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ADVANCES IN SCIENCE AND TECHNOLOGY

VOLUME IV

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PREFACE

The pursuit of knowledge is an intrinsic characteristic of the human species. Throughout history, we have endeavored to unravel the mysteries of the universe, to understand the intricate workings of our world, and to harness the forces of nature for the betterment of our lives. Science and technology have been the driving forces behind our progress, leading us to astonishing discoveries and revolutionary inventions.

In this rapidly evolving era, where boundaries are constantly being pushed and new frontiers are being explored, it is imperative that we stay abreast of the latest advancements in science and technology. This book, "Advances in Science and Technology," serves as a comprehensive compilation of cutting-edge research and breakthrough innovations that are shaping our present and defining our future.

The chapters in this book are a testament to the spirit of curiosity and intellectual curiosity that drives scientific inquiry. From exploring the depths of the cosmos to delving into the intricacies of the human brain, from harnessing renewable energy sources to revolutionizing healthcare, each chapter presents a unique perspective and a fresh perspective on the forefront of knowledge.

Furthermore, this book also acknowledges the interconnectedness of science and technology with society. It recognizes the ethical implications of scientific advancements and explores the potential impact on our lives, economy, and environment. It underscores the need for responsible innovation, sustainable development, and equitable access to the benefits of scientific progress.

The book is a valuable resource for anyone who is interested in learning more about the latest advances in science and technology. It is also a valuable resource for students, researchers, and professionals in a variety of fields.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

Editors

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ORGANIC – GREEN CHEMISTRY PERSPECTIVES FOR ACHIEVING SUSTAINABILITY

M. Tamilselvi* and S. Uma
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Abstract:

Green chemistry has been aiding the development of global sustainability for more than three decades. The use of toxic reactants and reagents by chemists has made the situation worse both from ethical and financial point of view. However sustainability in organic chemistry has been driving innovation. Redesigned green laboratory experiments provide the resource to the chemistry community and beyond for analyzing problems and arriving at solutions. This paper has identified crucial organic green chemistry experiments which can be readily integrated into the chemistry laboratory. This will in turn increase the awareness to minimize the effect and use of hazardous substances on the environment and human health.

Introduction:

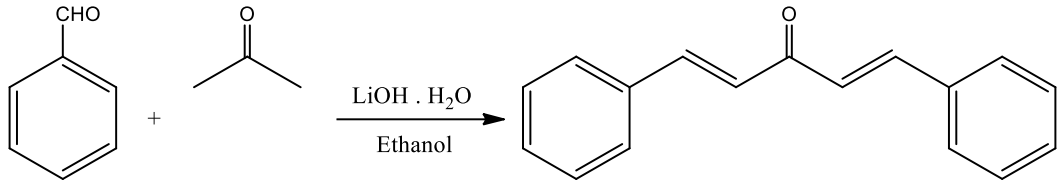
Nowadays with the amount of waste generated in many synthetic chemistry routes due to fast paced inventions, there develops a need for sustainability. Green chemistry plays a vital role in organic chemistry synthesis. The main goal of green chemistry is to use green solvents (PEG, water, acetone, alcohol) eliminate the toxicity, uses of small quantity of catalyst and minimize the potential for chemical accident during work.[1,2]

Green chemistry influences medical field by providing a new path for synthesizing safer chemical products. Synthesis of medicinal compounds like paracetamol is widely used in medicinal preparation. The conventional route of synthesis produces a huge amount of industrial waste. The greener synthesis of paracetamol in the presence of Titanium (IV) silicate catalyst is a controlled oxidation and gives oxime derivative of p-hydroxy acetophenone which undergoes Beckmann rearrangement giving paracetamol.[3]

A series of reductions are involved in green chemistry. Also many criteria or methods should be followed for synthesis chemical products during manufacturing condition. Some of these are prevention of waste, Atom economy, less hazardous chemical syntheses, designing safer chemicals, safer solvents, design for more energy efficient chemical, use of renewable feed stocks, reduce derivatives in any compounds, catalysis, design for degradation, real time analysis for pollution prevention, inherently safer for accident prevention, etc.[4]

Synthesis of benzalpropanone:

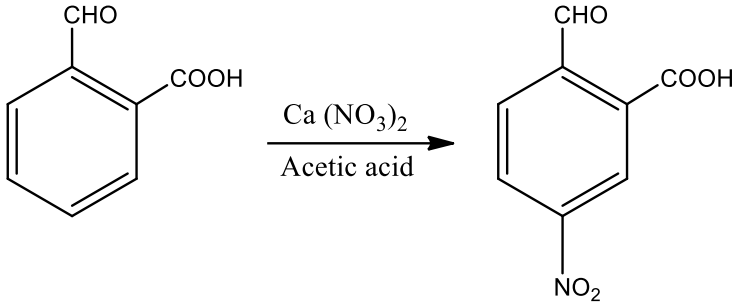
In the green context synthesis of benzalpropanone, hazardous organic solvents are avoided and reagents are non-toxic. Lithium hydroxide is easy to handle as it is comparatively less hygroscopic than other alkali metal hydroxide.[5]



benzaldehyde acetone dibenzalpropanone

Nitration of Phenol:

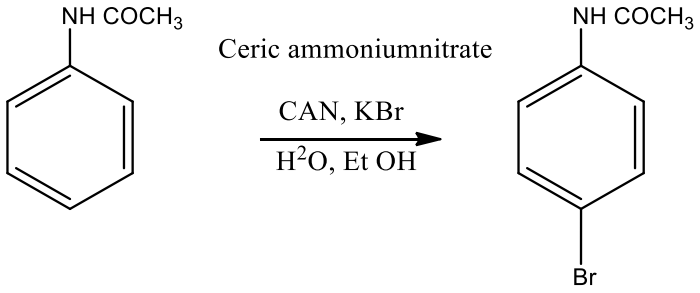
Conventional procedure for nitration of phenol involves the use of con.sulfuric acid and nitric acid. On the other hand alternative green procedure for nitration is rapid and eco-friendly. Reagents and byproducts (calcium acetate) in the green reaction are useful agrochemicals.[6]



Salicylic acid p-nitro Salicylic acid

Bromination of Acetanilide:

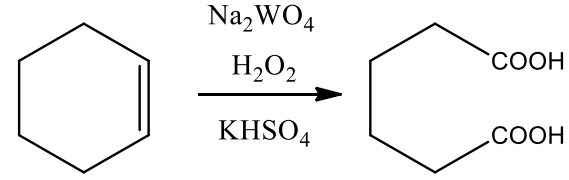
In conventional procedure liquid molecular bromine is used. In alternative green procedure corrosive molecular bromine is replaced with a novel brominating agent. Bromination is carried out in aqueous medium. Chlorinated solvents are avoided and the use of acetic acid as solvent is avoided. The reaction is also considerably fast.[7]



Acetanilide p-bromo acetanilide

Green Oxidation Reaction:

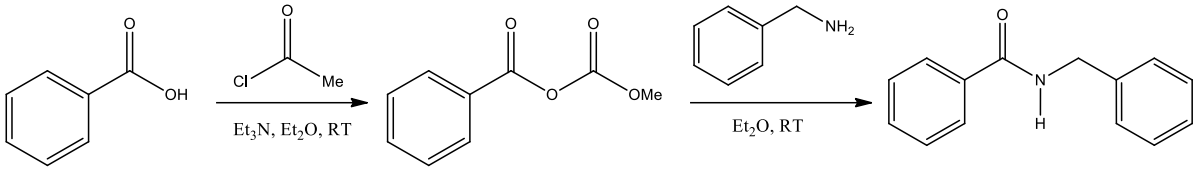
Conventional procedure involves corrosive con.nitric acid. This results in evolution of oxides of nitrogen. The reaction has to be carried out in fume cupboard, and oxides of nitrogen need to be absorbed in water. But green procedure eliminates the use of nitric acid and waste by product. The yield is also better.[8]



Cyclohexene Adipic acid

Preparation of a Derivative for Carboxylic acid:

In qualitative organic analysis, the preparation of a derivative of the identified organic compound is required for confirmation of its identity. For carboxylic acids the most common derivative is amide and acetanilide which are prepared through acid chlorides by treatment with phosphorus pentachloride or thionyl chloride which are highly toxic. Thus, an alternative method for preparation of a solid derivative or carboxylic acids avoiding these toxic chemicals in highly desirable. The N-benzyl benzamide can be a good alternative and it can be prepared in a greener way.[1]



Benzoic acid N-benzyl benzamide

Alternative Green Techniques:

Organic solvent is objectionable from the stand point of environmental hazard. This is why "solvent free reaction" condition is an important object of green chemistry. The no solvent condition however faces the problems like heat and mass transfer mixing of the reactants. To overcome these barriers techniques like grinding, Microwave irradiation are used.[9]

Microwave irradiation techniques do not require solvents and are considered "greener" than the conventional method. Wide range of applications of microwave chemistry has been extended to many aspects of organic synthesis under the principle of green chemistry. All these techniques aim at replacing toxic solvents in many chemical processes in the synthetic laboratory and in the chemical industry.[10]

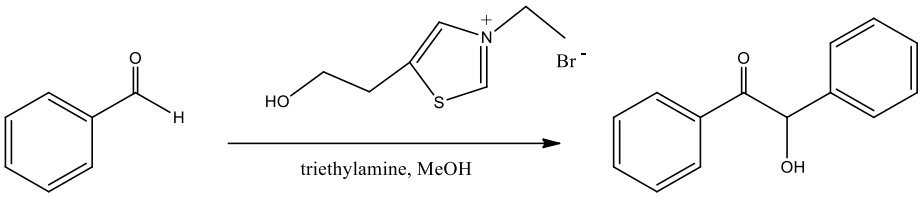
Sonali R. Sharma had reported Green Chemistry, Green Solvents and alternative techniques in organic synthesis. New methods have been developed, where organic synthesis can be performed without solvents, and using mild conditions and low energy consumption.[11]

Conventional solvents	**Green solvents**
Methylchlorides	Dimethyl carbonate
Dimethyl sulphate esters	Dimethyl carbonate
Benzene	Toluene
Carbon tetrachloride	Cyclohexane
Chloroform	Dichloromethane

Benzoin Condensation by Organocatalysis:

LEE, Dohyung et al., investigated Benzoin condensation by organocatalysis and consideration of its usage for waste management. Benzaldehyde, 3-Ethyl-5-(2-hydroxyethyl)-4-

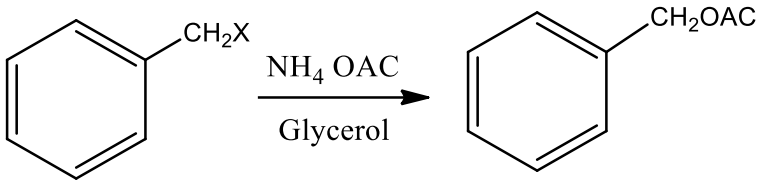
methylthiazolium bromide and triethylamine were used as reagent and benzoin condensation was carried out successfully.[12]



Benzaldehyde Benzoin

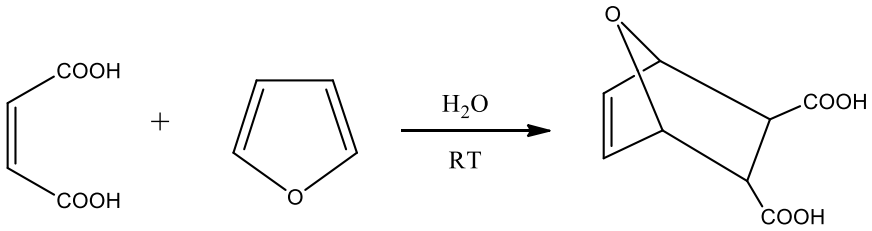
Nucleophilic Substitution of Benzyl Halides:

Woltson et al., had reported Nucleophilic substitution of Benzyl Halides with ammonium acetate in presence of glycerol as green solvent. Glycerol was employed as a green solvent in the nucleophilic substitution of benzyl halides and ammonium acetate.[13]



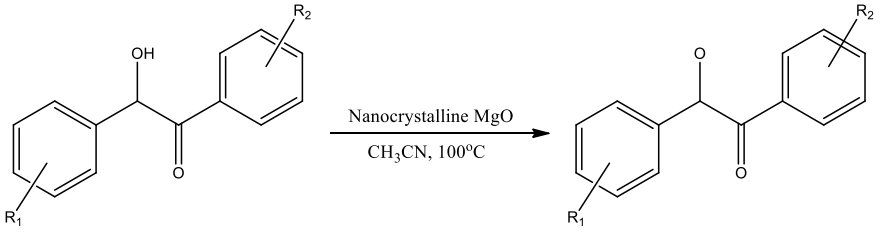
Green Procedure for Diels – Alder Reaction:

R.B. Woodward *et al.,* had reported green procedure for Diels – Alder reaction between furan and maleic acid. The reaction was carried out in aqueous medium avoiding benzene, efficient at room temperature with 100% atom efficiency.[14]



Green Chemistry – Mediated Synthesis Of Benzyl:

Zohre Zarnegar and javad safari had reported Green Chemistry – mediated synthesis of benzyl by using nano-MgO. Nanocrystalline magnesium oxide used was an efficient catalyst for conversion of benzoin to benzil, in the presence of acetonitrile as solvent. This reaction is green and requires milder conditions and shorter reaction time.[15]



Conclusion:

The green chemistry approach aids to present chemistry with the tools to tackle the dangers posed to the human health and the environment. The concept of Organic green chemistry helps to reduce harmful substances produced from many synthetic processes, by applying suitable green chemistry principles at certain stages of reactions. Some examples are as use of green solvents,

alternative reaction conditions (i.e. microwave activation), etc. Many such possibilities were discussed in this paper. Thus the promotion of Organic green chemistry will maintain economical balance and also protect the environment from chemical hazards and in turn contribute to global sustainability.

