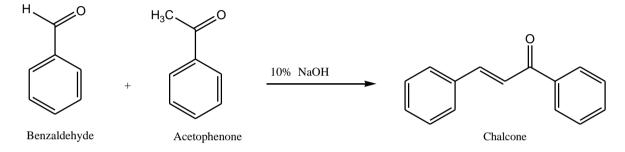
A clean beaker was taken and exactly (2.08gm - 0.01 mol) of Chalcone was weighed. Next Hydroxylamine hydrochloride (0.695gm - 0.01 mol) was weighed. (0.81gm) of Sodium Acetate was accurately weighed and was then dissolved in 12.5 ml of ethanol. All

three compounds were mixed in a beaker and was stirred well using a glass rod. This solution was kept to reflux for about 6 hours. After 6 hours the solution was removed, and ed into 25ml ice cold distilled water, the solution was mixed well. The solution was filtered and the residue was weighed. It was than recrystallized with alcohol and its melting point was found.

Reaction and mechaisium

1.Chalcone

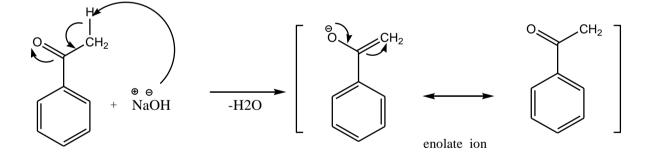
The Claisen-schmidt condensation is a coupling reaction between aldehyde and ketone that allows the preparation of chalcone in presence of aqueous alcoholic alkali like NaOH.



MECHANISMS :

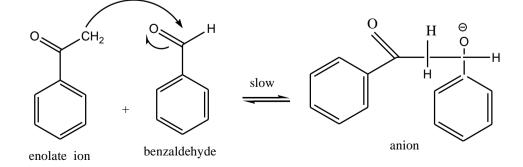
Step 1 : Formation of enolate ion

The base removes proton from acetophenone to form an enolate ion.



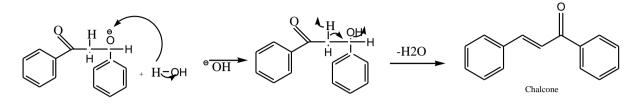
Step 2: Nucleophilic attack on benzaldehyde

The enolate ion being a strong nucleophile, attacks the carbonyl group of aldehyde.

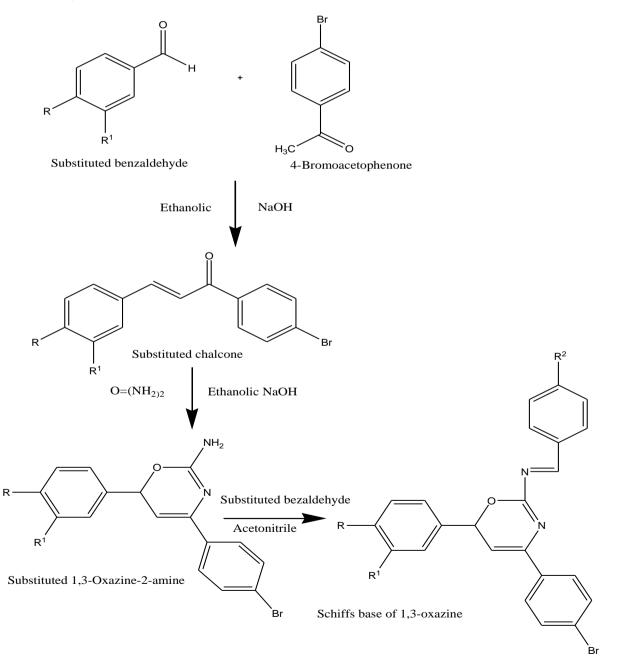


Step 3 : Formation of Chalcone

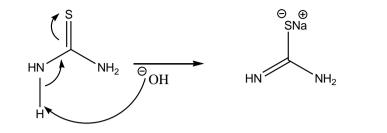
The anion formed in step 2 abstract a proton from water to form chalcone.