References:

1. P.T. Anastas, J.C. Warner; 1998: Green Chemistry: Theory and Practice; Oxford University Press, Oxford [England]; 30.
2. Ananya Das, Abir Sadhukhan, *et al.,* 2022: Role of Green Chemistry in Organic Synthesis and Protection of Environment, IJRASET, 10.(XII): 1850.
3. Asim K. Das, 2010: "Green synthesis of Paracetamol", Environmental chemistry with green chemistry, 722.
4. Krishna N. Ganesh, Deqing Zhang, *et al.,* 2021: Green Chemistry: A Framework for a Sustainable Future, Org. Process Res. Dev, 25. 1455-1459.
5. S. Bhagat, R.Sharma , *et al.,* 2006: "Base catalysed aldol condensation", J. Mol. Cat. A: Chemical .
6. A.K. Bose ,S.N. Ganguly, *et al.,* "2006: Electrophilic aromatic substitution reaction", Tetrahedron Lett.
7. P.F. Schatz, 2006: " Electrophilic aromatic substitution reaction", J. Chem. Education.
8. S.M. Reed and J.E. Hutchison, 2000: " Green oxidation reaction", J. Chem. Education, 77.
9. Herbert W. Rosky, Dietmar, *et al.,* 2009: "Experiments in green and sustainable chemistry".
10. K.R. Desai *et al.,* 2010: "Green chemistry microwave assisted synthesis", Global media, New York.
11. Sonali R. Sharma, 2015:"Green chemistry, Green solvents and alternative techniques in organic synthesis", International J. Chem. and physical Sci., 4, 516.
12. LEE, Dohyung, 2005:"Benzoin condensation by organo catalysis and consideration of its usage for waste management", United nation environment programe.
13. A. Wolfson, H. Kimchi *et al.,* 2011: "Nucleophilic substitution of benzyle halides with in Ammonium acetate in presence of glycerol as green solvent", Asian J. Chemistry, 23, 3, 1227-1229.
14. R.B. Woodward and H. Baer, 1948: "Cyclo addition reaction" J. Am. Chem. Soc, 70.

NOVEL THERAPEUTIC STRATEGIES FOR TREATMENT OF ALZHEIMER'S DISEASE

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Abstract:

Alzheimer's disease is a progressive neurodegenerative disease and accounts for most cases of dementia. The prevalence of AD has increased in the current rapidly aging society and contributes to a heavy burden on families and society. Despite the profound impact of AD, current treatments are unable to achieve satisfactory therapeutic effects or stop the progression of the disease. Finding novel treatments for AD has become urgent. In this paper, we reviewed novel therapeutic approaches in four categories: immunotherapy, gene therapy, Stem cell therapy and Cognitive Training Interventions. According to overview of the current literature, AD is a multi-factorial disorder with several pathogenic trajectories; hence, a multifunctional strategy to create effective neuroprotective agents is required to treat this disorder.

Keywords: Alzheimer disease; immunotherapy; therapeutic strategies; Gene therapy

Introduction:

Alzheimer's disease (AD) is a neurodegenerative disease that often manifests itself gradually and progressively worse over time. It is the cause of 60–70% of dementia cases. The most prevalent initial sign is trouble recalling recent events. Language difficulties, disorientation (including a tendency to get lost easily), mood swings, a lack of desire, self-neglect, and behavioural issues can all be indicators of advanced Alzheimer's disease. A person's illness worsens, and as a result, they frequently become more isolated from their family and society. The loss of bodily functioning progresses to death. The typical life expectancy following diagnosis is three to nine years, though the rate of progression can vary.[1]

The cause of Alzheimer's disease is poorly understood. There are many environmental and genetic risk factors associated with its development. The strongest genetic risk factor is from an allele of apolipoprotein-E (ApoE). Other risk factors include a history of head injury, clinical depression, and high blood pressure. The disease process is largely associated with amyloid plaques, neurofibrillary tangles, and loss of neuronal connections in the brain. A probable diagnosis is based on the history of the illness and cognitive testing with medical imaging and blood tests to rule out other possible causes. Initial symptoms are often mistaken for normal brain aging. Good nutrition, physical activity, and engaging socially are known to be of benefit generally in aging, and these may help in reducing the risk of cognitive decline and Alzheimer's. [2]

Despite the profound and chronic effects of AD, current treatments are unable to achieve satisfactory therapeutic effects or stop disease progression. Today, only five drugs have been approved by the FDA for AD treatment: donepezil, rivastigmine, galantamine, tacrine, and memantine. The first four drugs are acetylcholinesterase inhibitors (AChEIs), while the last one is an N-methyl-D-aspartate receptor (NMDAR) antagonist. American and European guidelines list AChEIs as first-line pharmacotherapies for mild to moderate AD. However, AChEIs only show modest efficacy on cognitive deficits and non-significant efficacy on functional capacity in mild to moderate AD. Memantine shows very limited efficacy on cognitive symptoms without functional improvement. [3] Finding novel treatments for AD has become urgent. The present review emphasizes on novel research on therapeutic strategies for management of AD which includes immunotherapy, gene therapy, Stem cell therapy and Cognitive Training Interventions.

Immunotherapy:

Amyloid-β (Aβ) plays a crucial part in the pathogenesis of Alzheimer disease (AD), making this peptide an attractive therapeutic target. Immunotherapy, which is the use of immunity-enhancing techniques as a medical treatment, has taken two basic forms in the fight against AD. In active immunization, a fragment of amyloid beta or a related antigen is administered in order to stimulate a response of both antibody-based and cellular immunity. Passive immunization, by contrast, relies upon the intravenous injection of pre-formed antibodies into an animal for the purpose of boosting resistance to the aggregation of amyloid beta or helping the immune system remove amyloid beta already aggregated into plaques. Although only a small fraction of intravenously administered antibodies passes the blood-brain barrier and enter the brain, their significant effects indicate the potential value of this treatment approach. A favourable aspect of active immunization is the capacity for a small number of vaccinations to generate a prolonged antibody response. A potential disadvantage is the variability in the antibody response across patients. The potential advantages of passive immunotherapy include the reproducible delivery of a known number of therapeutic antibodies to the patient and rapid clearance of those antibodies if side effects develop. A disadvantage is the requirement for repeated infusions of antibodies over time. While immunotherapy holds promise for the treatment of AD, it is not without potential risks and side effects. One of the potential side effects of immunotherapy is the development of brain inflammation, known as amyloid-related imaging abnormalities (ARIA). ARIA can cause headaches, confusion, and other neurological symptoms. However, the risk of ARIA can be minimized by careful monitoring and dose titration. [4]

Immunotherapy for AD involves the use of monoclonal antibodies that target beta-amyloid. These antibodies can bind to beta-amyloid and remove it from the brain. Some of the most promising immunotherapy drugs for AD are aducanumab, donanemab, and lecanemab. Aducanumab is an antibody that binds to beta-amyloid and clears it from the brain. In clinical trials, aducanumab has been shown to reduce beta-amyloid plaque levels in the brain and improve cognitive function in some AD patients. In 2021, the US Food and Drug Administration (FDA) approved aducanumab for the treatment of AD, making it the first disease-modifying treatment for the disease. Donanemab is another monoclonal antibody that targets a specific form

of beta-amyloid, known as N-terminal pyroglutamate (pGlu)-Aβ. In a phase 2 clinical trial, donanemab was found to significantly reduce the accumulation of pGlu-Aβ plaques and improve cognition in AD patients. Lecanemab is another monoclonal antibody that targets beta-amyloid. In a phase 2 clinical trial, lecanemab was found to reduce beta-amyloid plaque levels in the brain and slow cognitive decline in AD patients. In recent years, a number of drug candidates targeting amyloid-ß (Aß) peptide have advanced into randomized controlled clinical trials. These include tarenflurbil (Myriad Genetics, Salt Lake City, UT, USA), semagacestat (Eli Lilly and Company, Indianapolis, IN, USA), tramiprosate (Neurochem Inc., Laval, Canada), ELND006 and AN1792 (Elan Corporation, Dublin, Ireland) and ponezumab (Pfizer, New York, NY, USA). However, most have failed because of safety issues or lack of efficacy. [5]

Gene therapy:

Gene therapy for Alzheimer's disease is an experimental treatment that involves the modification of brain cells with specific genes that are programmed to produce therapeutic molecules. Putative targets of genetic therapies tested in pre-clinical trials include amyloid pathway intermediates and enzymes modulation, tau protein downregulation, APOE4 downregulation and APOE2 upregulation, neurotrophin expression (nerve growth factor (NGF) and brain-derived neurotrophic factor), and inflammatory cytokine alteration, among several other approaches. [6]

The main strategies for brain gene therapies involve the use of nanoparticles, such as metal nanoparticles coated with nucleic acids, or a combination of nanovectors coupled with antibodies for tissue selectivity. This methodology also allows for sustained release of payloads, potentially increasing therapeutic efficacy. Liposomes represent an effective way of crossing the blood–brain barrier, with the possibility of carrying nanoparticles or viruses inside. These systems can be combined with other forms of delivery to temporally disrupt the BBB as with focused ultrasound. Alternative routes for reaching the CNS are being developed such as intranasal delivery or by viruses crossing the BBB and targeting the brain. Additionally, reprogrammed and modified cells can be introduced into the brain to improve different aspects of AD pathology. Currently, many somatic cells can be derived from the patient's various sources and induced to neural precursors, neural and astroglial phenotypes for transplantation into the brain. Transduction of transcription factors directly to the brain can be also complemented with cell therapies to improve survival of the transplants.

Gene therapy approaches targeting the tau pathology in tauopathies including AD. Alternative splicing of the MAPT pre-mRNA results in two isoforms of tau: 3R-Tau and 4R-Tau. Retention of exon 10 generates 4R-Tau. In the healthy state, the 3R and 4R-Tau isoform ratio is close to one. However, in tauopathies, the 3R-Tau/4R-Tau ratio is altered which results in hyperphosphorylated tau aggregation into neurofibrillary tangles (NFTs), which impair cell trafficking and lead to cell death. Proposed gene therapies include (1) Spliceosome-mediated RNA trans-splicing (SMaRT) restoring 3R/4R-Tau balance by skipping exon 10, thus reducing 4R-Tau abundance. (2) A similar approach can be achieved using antisense oligonucleotides (ASOs), restoring the 3R:4R ratio by either exon 10-skipping or by inducing degradation of the

4R-Tau by RNAi silencing complex. (3) Resident cells in the brain can be transfected with AAVs to encode anti-tau antibodies, reducing total soluble tau and thereby preventing tauopathies.

Cell therapies approaches for Alzheimer's disease therapeutics. Patient-derived neural precursor stem cells, induced neurons or induced astrocytes can be generated from somatic cells and modified to withstand the stressful environment of AD brains. Predefining cell identity is crucial for NPSCs to avoid tumorigenesis or divergence in cell specification after transplants. Experiments have shown that transplanted cells have the ability to differentiate and integrate into neural circuits in the hippocampus. Combining cell types into a transplant for treating AD is possible although neuron to astrocyte ratios should be adjusted to ensure efficient synaptic connectivity. Together with cell therapy, it is possible to inject plasmids containing transcription factors to improve transplant survival, because the transcription factors (TFs) improve the extracellular environment by altering miRNA signalling and other unknown mechanisms. Other gene therapy approaches can be used in combination with the cell therapy strategies proposed in this review depending on the personalized needs of the patients. [7]

Stem cell therapy:

The capacity for regeneration of stem cells and their efficacy and safety when applied in therapy have already been reported by several studies in the past, making it a potential option for targeting neurodegeneration in AD. Because of their potential to multiply and give rise to multiple cell types, stem cells are an intriguing prospect for repairing damaged tissues. Stem cell therapy is increasingly being researched as a treatment option in neurocognitive disorders presenting with dementia, such as AD. Majority of the available research at present focuses on mesenchymal stem cells (MSC), which are pluripotent and aid in neurogenesis and angiogenesis. They also prevent the loss of neurons by exerting anti-apoptotic effects. This is brought about by releasing growth factors, neurotrophins, and cytokines. Thus, they aid in remyelination and regeneration. Additionally, they also interact with several immune cells, thus giving rise to anti-inflammatory effects. There are different types of mesenchymal stem cells based on the tissue that they are sourced. Several clinical trials studied and compared bone marrow-derived MSC (BMMSC), adipose-derived MSC (ADMSC), umbilical cord-derived MSC (UC-MSC), and placenta-derived MSC (PD-MSC). BMMSC and ADMSC were the most dependable and widely utilized among them. [8]

Processes involved in the pathogenesis of AD include proliferation, apoptosis, angiogenesis, inflammation, immunomodulation and so on. It is proposed that stem cell transplantation may alter these processes, thus repairing the neurological dysfunction and bringing about improvement in neurobehavioral function. [9]

Cognitive training interventions:

About 90 % of all persons with mild Alzheimer’s disease experience neuropsychiatric symptoms, most frequently apathy, depression, anxiety and irritability. These symptoms are associated with greater morbidity, a reduced quality of life for the patient, an increased burden and depression for the caregiver, and higher costs of care and nursing home placement.

Psychosocial interventions based on behaviour therapy represent the most efficacious treatment of neuropsychiatric symptoms. [10]

Cognitive training interventions refers to a series of standardized tasks with inherent challenges that target specific cognitive domains. Positive outcome of cognitive training in persons with Alzheimer's disease has been reported. The interventions are typically classified into three broad categories: cognitive stimulation, cognitive training, and cognitive rehabilitation, which are based on different theoretical constructs of restoration and compensation. Cognitive stimulation is usually administered in a group setting, is often recreational in nature, and involves non-specific cognitive activities. Group discussions, reality orientation, and reminiscence therapy are examples of cognitive stimulation techniques. Cognitive training is defined as guided practice on a set of standard tasks designed to reflect particular cognitive functions such as memory, attention, or executive functions. Training is assumed to improve, or at least stabilize, performance in a given cognitive domain (i.e., near transfer effect). Cognitive training is based on the principles of neuronal plasticity and restoration of cognitive abilities, but also generalized effects beyond the immediate training context are expected (i.e., far transfer effects). Cognitive rehabilitation refers to more individualized approaches in which personally relevant goals are identified, and inclusive treatments and compensatory strategies are adopted to manage symptoms, and increase daily functioning. The focus in Cognitive rehabilitation is more on far transfer effects of rehabilitation. These three intervention concepts have been used almost interchangeably in the past, and lack of precision in categorizing cognitive interventions is still present in many trials. Furthermore, many intervention programs combine Cognitive training techniques with other methods of rehabilitation, adding to the ambiguity. [11]

Conclusion:

AD is a complex multifactorial disorder which may require equally complex approaches to treatment. Therefore, research efforts should be focused on the development of more targeted pharmacological and genetic therapies, which can reduce the burden or deleterious effects of this disease in affected patients.

References:

1. Beera, A. M., Seethamraju, S. M., & Nori, L. P. (2021). Alzheimer’s Disease: Perspective on Therapeutic Options and Recent Hallmarks in Clinical Research. International Journal of Pharmaceutical Research and Allied Sciences, 10(4), 110-120. https://doi.org/10.51847/ViC6sAGCyq
2. Lane, C. A., Hardy, J., & Schott, J. M. (2018). Alzheimer's disease. *European journal of neurology*, *25*(1), 59–70. https://doi.org/10.1111/ene.13439
3. Yu, T. W., Lane, H. Y., & Lin, C. H. (2021). Novel Therapeutic Approaches for Alzheimer's Disease: An Updated Review. *International journal of molecular sciences*, *22*(15), 8208. https://doi.org/10.3390/ijms22158208
4. Immunotherapy and Alzheimer’s Disease: Helping the Body to Help Itself | Bright Focus Foundation. (2021, August 31).

https://www.brightfocus.org/alzheimers/article/immunotherapy-and-alzheimers-disease-helping-body-help-itself

5. Lannfelt, L, Relkin, NR, Siemers, ER Uppsala University, Uppsala, Sweden; Weill Cornell Medical College, New York, NY; and Eli Lilly and Co., Indianapolis, IN, USA. Amyloid-ß-directed immunotherapy for Alzheimer's disease (Key Symposium). *J Intern Med* 2014; 275: 284– 295.
6. Lennon, M. J., Rigney, G., Raymont, V., & Sachdev, P. (2021). Genetic Therapies for Alzheimer's Disease: A Scoping Review. *Journal of Alzheimer's disease : JAD*, *84*(2), 491–504. https://doi.org/10.3233/JAD-215145
7. Loera-Valencia, R, Piras, A, Ismail, MAM, Manchanda, S, Eyjolfsdottir, H, Saido, TC, Johansson, J, Eriksdotter, M, Winblad, B, Nilsson, P (Karolinska Institutet, Solna, Sweden; Karolinska University Hospital, Huddinge, Sweden; Karolinska Institutet, Huddinge, Sweden; Karolinska University Hospital, Huddinge, Sweden; RIKEN Brain Science Institute, Wako, Saitama, Japan). Targeting Alzheimer's disease with gene and cell therapies (Review). *J Intern Med* 2018; 284: 2– 36.
8. Lee, H. J., Suk, J. E., Patrick, C., Bae, E. J., Cho, J. H., Rho, S., Hwang, D., Masliah, E., & Lee, S. J. (2010). Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. The Journal of Biological Chemistry, 285(12), 9262–9272.
9. Pradhan, A. U., Uwishema, O., Onyeaka, H., Adanur, I., & Dost, B. (2022). A review of stem cell therapy: An emerging treatment for dementia in Alzheimer's and Parkinson's disease. Brain and Behavior, 12, e2740. https://doi.org/10.1002/brb3.2740
10. Forstmeier, S., Maercker, A., Savaskan, E., & Roth, T. (2015). Cognitive behavioural treatment for mild Alzheimer's patients and their caregivers (CBTAC): study protocol for a randomized controlled trial. *Trials*, *16*, 526. https://doi.org/10.1186/s13063-015-1043-0
11. Kallio, E.-L., Öhman, H., Kautiainen, H., Hietanen, M., & Pitkälä, K. (2017). Cognitive Training Interventions for Patients with Alzheimer’s Disease: A Systematic Review. Journal of Alzheimer’s Disease, 56(4), 1349–1372. doi:10.3233/jad-160810

GENOME ANNOTATION- A PARADIGM IN GENOMICS

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Abstract:

Development of high-throughput and next generation sequencing technologies, has paved a way into the core of an organism's genome. Bioinformatics has taken biological aspects into a different level. Earlier forward genetics and lately reverse genetics playing a vital role and in much need of in-depth knowledge about functions of existing flora and fauna. Annotation of genome elements has befallen as one of the prime research areas in computational biology. This chapter provides an insight into the basics of genome annotation, its pipelines such as homology-based approaches, *ab* initio predictions and also about different software holding an upper hand in terms of their efficiency level.

Introduction:

Large-scale DNA sequence information has been efficiently generated from a diverse range of lifeforms thanks to Next-Generation Sequencing (NGS). To comprehend the organism and its evolution, we must extract the information contained in each sequence. Annotation retrieves data that is encoded within the different sequence patterns of the four nucleotides (A, T, C, and G).

The readiness of first-class genome assemblies has offered an essential source of evolutionary info, which has been used to enhance whole-genome alignments and annotations, which had previously depended mainly on models from humans and mice. A significant portion of genomic annotation involves uncovering and identifying genes. Therefore, a thorough and precise method of gene discovery is essential. It requires the use of a array of independent and complimentary analytical tools and techniques. Thus, the methodologies should use information, such as knowledge of proteins and transcripts, and intrinsic information, such as *Ab* initio predictions.

Although many software tools and procedures have been created to address the different issues related to annotation, the difficulty persists as technology and understanding advance.

What is genome annotation?

Genome: is the complete genetic material of an organism or a species

Annotation: a note of or an explanation given.

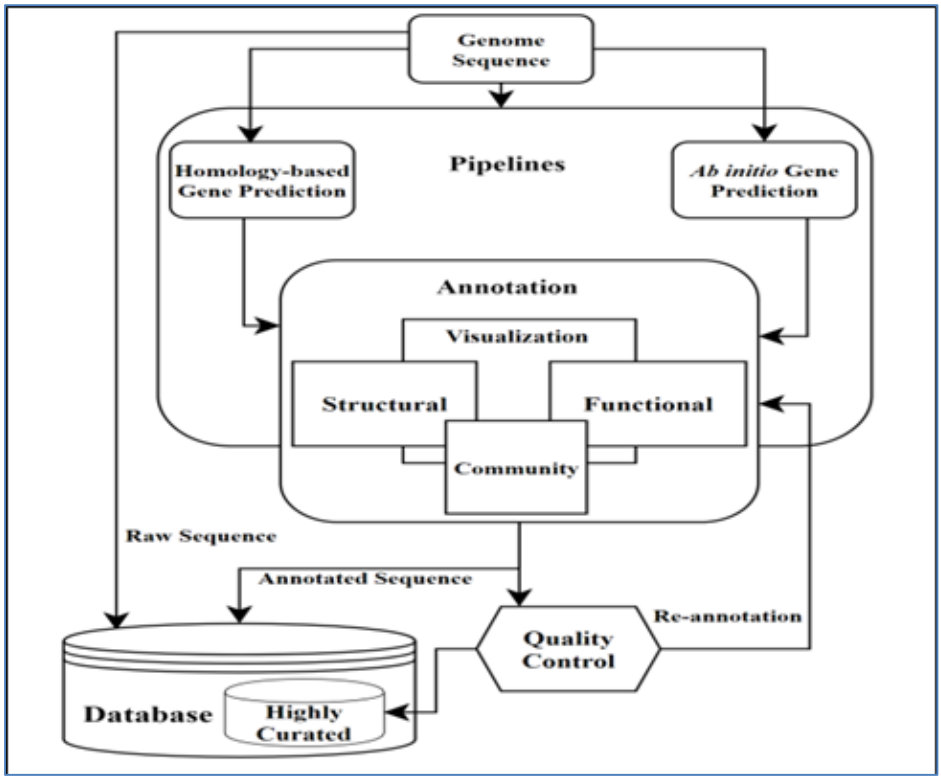
Genome annotation stands for the process of predicting structural elements which constitutes the genome and asserting related functions to them.

There are two major types in genome annotation:

Kinds of Annotation

1) Structural Annotation

Structural annotation identifies DNA components like exons, promoters, introns, transposons, and other elements. Gene delineation has changed along with the advancements in contemporary genomics, whereas structural annotation looks for genes in a genomic sequence. An "essential sequence region for producing functional products" is a gene. Proteins and RNAs are the by-products of genes that possess functional properties. Protein-coding genes are the ones responsible for synthesising proteins. Noncoding genes are distinct genes that produce active RNA molecules rather than proteins. Ribosomal RNA (rRNA), microRNA (miRNA), transfer RNA (tRNA), small nuclear RNA and nucleolar RNA (snoRNA and snRNA, respectively), and long noncoding RNA (lncRNA) are illustrations of noncoding RNA genes.



Genome annotation workflow

Structural annotations also detect pseudogenes. Early on, they had little value and were evolutionary dead ends.

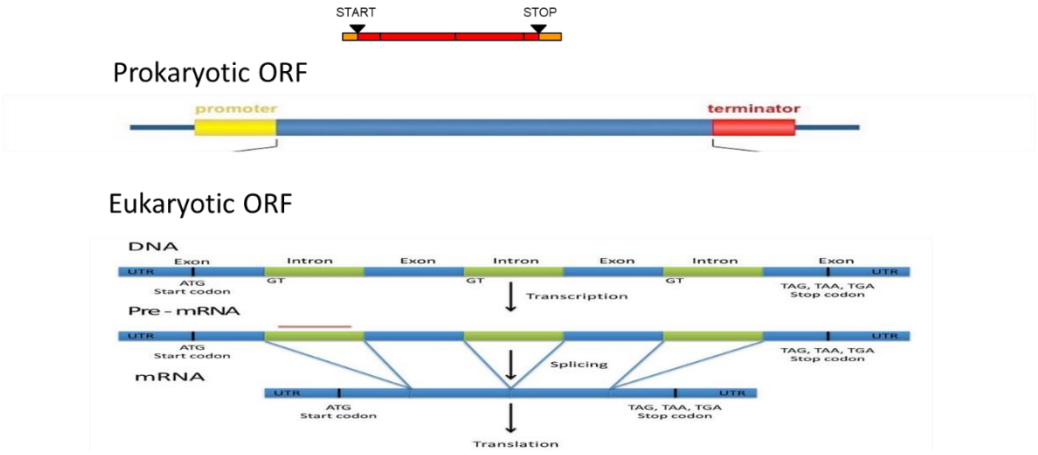
The different structural elements of genome are:

Open reading frame (ORF) is the part of a reading frame that gets translated to protein. An ORF is a continuous stretch of codons that begins with a start codon and ends at a stop codon.

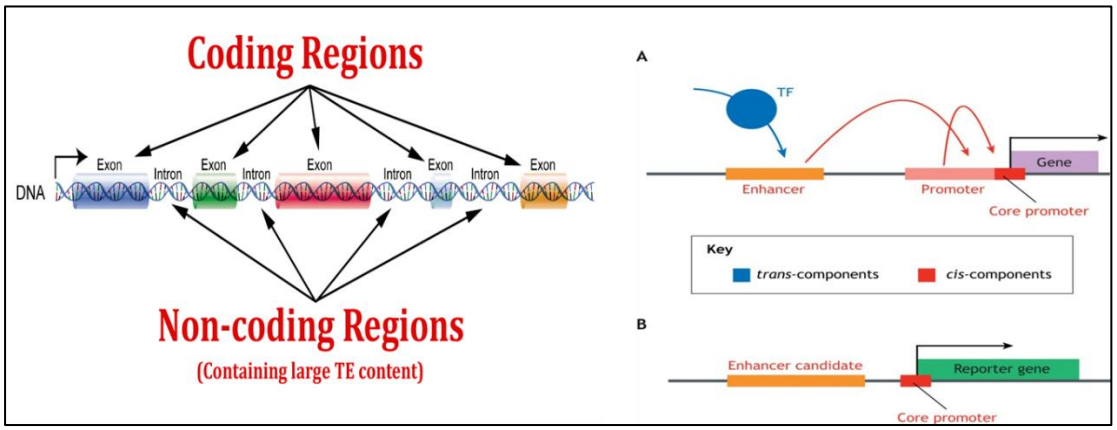
Coding regions: The coding region of a gene, also told as the CDS (coding sequence), is the portion of a gene's DNA or RNA that codes for protein.

Regulatory motifs: are the short nucleotide sequences present upstream or downstream to the genes, which affect transcription.

Ex: Promoters, enhancers, cis and trans acting genes etc.