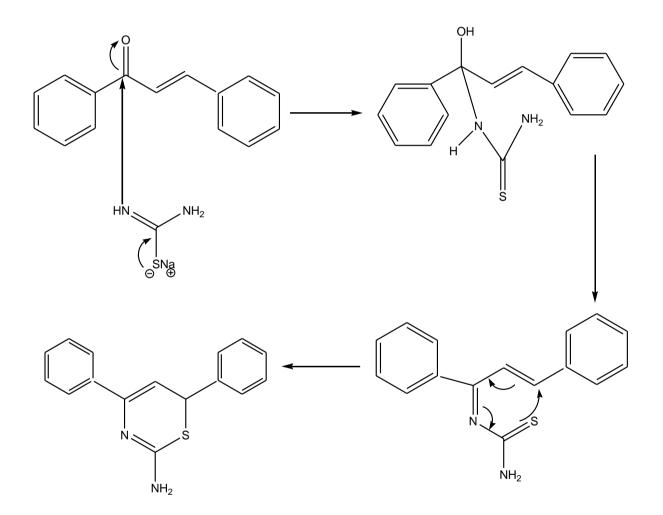


2. Oxazine Synthesis mechaisium

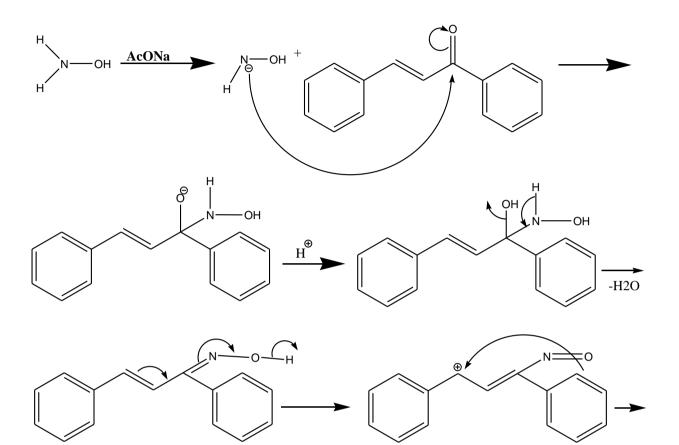


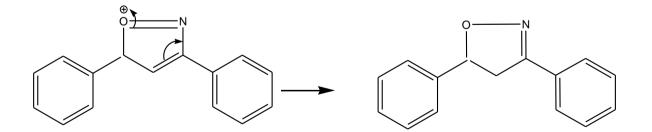
3. Thiazine Synthesis mechaisium





4.Isoxazole Synthesis mechaisium





CALCULATION

1. To find the theoretical yield of Chalcone

Molecular weight of Benzaldehyde= Molecular weight of Chalcone

Weight of the Benzaldehyde taken= Weight of Chalcone required

$$106.12g = 208.26g$$

$$3.493g = X g$$

X g × 106.12 g = 3.493 g × 208.26 g
X = 3.493 × 208.26 / 106.1
X = 727.4522 / 106.12
X = 6.855 g

- 2. The crude yield of the Chalcone = 3.764 g
- 3. To find the percentage (%) yield

% yield = crude yield / theoretical yield \times 100

$$= 3.764 / 6.855 \times 100$$
$$= 54.90 \%$$

1. To find the theoretical yield of oxazine

Molecular weight of Urea = Molecular weight of Oxazine

Weight of the Urea taken = Weight of Oxazine required

$$60.06g = 252.3154g$$
$$0.67g = X g$$
$$X g \times 60.06 g = 0.67 g \times 252.3154 g$$
$$X = 0.67 \times 252.3154 / 60.06$$
$$X = 160.051 / 60.06$$
$$X = 2.81 g$$

- 2. The crude yield of the oxazine = 1.833 g
- 3. To find the percentage (%) yield

% yield = crude yield / theoretical yield $\times 100$

 $= 1.833 / 2.81 \times 100$

1. To find the theoretical yield of thiazine

Molecular weight of Thiourea = Molecular weight of Thiazine

Weight of the Thiourea taken= Weight of thiazine required

$$76.12g = 268g$$

$$0.795g = X g$$

$$X g \times 76.12 g = 0.795 g \times 268 g$$

$$X = 0.795 \times 268 / 76.12$$

$$X = 213.06 / 76.12$$

$$X = 2.79g$$

- 2. The crude yield of the chalcone = 2.543 g
- 3. To find the percentage (%) yield

% yield = crude yield / theoretical yield $\times 100$ = 2.543 / 2.79 $\times 100$ = 91.14 %

1. To find the theoretical yield of Isoxazole

Molecular weight of hydroxylamine hydrochloride= Molecular weight of isoxazole Weight of the hydroxylamine hydrochloride taken= Weight of isoxazole required

$$64.49g = 223.2737g$$

$$0.695g = X g$$

$$X g \times 64.49 g = 0.695 g \times 223.2737 g$$

$$X = 0.695 \times 223.2737 / 64.49$$

$$X = 155.175 / 64.49$$

$$X = 2.406 g$$

- 2. The crude yield of the isoxazole = 1.204 g
- 3. To find the percentage (%) yield