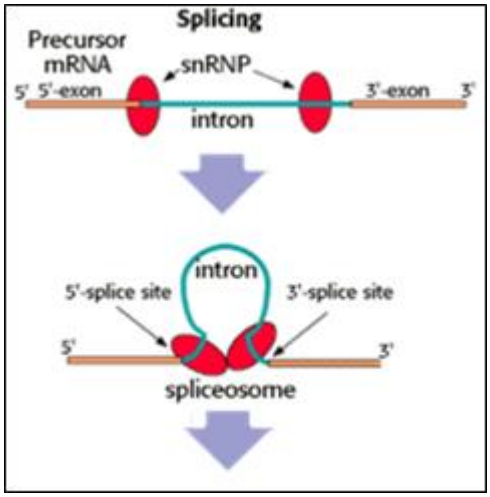
Difference between prokaryotic and eukaryotic ORF



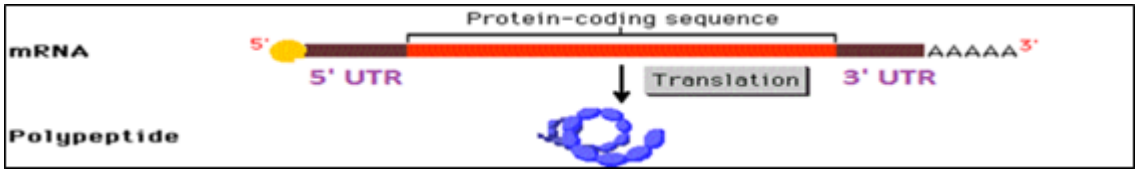
Representation of coding regions, non-coding regions and regulatory motifs

Splice sites: The location on a strand of mRNA where the molecule can be cut and reannealed during the regulation of protein synthesis by cells.

Ex: Software like SPLICEPREDICTOR, GENEID etc.



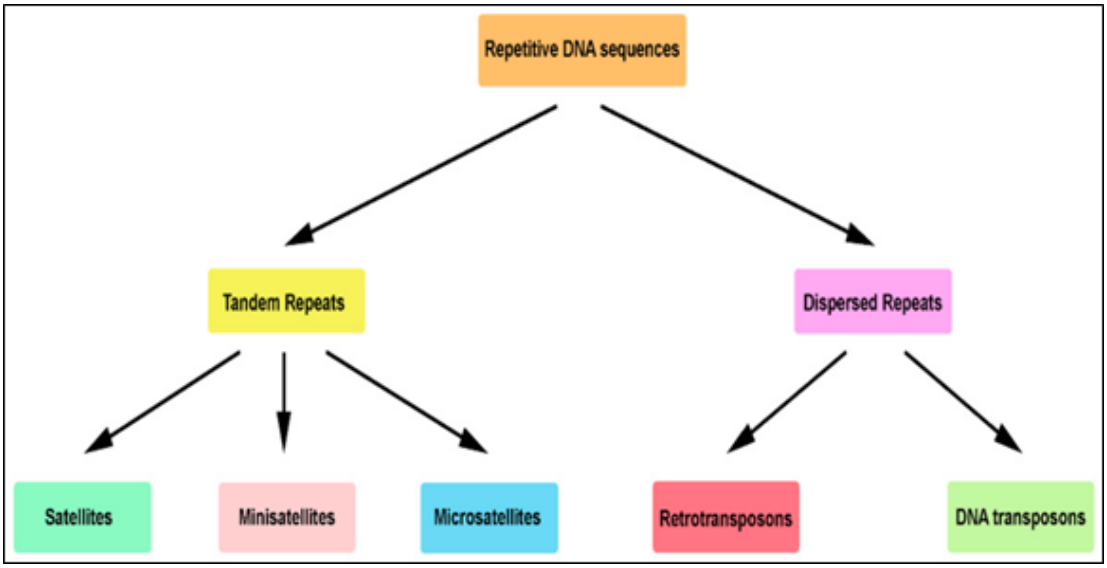
Non coding Regions: are components of an organism's DNA that do not encode protein sequences.



Introns: Segments of DNA or RNA molecule which won't code for proteins and interrupts the gene sequence.

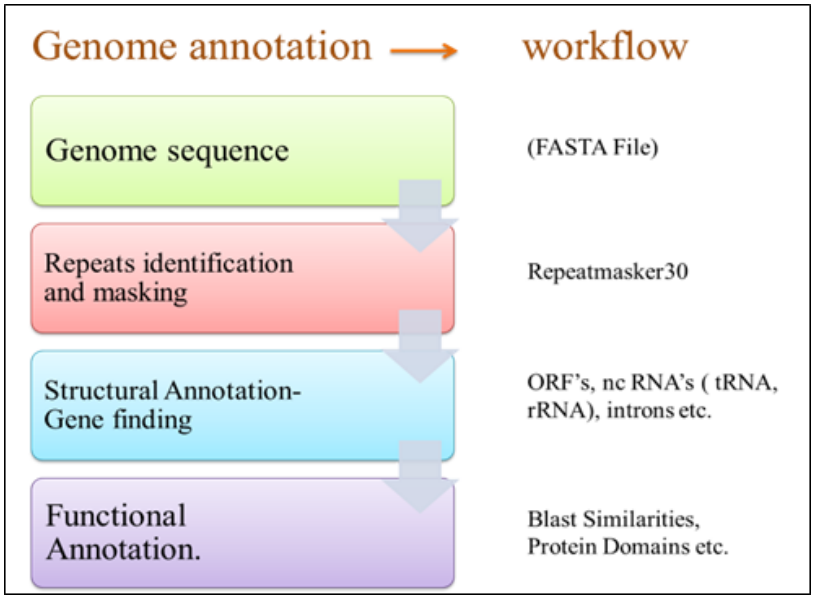
Repeats

These are the pattern of nucleic acids that occur in multiple copies throughout the genome.



Flow chart of DNA repeats

0 to 42% of the bacterial genome comprises DNA repeats. Eukaryotic genomes also include a large number of repetitions. Repeat sequences can be found in tandem, that is, contiguous to one another, or they can be found in the centromere and telomere regions.



Gene prediction and its different features

Many programs exist for the process of gene prediction. They can be sorted into two groups:

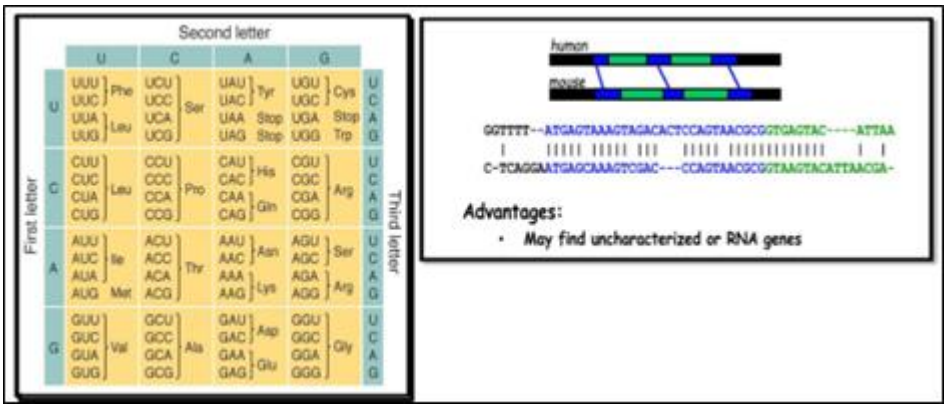
homology-based method and *ab initio* method.

***Ab* initio methods:**

Ab initio approaches are strategies for gene prediction based on the nucleotide sequence. They rely on statistical models, like the hidden Markov model (HMM), to locate promoters, intron-exon junctions, and coding or noncoding regions in the genome.

Homology-based methods:

They match the sequence with complementary DNA (cDNA), protein evidence, expressed sequence tags (ESTs), or both to predict genes. Additionally, as splicing is a critical player in controlling gene expression and transcriptome and proteome variety, gene prediction programmes should be able to predict alternative splicing locations. To anticipate splice sites, gene prediction programmes use a variety of models. As a result, over 99% of the introns in sequenced genomes start with GT and conclude with AG; most gene prediction methods consider these characteristics necessary to identify splice sites.



***Ab initio* vs homology-based approach – an illustration**

	Gene Prediction	Source of Data	Evolutionary Distance Effect	Strength
Ab initio	Rely on statistical model and gene signal	Models (HMM, GHMM, WAM) that can be trained supervised or unsupervised	Medium	Fast and easy means to identify and novel genes
Homology	Rely on sequence alignment	Proteins, EST, cDNA	High	Better accuracy, suitable for functional annotations

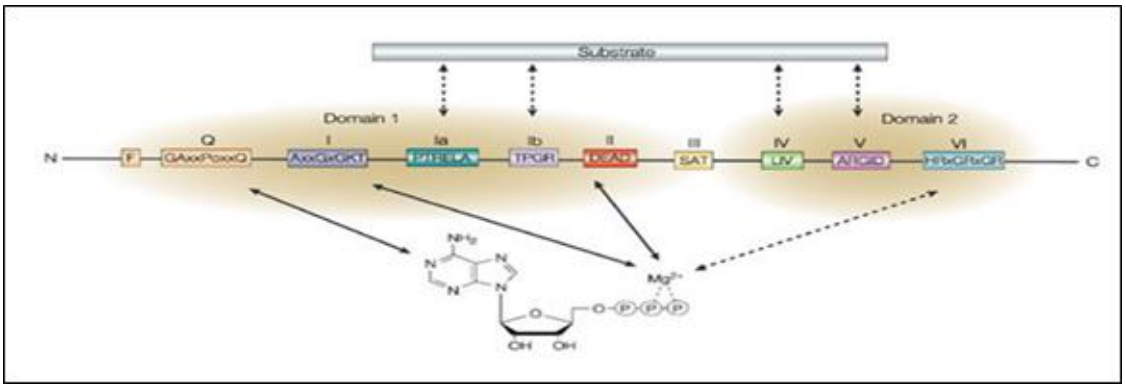
Databases for Structural Annotation

Annotations demand supportive data that can be employed or exhibited as evidence of predicted assignments. Databases swiftly deliver such data. Protein and nucleotide sequences or structures can be discovered in comprehensive public databases

e.g., the GenBank, DNA Databank of Japan (DDBJ) and European Nucleotide Archive (ENA), UniProt, are protein sequence-based databases that intermixes UniProtKB/Swiss-Prot and UniProtKB/TrEMBL, offers the scientific communal with high-quality and freely accessibility to protein sequences coupled with functional information.

Another excellent database for annotation of protein is InterPro, which imparts info on protein domains, families and prominent sites such as active sites, binding sites, conserved sites, and DNA repeats.

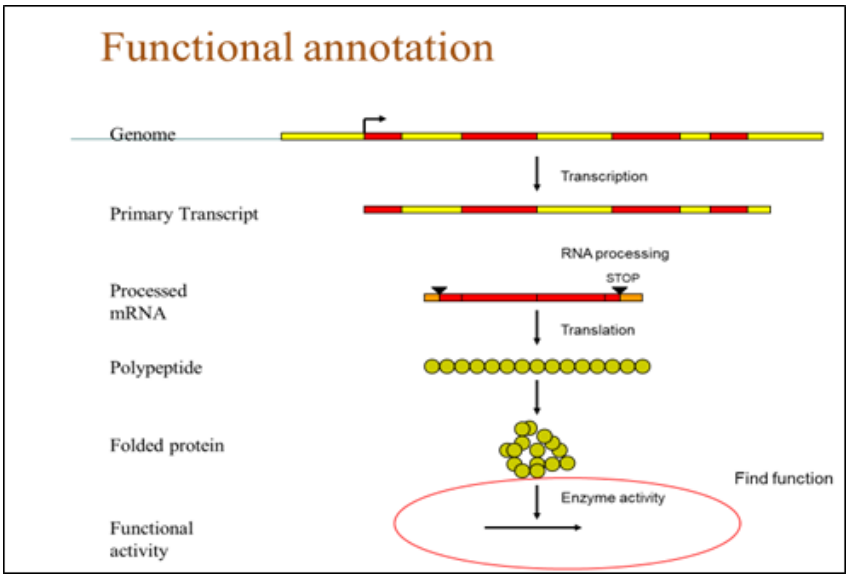
The InterPro Group has fourteen member databases, including Pfam, PROSITE, TIGRFAM, CATH-Gene3D, and PANTHER.



In addition, some specific databases are constructed as comprehensive one-stop information points on particular topics of interest. For example, databases such as NONCODE, Pseudogene.org, Dfam, and miRbase have provided to the structural annotation of noncoding RNAs, transposable elements, pseudogenes, and microRNAs.

2) Functional Annotation

The connotation of biological info with gene or protein sequences recognized by structural annotation is termed as functional annotation. conventional functional annotation focused mainly on protein-coding genes. Yet, numerous different functions of non-coding genes and untranslated transcripts are currently identified. The primary part of functional annotation comprises associating an active description with a gene after finding a parallel sequence using tools such as BLAST. This annotation is likewise employed to assess the dissimilarity in genes on their functional effects. The practical significance of variants can be explored in databases with functional annotation info of novel discerned variants.



Basics of central dogma to carryout functional annotation

Automated Functional Annotation

The typical annotation method is manual; however, scaling this method needs to be revised. Manual annotation is required to scale up automatic annotation methods to match the genomic data produced by NGS technology. Local alignment methods, like BLAST, where a protein database is utilised for high-scoring alignments and automatic function prediction, may be accomplished directly.

Functional Annotation Databases

Gene Ontology (GO): This source is the utmost comprehensive and broadly used knowledge basis for gene function. It encompasses three features of gene function: the cellular component, the molecular function, and the biological process. Gene products should be reliably described to permit coverage of biological concepts.

Program	Description
BLAST2GO	A comprehensive bioinformatics tool for functional annotation of sequences and data mining on annotation results
FastAnnotator	An integration of well-established annotation tools for annotation of transcripts, which assigns GO terms, enzyme commission numbers, and functional domains
GO FEAT	Homology-based functional annotation tool for genomic and transcriptomic data
GOtcha	A method that predicts gene product function by annotation with GO terms
PANNZER2	A fully automated service for functional annotation of prokaryotic and eukaryotic proteins of unknown function that provides both GO annotations and free text description predictions
PoGO	A statistical pattern recognition method that assigns GO terms for fungal proteins

The Kyoto Encyclopaedia of Gene and Genomes (KEGG) connects high-order functional data and genomic information, which reside in databases for genes and pathways, respectively. The Reactome Pathway Knowledge database focuses on Homo sapiens, relating human proteins to their molecular functions.

The data of chemical entities in ChEBI has been carefully vetted and is divided into two sub-ontologies. The chemical entity ontology grouping is based on typical structural traits, but the role ontology examines activities in chemical and biological systems or applicability. The Conserved Domain Database (CDD) at the National Centre for Biotechnology Information (NCBI) contains protein domains that have remained constant throughout molecular evolution and include annotations of protein sequences for conserved domain footprints and conserved functional sites.

Structural Pipelines

An annotation and data management programme called MAKER2 is a multi-threaded tool built on MAKER. It works good with first-generation genome projects but is intended for sec-generation genome projects, which need more pre-existing gene models to train gene finders. MAKER2 incorporates the *Ab* initio gene prediction tools AUGUSTUS, SNAP, and GenMark-ES.

The NCBI Eukaryotic Annotation tool is an automated process for eukaryotes that allows the annotation of both finished and unfinished genomes' non-coding and coding genes,

transcripts, and proteins. The Comparative Annotation Toolkit (CAT) is an end-to-end annotation software toolkit that is entirely open-source.

For eukaryotic genomes, BRAKER1 is a completely automated and exceptionally precise unsubstantiated RNA-seq-based gene annotation workflow. It combines GeneMark-ET and AUGUSTUS' complimentary qualities.

Functional Pipelines

Rapid annotation of bacterial genomes is possible with the Unix-based command-line programme Prokka. RAST is a pipeline for completely automated annotation of archeal and bacterial genomes.

Difference between Manual and Automated annotation

Accuracy	Will be more then Automated	Less than Manual
Time Required	More time Consuming	Less
Cost	More	Initial cost is more
Errors	Less than automated	More compared to manual
Examples	MAKER, APPOLO etc.	SwissProt, UniProt, InterProScan , Ensembl etc.

Annotation Visualization

File Formats

The FASTA format is a accepted for transferring sequence data across bioinformatic tools. Sequence databases may be searched, similarity scores can be calculated, and periodic similarity scores can be found using the FASTA format. The straightforward data file format FASTA cannot accommodate all the data that could be included during an annotation. The GenBank file format of NCBI, , EMBL format of ENA, DDBJ format of DDBJ, general feature format, and GTF are other formats.

Genome Browsers

Utilising a graphical user interface, genome browsers are often used to quickly and easily explore, search, recover, and analyse gene sequence and annotation data. General genome browsers offer several genomes with extensive annotations from various species and allow for comparative study.

Most visualisation tools are designed after the UCSC Genome Browser, the most widely used tool. The UCSC database, established in 2001, already houses more than 105 species, although its user base is mainly focused on mouse and human research.

Another popular browser for vertebrate genomes is Ensembl, which offers sequence variation analysis, comparative genomics, and investigation of transcriptional control.

A browser that offers visual exploration and study of eukaryotic Reference sequence (RefSeq) genome assemblies is the NCBI Genome Data Viewer (GDV), formerly the Map Viewer.

Species-specific areas require more customised visualisation, which generalised browsers cannot manage. The genomic, epigenomic, and transcriptome information for a single model

organism is the focus of species-specific genome browsers, which aid in visualising this data. For instance, the Generic Model Organism Database (GMOD)'s Flybase, Wormbase, and MaizeGDB offer species-specific browsers built on its GBrowse architecture.

Quality Control for Annotation

The input sequence quality has a direct impact on the quality of the annotation. Although NGS technologies have made it possible to generate sequences in a timely and cost-effective manner, they also create reads that range from a few to many thousands of consecutive bases and must be put together to form a whole sequence. Therefore, evaluating a sequence assembly's quality is essential before further annotation.

The MaGuS, QUAST, and BUSCO tools evaluate genome assemblies' accuracy, continuity, and completeness. The annotation confidence score (ACS) is one suggested solution that employs a genome comparison strategy. ACS combines textual and sequence homology. Sequence homology, textual similarity, taxonomic distance and analysis provide the quality score. A semi-automated gene annotation comparison and integration approach is another illustration. This method compares the annotations and creates a hormonal annotation depending on the comparison's results.

A fully automated, straightforward, and rapid comparison of genome annotations produced by various annotation methods is possible with the help of the automated tool for bacterial genome annotation comparison (BEACON).

Annotation edit distance (AED) is a new annotation comparison metric that tries to quantify changes across annotation releases. It evaluates structural modifications to an annotation—such as alternative splicing—that cannot be reported using more traditional metrics like sensitivity and specificity.

Re-Annotation

Genome annotations now include a sizable amount of computational analysis methods due to the growing amount of data from sequencing initiatives. Nevertheless, high amounts of miss annotation in public databases have resulted from this. Researchers must provide them with high-quality data since annotations are a resource for other annotation initiatives.

NCBI and other sequencing centres have created international annotation standards to guarantee high-quality data. Re-annotation is essential for fixing specific incorrect annotations. Still, it is also done for various reasons, including finding novel genes or protein activities, contrasting new and old annotation techniques, and determining the repeatability of annotations—the end-user benefits from re-annotation by getting the most recent resources. Genome annotations must be updated and re-annotated to remain accurate and valuable since downstream research in comparative genomics, transcriptomics, proteomics, and metabolomics always adds to our understanding of gene products. Large entire genomes may be created by re-annotation; specific methods can be utilised.

Restauro-G is bacterial genome re-annotation software that uses a BLAST-like alignment method for re-annotation.

MAKER2 has a method for external annotation pass-through that will accept as inputs aligned experimental data in GFF3 format and pre-existing genome annotations

Applications of genome annotation in Crop breeding

- ❖ Molecular sequence of a particular phenotype and also identifying the gene sequence by knowing its function.
- ❖ Development of primers using annotated data.
- ❖ To knock down or knockout gene function by means of CRISPR-Cas technology.
- ❖ Development of resistant or superior events for abiotic and biotic stress through transgenic or cisgenic approaches.

Limitations

- The assembly quality will heavily influence the quality of the annotation
- SNP errors can change start/stop-codons.
- If a gene is *missing*, it will not be annotated.
- Poor conservation of repeats produces false annotation.
- Alternative isoforms, Pseudogenes, do not have suitable gene prediction methods

Conclusion:

Understanding a gene's structure is vital for comprehending its function and the importance of variants. Such endeavours can be carried out automatically using computational annotation methodologies like Ab initio and homology-based annotations. Given the vast volumes of sequence data generated by NGS, using automated annotation tools and processes is essential. Genome annotation is crucial for a variety of purposes, including addressing complex issues about evolution and developing new drugs and medical treatments. As mistakes can spread readily farther downstream, this calls for annotation quality control. Such errors can be prevented with the use of community annotations and quality-control techniques. Furthermore, re-annotation is required to uncover novel characteristics that earlier technologies missed, correct incorrect annotations, update previous annotations, and even identify well-studied genes with unique traits in some situations. These aspects cast annotation as a never-ending adventure as fresh viewpoints and technological advancements appear daily.

References

1. Ejigu, G. F. and Jung, J., 2020, Review on the computational genome annotation of sequences obtained by next-generation sequencing. *Biology*, 9(9): 295.
2. Keshri, R., Mir, T. T. and Kaur, H., 2020, Annotation of plant genome: A case study of *Oryza sativa*.
3. Jegadeesan, S., Raizada, A., Dhanasekar, P. and Suprasanna, P., 2021, Draft genome sequence of the pulse crop blackgram [*Vigna mungo* (L.) Hepper] reveals potential R-genes. *Scientific reports*, 11(1):1-10.

NON-CODING RNAs – CONCEALED BUT VITAL!

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Abstract:

According to the central dogma of molecular biology, the primary role of RNA is to convert the genetic information stored in DNA into proteins. In reality, there is much more to the RNA story. A large and significant portion of eukaryotic transcriptomes consists of noncoding RNAs (ncRNAs), which are not translated into functional protein. Although many challenges remain to be solved, application of regulatory ncRNAs can be used as tools to develop high yielding, stress-tolerant crop cultivars to address food insecurity problems. Some of its features, applications and short comes are discussed in this book chapter.

Introduction:

Following important findings like the identification of DNA as genetic material in 1940s and in early 1950s discovery of DNA's double helical structure paved the way for the development of molecular biology. Cell biologists have concurrently examined the relationship between the rate of protein production in a cell and the quantity of cytoplasmic RNA. Following the physicist Gamov's hypothesis regarding the triplet genetic code and the potential role of RNA in the transfer of information from DNA to proteins, Crick proposed the central dogma of molecular biology, which suggests the path of information transfer between nucleic acids and proteins with the restriction that the information cannot flow back from protein to nucleic acids.

In the 1960s, many observations of a wide diversity of heterogeneous nuclear RNAs were against the favour on proteins as the prioritized phenotypic factors. They have been stifled by the ideas of selfish and junk DNA, which have hindered understanding of the multifaceted roles that RNAs actually play in maintaining complex biological networks.

The discovery of RNA led scientists to develop the RNA World theory, which states that prebiotic life revolved around RNA, since it appeared before DNA and protein. Indeed, the extensive studies of its roles in cell biology revealed that RNA is necessary for DNA replication and that its ribonucleotides are precursors for DNA’s deoxyribonucleotides. Moreover, RNA plays an important role in every step of protein synthesis. A large and significant portion of eukaryotic transcriptomes consists of noncoding RNAs (ncRNAs), is an RNA molecule that is not translated into a protein.

Thus, for almost two decades, these non-coding RNAs were generally disregarded. In the new millennium, discussions about the potential significance of non-coding RNAs were revived in response to the discovery of RNA interference and the sequencing of several eukaryotic genomes. Therefore, there is an urgent need for more productive research into the roles played by these unique classes of genes.

Different classes of RNA

1. **Coding RNA:** Those RNA molecules which are translated into functional protein generally refers to mRNA
2. **Non coding RNA:** RNA molecule that is not translated into functional protein
    - Different classes of RNA are depicted in the Figure 1

➢ Two types of non-coding RNAs have been recognized

1. House-keeping non-coding RNAs
2. Regulatory non-coding RNAs
    - Housekeeping non-coding RNAs includes

(i) transfer RNA (tRNA)
(ii) ribosomal RNA (rRNA)
(iii) small nucleolar RNA (snoRNA)
(iv) small nuclear RNA (snRNA)

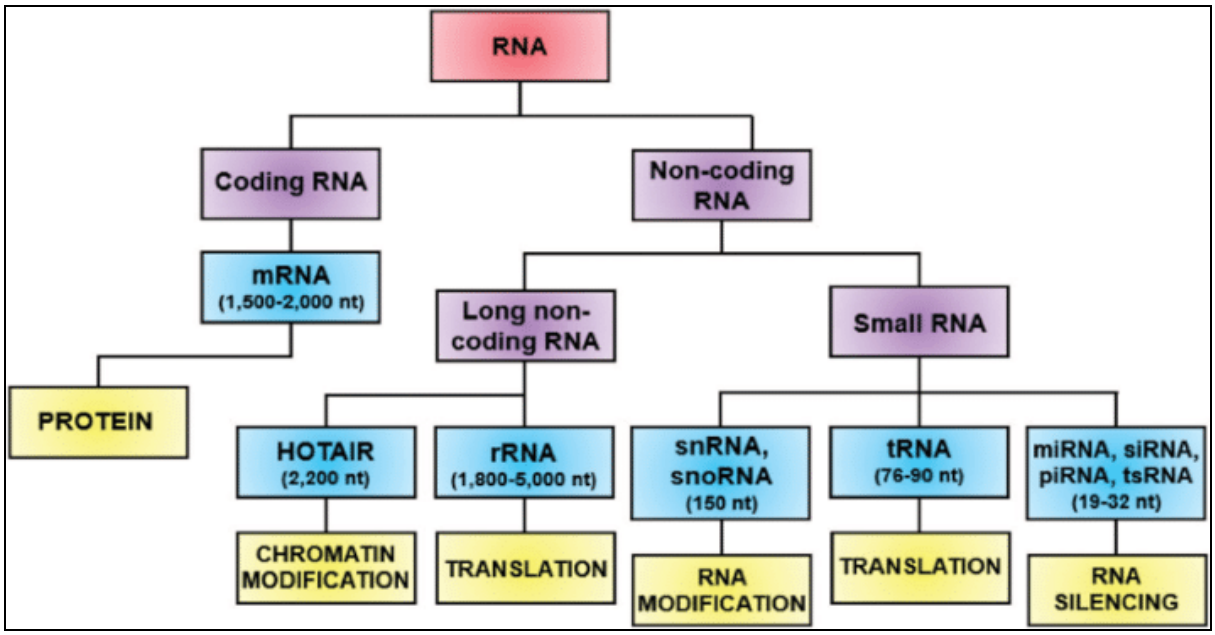


Fig. 1: Different classes of RNAs

- Regulatory non coding RNAs: These are non-coding RNAs which play essential regulatory roles in biological processes at the transcriptional and posttranscriptional levels. There are two kinds of regulatory non-coding RNAs which includes,

1. Small non-coding RNA: These are regulatory non-coding RNAs which are having size less than 200 nucleotides length. There are different kinds of small non-coding RNAs which includes:

a. microRNAs (miRNA
b. small interfering (siRNA)
c. piwi interacting RNAs (piRNA)

2. long non-coding RNAs: These are regulatory non-coding RNAs which are of length more than 200 nucleotides.