% yield = crude yield / theoretical yield $\times 100$

$$=$$
 1.204 / 2.406 × 100

RESULT & DISSCUSSION

- 1. Chalcone
- Theoretical yield of Chalcone is = 6.855g
- Percentage yield of Chalcone is = 54.90 %
- Melting point of Chalcone is = 57°C
- 2. Oxazine
- Theoretical yield of Oxazine is = 2.81g
- Percentage yield of Oxazine is = 65.23 %
- Melting point of Oxazine is = 160°C
- 3. Thiazine
- Theoretical yield of thiazine is = 2.79g
- Percentage yield of thiazine is = 91.14 %
- Melting point of thiazine is = 152°C
- 4. Isoxazole
- Theoretical yield of Isoxazole is = 2.406g
- Percentage yield of Isoxazole is = 50.04 %
- Melting point of Isoxazole is = 260°C

CONCLUSION

Chalcone were synthesized by Claisen-schmidt condensation method by using two alkaline base that are sodium hydroxide (NaOH) and barium hydroxide (Ba(OH)₂). The technique such as physical constant determination was used to check the purity of chalcones or to confirm the structure of chalcones that were obtained successfully. Oxazine, Thiazines and Isoxazole derivatives can be prepared from Chalcone as starting materials.

REFERENCES

- Paula Boeck, Camila Alves Bandera Falcão, Paulo Cèsar Leal. Rose do Augusto Yunes. Valdir. *Bioorganic & medic-inal chemistry* 14(5), 1538-1545, 2006. Synthesis of Chalcone analogues with increased antileishmanial activity.
- Hari Om Saxena, Uzma Faridi, JK Kumar, Suaib Lugman. MP Darokar. Karuna Shankar. Chandan S. Steroids 72(13),892-900, 2007. Synthesis of chalcone derivatives on steroidal framework and their anticancer activities.

- Alka N Choudhary, Vijay Juyal. *International journal of pharmaceutical Sciences* 3(3), 125-128, 2011. Synthesis of chalcone and their derivatives as antimicrobial agents.
- A Solhy, R Tahir, S Sebti, R Skouta, M Bousmina, M Zahouily, M Larzek. *Applied Catalysis* A: General 374(1-2), 189-193, 2010. Efficient synthesis of chalcone derivatives catalysed by re-usable hydroxyapatite.
- Jae-Chul Jung, Yong am Lee, Min Dongguk, Mankil Jung. November 2017. Molecules 22(11):1872 DOI:10.3390/molecules 22111872. Practical Synthesis of Chalcone Derivatives and Their Biological Activities.
- Aluru Rammohan, Satyanarayana Reddy Julakanti, Sravya Gundala, Chittluri. January 2020. Environmental Chemistry Letters 18(1)DOI:10.1007/s10311-019-00959-w. Chalcone synthesis, properties and medicinal applications: a review.

Quality Policy

- 1. Contribute to academic standing and overall knowledge development of the students.
- 2. Providing creative enriching learning experience through student centric activities.
- 3. Incorporating value added programs to curriculum and improving prospects of the students.
- 4. Focusing on overall development of the students and strengthening the capabilities and leadership abilities.
- 5. Inculcating moral and ethical values amongst the student and staff.
- Enhancing the competence of the faculty through faculty development program and add to make them adopt all modern and innovative methods of teaching and learning process.
- 7. Adoption of innovative teaching methods.
- 8. Disseminating knowledge in the region through continuing education program.
- 9. Ensuring continual improvement of Quality Management System.
- 10. Creating a conductive environment for effective teaching, active learning and purposeful research.
- 11. Collaborating with industry, other institutions and organisations for mutual benefits.
- 12. Maintaining state of art infrastructure and congenial learning environment.

Advisory Board Members

Prof. Manoj Kamat

Chairman Principal, Shree Mallikarjun and Shree Chetan Manju Desai College, Canacona Goa

Dr. Joydeep Bhattacharjee

Member Principal, Govt. College of Arts Science and Commerce, Quepem-Goa

Dr. Nandini Vaz Fernandes

Parvatibai Chowgule College of Arts and Science, Goa Dr. Ramrao S. Wagh Member Goa Business School Goa University, Goa

Ms. Siddhali V. Girkar Member School of Chemical Sciences Goa University, Goa

Disclaimer

A significant part of this publication is from the M. Sc. project conducted at Goa University. Humble credits are due to tic oncer supervisor, fellow researchers and other peers associated with the article. No infringement of any copyright or credits is implied to the editor or publisher.

> Dr. Mithil Fal Desai Editor Science Viewpoint August 2021 Shree Mallikarjun and Shri Chetan Manju Desai College Canacona Goa