- **House-keeping non-coding RNAs:**

These are constitutively and abundantly expressed in cells, primarily they will regulate genetic cellular functions

i. Transfer RNA (tRNA)

A transfer RNA (abbreviated tRNA and formerly referred to as sRNA, for soluble RNA) is an adaptor molecule composed of RNA, typically 76 to 90 nucleotides in length.

- The tRNA structure can be divided into three parts: the primary structure, the secondary structure (which is sometimes represented as the cloverleaf structure), and the tertiary structure, which is the L-shaped three-dimensional structure that enables tRNAs to fit into the P and A sites of the ribosome (FIGURE 2). A frequent RNA tertiary structure motif, coaxial stacking of the helices transforms the cloverleaf structure into the three-dimensional (3D) L-shaped structure. Each arm's length and the loop's "diameter" in a tRNA molecule differ depending on the species.

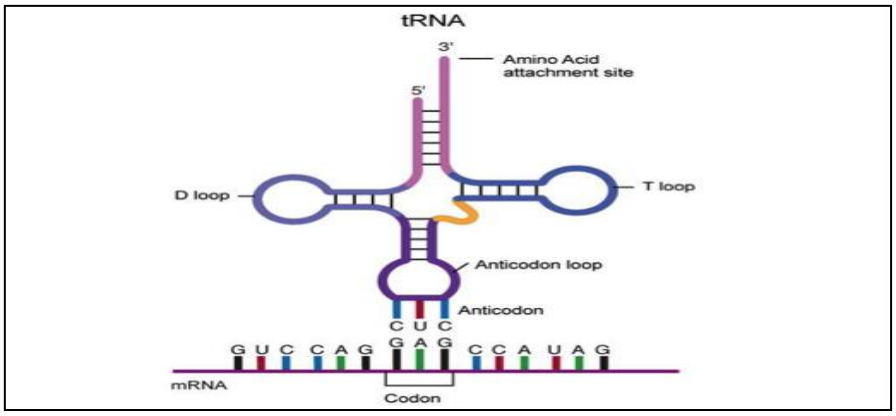


Fig. 2: Structure of tRNA

- The mRNA and the proteins' amino acid sequence are connected physically through tRNA. Transfer RNA accomplishes this by transporting an amino acid to a cell's ribosome, which is controlled by the complementary recognition of a mRNA 3-nucleotide sequence (codon) by a tRNA 3-nucleotide sequence (anticodon) of the tRNA. tRNAs are thus an essential part of translation, which is the biological production of new proteins in line with the genetic code.

ii. Ribosomal RNA (rRNA)

Ribosomal ribonucleic acid (rRNA) is a type of non-coding RNA which is the primary component of ribosomes, essential to all cells. rRNA is a ribozyme which carries out protein synthesis in ribosomes.

To create the small and large ribosome subunits, ribosomal RNA must be transcribed from ribosomal DNA (rDNA). Transfer RNA (tRNA) and messenger RNA (mRNA) must be processed and translated into proteins by the ribosome, which is physically and mechanically governed by rRNA. The most common type of RNA present in most cells is ribosomal RNA, which accounts for around 80% of cellular RNA despite never being translated into proteins by itself. By mass, ribosomes are made up of roughly 60% rRNA and 40% ribosomal proteins, eukaryotic and prokaryotic rRNA are shown in figure 3.

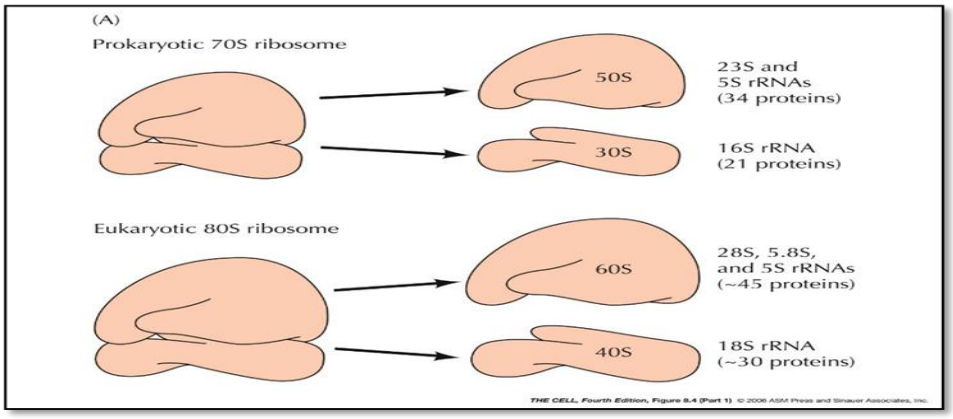
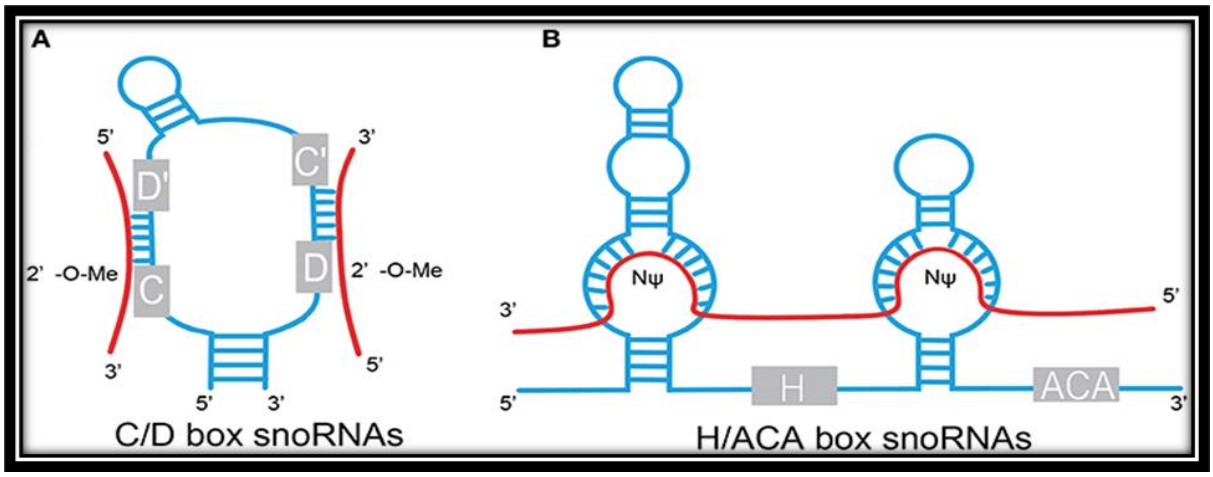


Fig. 3: prokaryotic and eukaryotic rRNA

iii. Small nucleolar RNAs (snoRNAs)

These are extensively studied non-coding RNAs that are primarily accumulated in nucleoli. These are mostly responsible for post-transcriptional modification and maturation of ribosomal RNAs, tRNAs, SnRNAs and circular RNAs.



There are mainly two classes of SnoRNA which are classified based on conserved motifs (depicted in the figure above):

(i) C/D box SnoRNAs: Which are associated with methylation

(ii) H/ACA box: Which are associated with pseudouridylation.

- Each snoRNA molecule acts as a guide for only one (or two) individual modifications in a target RNA. In order to carry out modification, each snoRNA associates with at least four core proteins in an RNA/protein complex referred to as a small nucleolar ribonucleoprotein particle (snoRNP). The snoRNA molecule contains an antisense element (a stretch of 10–20 nucleotides), which

  are base complementary to the sequence surrounding the base (nucleotide) targeted for modification in the pre-RNA molecule. This enables the snoRNP to recognize and bind to the target RNA. Once the snoRNP has bound to the target site, the associated proteins are in the correct physical location to catalyse the chemical modification of the target base.

iv. Small nuclear RNAs (SnRNAs)

Many of the functions of the spliceosomes are performed by RNAs which are called SnRNAs that is catalysers of the splicing reaction.

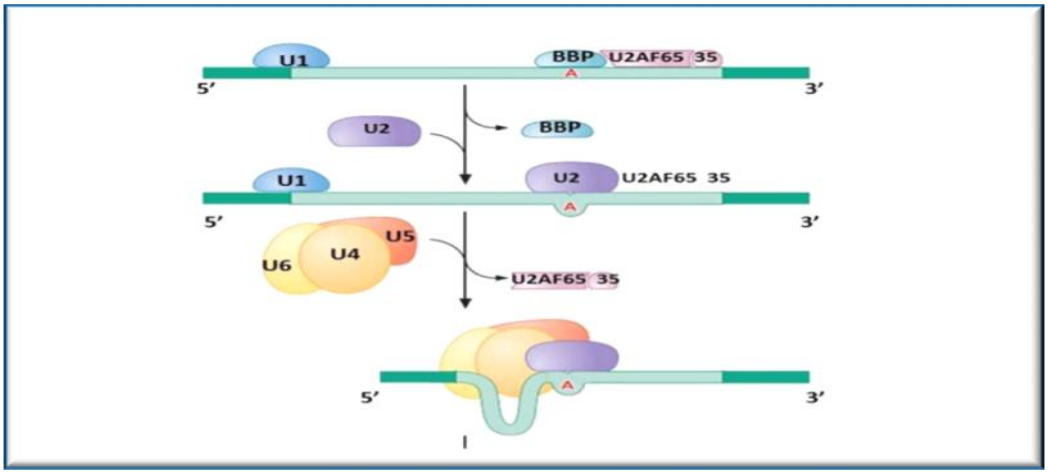


Fig. 4: Types of SnRNAs

SnRNAs come in five different types: U1, U2, U4, U5, and U6. These are complexed with numerous proteins and range in length from 100 to 300 nucleotides. Small nuclear ribonuclear proteins (SnRNPs) are the RNA-protein complexes, which appear and disappear at various periods or stages of the splicing reaction (FIGURE 4).

It serves a crucial function in identifying the branch and 5' splice sites, which means that they catalyse the RNA cleavage and joining events. Interaction between SnRNPs and mRNA occurs during splicing. U1 first interacts with the 5' splice site through complementary base pairing, and U6 SnRNA then recognizes the splice site. These rearrangements bring the splice site and branch site together, which drives the splicing process and improves its precision.

- **Regulatory non coding RNAs (ncRNAs)**

Regulatory non-coding RNAs (ncRNAs) regulate target gene expression through a variety of molecular processes and are composed of various subgroups of small RNAs and long non-coding RNAs. Recent research on regulatory ncRNAs in plants has revealed that these RNAs mainly serve as master regulators, modulating a number of gene regulatory networks by regulating a specific subset of downstream genes that are closely related to agricultural traits, such as seed maturation, floral development, pathogen resistance, and other abiotic stress resistance.

These non-coding RNAs may regulate the gene expression through two different mechanisms:

1. **Post-transcriptional gene silencing:** Involves sequence-specific RNA degradation through RNA interference
2. **Transcriptional gene silencing:** Through RNA directed DNA methylation

Components of gene regulation pathways:

The production of regulatory non-coding RNAs that are 20 to 26 nucleotides long is the first step in the suggested RNA silencing mechanism, such as

1. Dicer- Like Proteins (DCLs)
2. Argonaute (AGO) proteins

3. RNA- Dependent RNA Polymerase (RDR) enzymes
4. Double stranded RNA binding proteins (dsRBPs)

1.Dicer

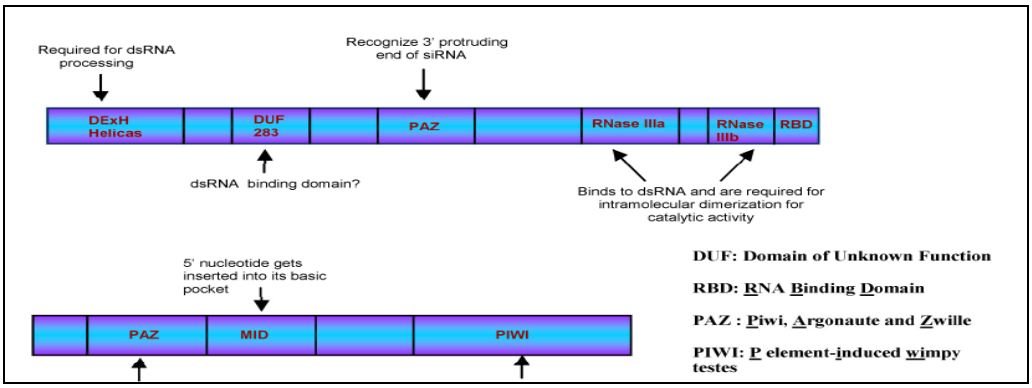


Fig. 5: Dicer and its structure

- Dicer also known as endoribonuclease dicer or helicase with RNase motif which is encoded by RNase III endonuclease enzyme. Dicers are the prime molecules in the generation of these small non-coding RNAs by cleavage of double stranded RNA into small non-coding RNAs. These proteins generate dsRNAs suitable for loading onto an Argonaute protein
- There are mainly four distinct domains namely, an amino terminal helicase domain, dual RNase III motifs, a dsRNAbinding domain and a PAZ domain

  RNA binding domain: recognizes duplex RNA structure

  PAZ domain: Binds to 3^1 2nt overhangs of the cleaved RNA substrate RBD and PAZ domains participate in association with RNA molecule and assist both the RNAse III domain to form intramolecular dimer. These RNase III juxtapose in a manner to cleave the 21nt duplex RNA molecules from the dsRNA precursors. In case of animals usually they encode a single type of dicer to generate various classes of small RNAs with exceptions of drosophila and *Caenorhabditis elegans* each encoding two dicers.

DCL family proteins (DCL1-4) are present in the genomes of plants in at least four different classes. Because defects in one class of DCL can occasionally be made up by compensating in other classes, each class of DCL has evolved to engage in its principal route, although the three siRNA-producing DCLs (DCL2-4) also perform redundant functions (FIGURE 6).

1. **DCL1:** only protein that creates the majority of 21-nt miRNAs is DCL 1
2. **DCL2:** is necessary for secondary siRNA mediated transitive silencing and can replace DCL4 loss.
3. **DCL3:** produces 24-nt repeat-associated siRNAs generated from transposons and DNA repetitive elements, and engages in transcriptional gene silencing (TGS) through RNA-dependent DNA methylation.
4. **DCL4:** a significant producer of endogenous siRNAs, including trans-acting siRNA (tasiRNA) and phased siRNA (phasiRNA), and 21-nt antiviral siRNA

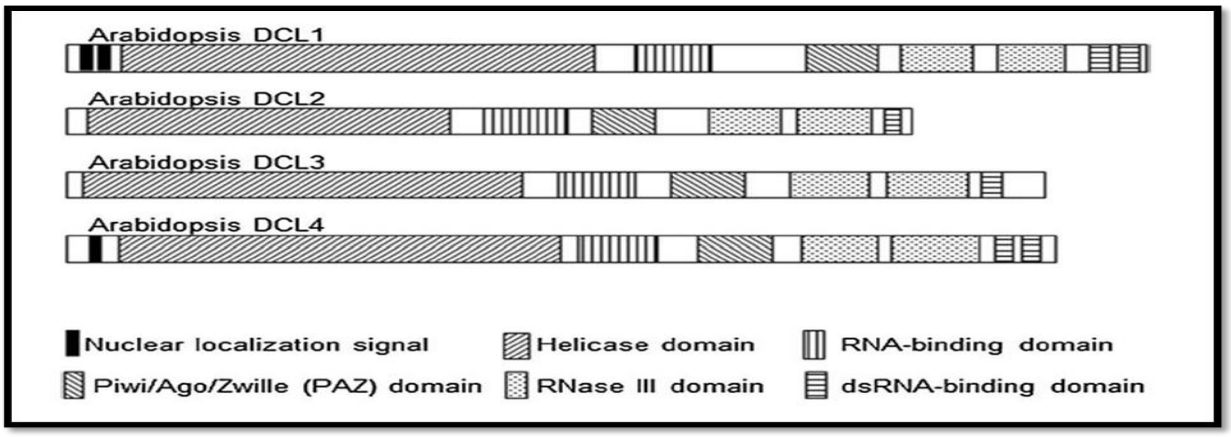


Fig. 6: Different classes of DCL proteins in *Arabidopsis thaliana*

2. Argonautes

- These are the proteins recruited by dicers. Which plays a central role in silencing process and it is an essential component of RNA induced silencing complex.
- • AGO proteins adopt a bilobal design that consists of a middle (MID) domain and a PIWI domain in the C terminal lobe and an N-terminal lobe with an N-terminal domain and a PAZ domain.
- • Argonautes helicase activity unwinds the duplex RNAs. To create a mature RISC, pre-RISC (in which AGO proteins bind with RNA duplexes) quickly eliminates the passenger strand.

3. Double stranded RNA binding proteins (dsRBPs)

- Dicing and RISC loading are aided to varying degrees by dsRBPs, which associate with a Dicer protein and typically comprise two or three dsRBDs.
- The dsRBD is widespread and typically recognizes dsRNA on the basis of its A-form helical shape with moderate to high affinity and in a sequence-nonspecific manner
- Proteins involved in a dsRNA handoff between Dicer and Argonaute

4. RNA-Dependent RNA Polymerase

Play a role in both triggering and amplifying the silencing effect. These aberrant RNAs may be recognised by the RdRP enzymes as templates, leading to the synthesis of antisense RNAs that ultimately serve as the targets for sequence-specific RNA destruction. In Arabidopsis, RDR1, RDR2, RDR6 have been implicated in the biogenesis of SiRNAs. RDR2 is a crucial player in biogenesis of heterochromatic SiRNAs.

RNA Interference (RNAi)

RNAi is a powerful, conserved biological process through which small, double-stranded RNAs specifically silence the expression of homologous genes, largely through degradation of their cognate mRNA.

Historically RNAi known by other names, including

- **PTGS** in plants
- **Transgene silencing** in animals and
- **Quelling** in fungi

Evolution of RNAi

In 1990- Napoli and coworkers first discovered **RNAi phenomenon** in transgenic plant *Petunia hybrida L.* by enhancing **anthocyanin pigments** in Petunia by the introduction of **chalcone synthase gene (CHS A)** encoding key enzymes in anthocyanin biosynthesis pathway.

- Unexpectedly, 42 % of plants with the introduced CHS gene produced totally white flowers and/or patterned flowers with white or pale nonclonal sectors on a wild-type pigmented background due to the silencing of endogenous homologous gene and this phenomenon was termed as **"Co-suppression.**

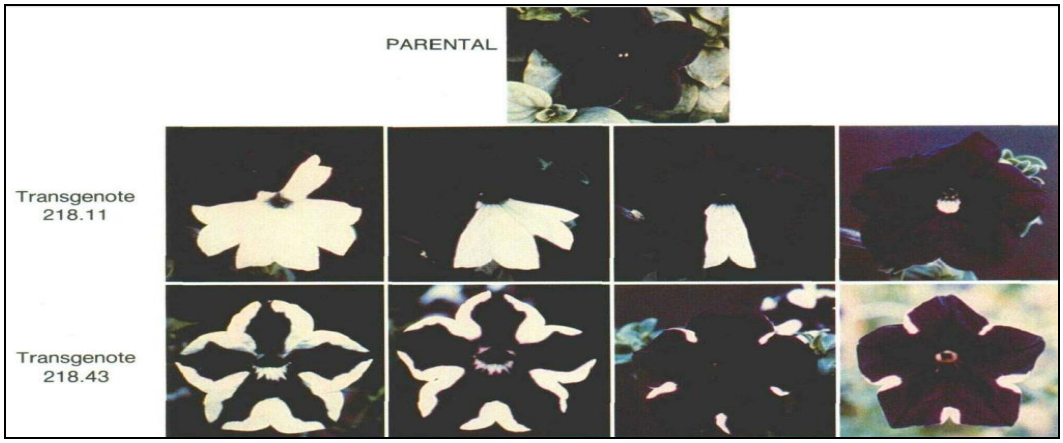
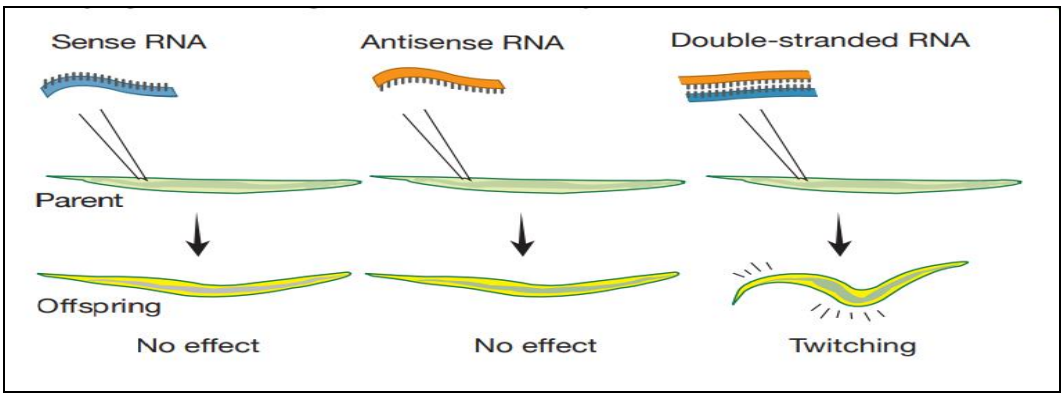


Fig. 7: Anthocyanin pigments in Petunia

- They concluded that Transgene loci often directly produced dsRNA as a consequence of imperfect integration events that included juxtaposed sense–antisense transgenes. PTGS efficacy was greatly enhanced by simultaneous sense and antisense expression or by direct production of long dsRNA from inverted-repeat (IR) transgenes

1998 – RNAi in nematodes (*C. elegans)* was discovered by Fire and coworkers working with the introduction of antisense RNA to silence gene that regulate embryo symmetry.



Simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA that interfere with the function of an endogenous gene.

They have injected sense strand, antisense strand and mixture of both sense and antisense strand into the body of adult nematodes, they found that double stranded RNA was substantially producing interference than either strand individually. After purification they found purified single strands had atmost a modest effect whereas double strand mixtures caused potent and

specific interference, depicted in above figure. For this achievement they are honoured with Nobel prize in physiology or medicine in the year 2006.

1. microRNA (miRNA)

Endogenous non-coding short RNAs like microRNA (miRNA), which have a length of 20 to 24 nt, post-transcriptionally suppress the expression of their target genes. Most plant genomes contain 100–300 miRNA (MIR) genes, and miRNAs are produced from single-stranded hairpin precursors with lengths of 64–303 nt (Figure 8).

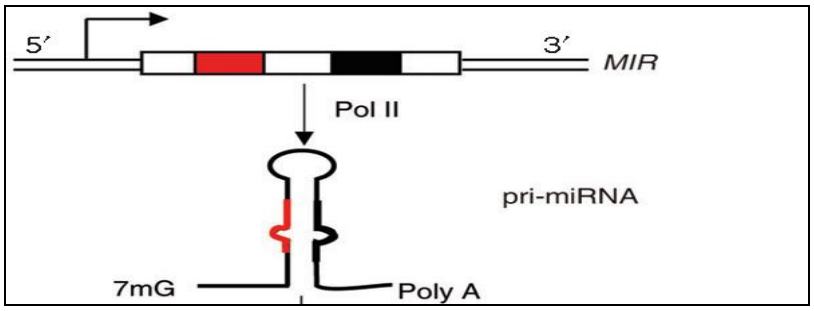


Fig. 8: Structure of miRNA

The pri-miRNAs are folded into hairpin-like structures consisting of a terminal loop, an upper stem, the miRNA/miRNA* region, a lower stem, and two arms (Pathak and Gogoi, 2016).

Origin:

1. The target gene sequences that make up miRNAs undergo inverted duplication events.
2. Spontaneous evolution - Small to medium-sized fold-back sequences found throughout the genome give rise to miRNAs.
3. Miniature inverted-repeat transposable elements (MITEs), which are non-autonomous elements of the DNA type, can easily fold into the defective stem-loop structures of miRNA precursors.

miRNA Pathway

The biosynthesis of miRNAs occurs in the nucleus, where specific mir genes are transcribed by RNA polymerase II into protracted primary transcripts, or pri miRNAs, which are typical transcripts with caps at the 5' and polyadenylated at 3' ends.

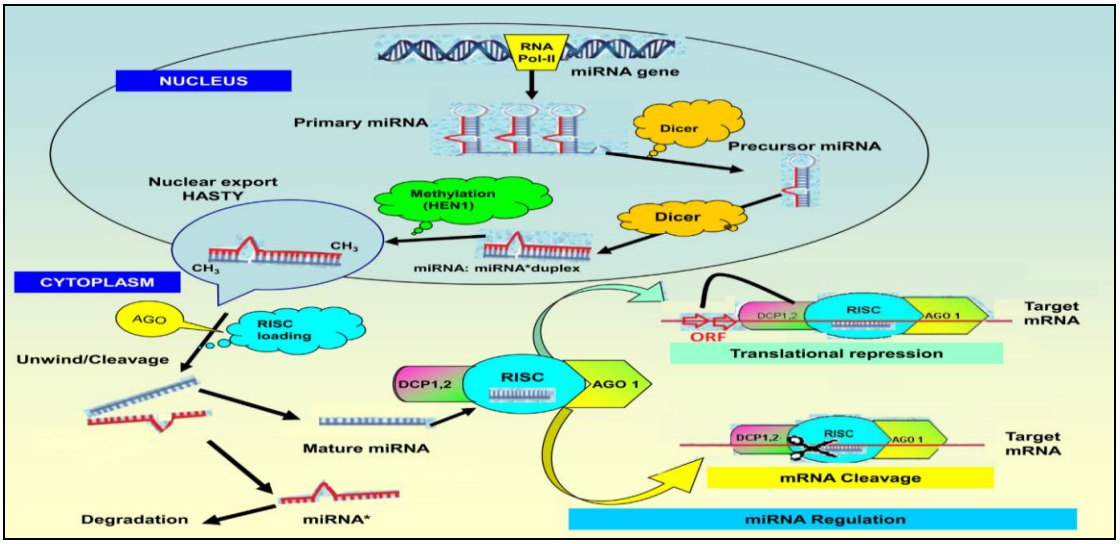


Fig. 9: microRNA (miRNA) pathway

The pri-miRNAs are broken down into pre-miRNAs by RNase III-like enzymes termed dicer like (DCL1) in collaboration with other proteins like HYL1 and serrate (SE) proteins

⬇

DCL1 performs additional processing on hairpin looping structures to produce miRNA: miRNA* duplexes in the nucleus

⬇

The methyltransferase enzyme methylates the duplexes at the 3' end

⬇

The exportin protein HASTY transports the miRNA duplexes to the cytoplasm

⬇

After the duplexes are incorporated into the RNA-induced silencing complex (RISC), which contains AGO proteins, the miRNA duplexes are largely unravelled by AGO 1

⬇

While mature miRNA is integrated into RISC that contains AGO, other strand of the duplex miRNA is sent to exosomes for degradation. Finally, mature miRNA directs the RISC to target complementary Mrna

Modes of action

Target cleavage: Degradation of the target mRNA occurs as a result of perfect miRNA-target pairing.

Translational inhibition: Target mRNA may be miRNA mismatched or poorly matched to the target mRNA, which results in decapping and destruction of the target mRNA, or it may be deadenylated.

Classification of miRNA

1. **Conserved miRNA:** These are abundantly expressed and targets transcription factors that directly regulate the gene expression. These are considered to be stable during the process of evolution
2. **Non-conserved miRNA:** These are weakly expressed and imperfectly processed without tractable targets and hence they are considered to be randomly evolved with a limited number of biological functions.

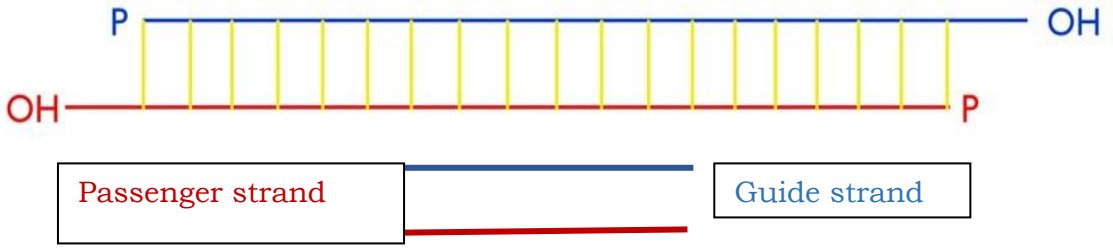
Biological functions of miRNAs in plants

1. **Phase transition:** miR156 is one of the most ancient miRNA family present in almost all plant lineages. In case of Arabidopsis, SPL genes are targeted by these miRNAs and promote vegetative phase change as well as floral transition.
2. **Lateral root formation:** miR172 in Arabidopsis and maize target NAC1 domain which are involved in the formation of lateral roots
3. **Grain formation:** miR156 SPL is responsible for grain formation in maize and also regulates tillering and panicle branching in rice
4. **Floral development:** miR172 is also one of the important microRNA family which promotes flowering in Arabidopsis by repressing TOE1 and TOE2

5. **Lodging resistance:** miR528 is a monocot specific miRNA which is induced by nitrogen luxury conditions in maize and regulates lodging resistance by targeting lignin biosynthesis gene *ZmLACCASE3* and *ZmLACCASE5*.

2. Small interferring RNAs

The small double stranded RNAs (21–25nt) were produced from double stranded RNA (500bp). A siRNA is a synthetic RNA duplex that is intended to target a specific mRNA for destruction. Since small RNAs are the direct byproducts of genes, they have the ability to attach to particular other RNAs and alter their activity by blocking messenger RNA from translating to protein. The most popular RNA interference (RNAi) technique for short-term protein coding gene silencing is siRNA (Chen, 2009).



Biogenesis

- ✓ siRNAs are generated from dsRNAs derived from RNA virus replication
- ✓ dsRNAs synthesized by plant endogenous RNA-dependent RNA polymerase (ra-siRNAs, ta-siRNAs)
- ✓ dsRNAs of natural sense and antisense transcripts (Nat-siRNAs)
- ✓ Single-stranded RNAs that form hairpin-loop secondary structures

siRNA mediated RNAi

The siRNA provides target specificity to RISC through base pairing of guide strand with the target mRNA. Only one of two strands which is known as guide strand, directs the gene silencing and the other is degraded during RISC actvation. Active components of an RNA-induced silencing complex (RISC) are endonucleases called argounate proteins, which cleave target mRNA strand complementary to their bound siRNA.

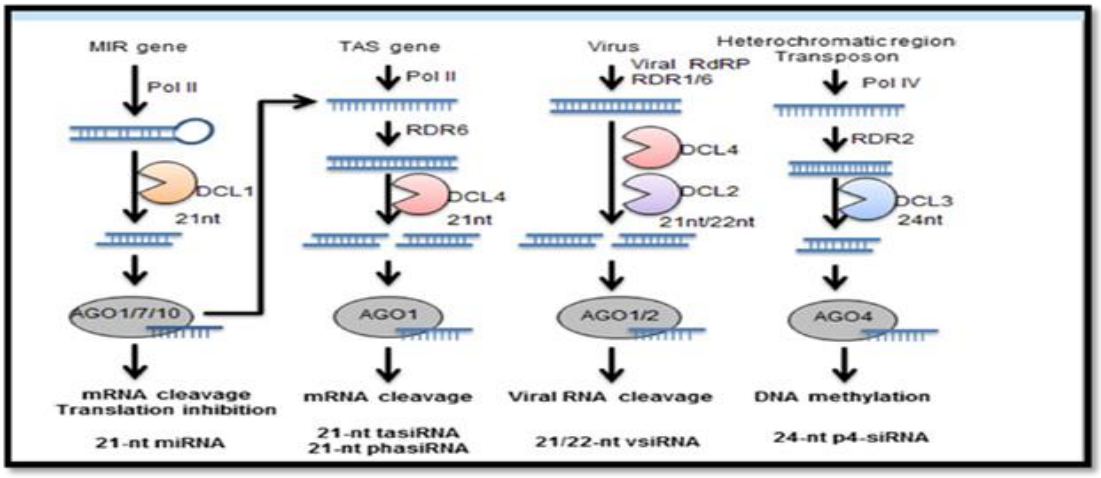


Fig. 10: Different kinds of siRNA pathways by different biogenesis

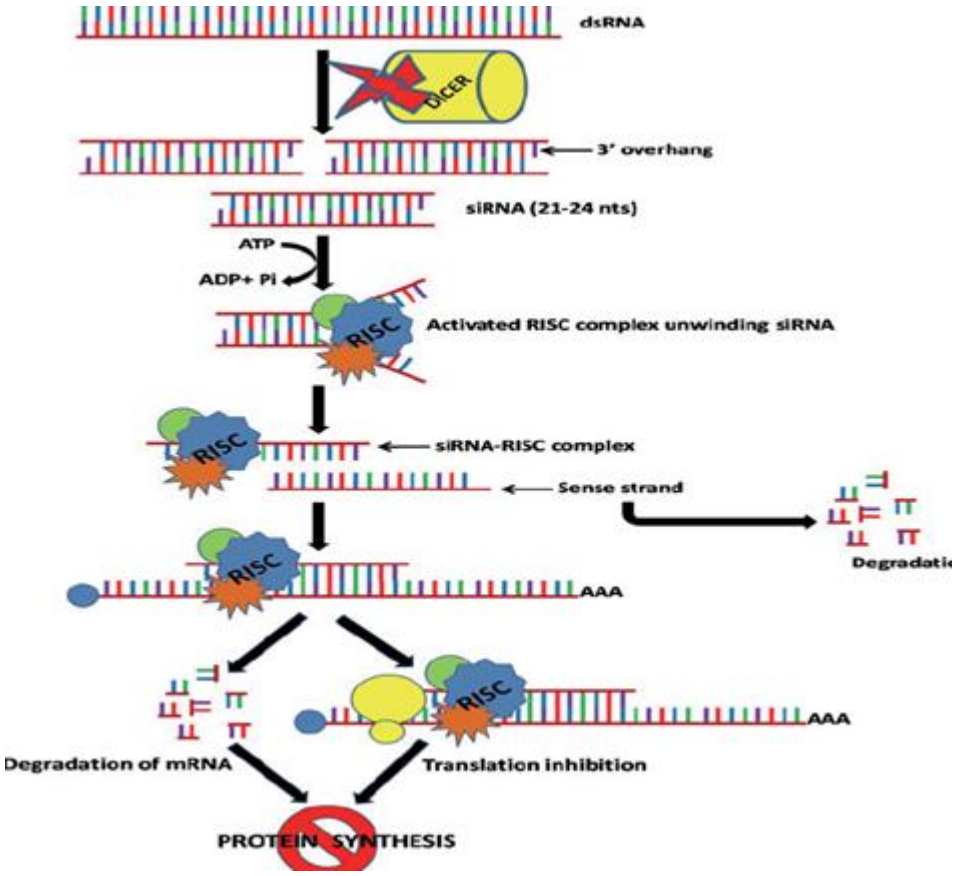


Fig. 11: Small interfering RNA (siRNA) pathway

There are different kinds of siRNA pathways that is by different biogenesis.

- **Trans acting siRNA:** Endogenous ta-siRNAs act via hetero-silencing, which means that the genes they target for cleavage and repression do not have much resemblance to the genes from which the siRNAs derive. This differs from other endogenous siRNAs which are cis-acting and perform auto-silencing, repressing the expression of genes that are the same as or have a lot of resemblance to the genes from which they derive. It was previously thought that only miRNAs exhibited hetero-silencing. Like other siRNAs, the ta-siRNAs are incorporated into RNA-induced silencing complexes (RISCs), where they guide the complex to cleave the target mRNAs in the middle of a single complementary site and repress translation.

➢ **Virus mediated gene silencing:** One of the novel reverse genetic approaches. One of the most significant defense mechanisms used by plants against viral infections is siRNA-mediated gene silencing.

Long dsRNAs are produced from viral RNA intermediates. Once these dsRNAs have been identified, dicer cleaves them into duplex short RNAs. Origin of foreign mRNA is encountered by the RISC complex.

Viral counter defense

Two mechanisms governed by the inhibition of RNA silencing by VSR are.,

(1) Some VSRs bind to short or long dsRNAs to sequester tiny RNA duplexes, which suppresses the assembly of AGOs into RISC.

(2) Some VSRs physically bind with AGO1 in order to block the loading of siRNA or miRNA, obstruct slicing activity, or destroy the AGO1 protein.

Functions of siRNA

Major function of siRNA is that it helps in viral defense

1. In Arabidopsis trans-acting siRNAs such as TAS1, TAS2 are targeted by miR173 to produce HTT1 and HTT2 to regulate plant thermotolerance**.**

2. TAS1 and TAS4 are produced by miR828 which repress MYB genes regulating anthocyanin biosynthesis

3. TAS3 target ARF embryo development, developmental transitions, leaf morphology, flower and root architecture, stress responses

RNA-directed DNA methylation (RdDM)

It is another important regulatory pathway which has involved in transcriptional gene silencing. It is a conserved phenomenon in case of fungi, plants and animals and it is best characterized in angiosperms particularly Arabidopsis. It is a biological process in which non-coding RNA molecules direct the addition of methyl group to specific DNA sequence.

In transposons and other DNA repeat regions aberrant SSRNAs are produced by DNA dependent RNA polymerase IV. The chromatin remodeling protein CLSY facilitate pol IV transcription

↓

RDR2 converts aberrant SSRNAs into dsRNAs which are then cleaved into 24nt siRNAs by DCL3 and these are bound by argonoutes

↓

siRNAs colonize with IGN transcripts produced by pol V generates single stranded RNA transcripts. Transcription requires DRD1, DMS3, RDM1 form a stable protein complex named DDR

↓

KTF1 tethers AGO4, Polymarase V and RNA transcripts to form Rddm effector complex

↓

The effector complex directs denovo DNA methylation transferase DRM2 to specific chromatin regions to catalyse new DNA methylation

RNA dependent DNA methylation implicated in a number of regulatory processes in plants. Since DNA methylation patterns are in plants are heritable, these are stably transmitted to the progeny. Hence one of the prominent roles of RDDM is transgenerational suppression.

3. PIWI interacting RNAs (PiRNAs)

- These are small non-coding RNA molecules derived from long single stranded precursor molecules. 21 to 35 nucleotides length and were first identified in *Drosophila melanogaster*.
- piRNA complexes are mostly involved in the epigenetic and post-transcriptional silencing of transposable elements and other spurious or repeat-derived transcripts especially silencing of transposable elements in germlines.

4. Long non coding RNAs (lncRNAs)

These are also regulatory non-coding RNAs which are having size more than 200 nucleotides in length. They have similar characteristics with mRNAs transcribed by RNA pol II, there are another class of ncRNAs transcribed by pol IV and pol V which plays a role in transcriptional gene silencing mediated by RNA dependent DNA methylation (Zhang and Chen, 2013).

- Long ncRNAs can originate from any location throughout the genome. Some lncRNAs are transcribed from introns or intergenic regions, and others overlap with protein-coding regions.
- 9000 plant lncRNAs have been annotated but only less than 1 % Characterized.

Classification of long non-coding RNA:

- **Sense long ncRNA:** These are long ncRNA which are present on the same strand of protein coding regions
- **Anti-sense long ncRNA:** which are present on the opposite strand of the protein coding regions
- **Bidirectional long ncRNA:** located on the opposite strand of the protein coding genes whose transcription is initiated 1000bp away
- **Intronic long ncRNA:** which are transcribed from intronic region
- **Intergenic long ncRNA:** which are transcribed from that particular region which is located between the two genes

Regulation pathways:

1. **Chromatin remodeling:** The most notable way that mammal lncRNAs control gene expression is through mediating chromatin remodelling. LncRNA COLDAIR also performs this regulatory pathway's function in plants.
2. **Endogenous target mimics (eTMs):** Plants' second regulatory mechanism for lncRNAs may act as a miRNA decoy. To prevent a specific transcript from binding, eTMs bind to the appropriate miRNA, which increases mRNA expression.

Biological functions of long non-coding RNAs

1. **Vernalization:** FLC locus is repressor of flowering, suppression of this gene by long ncRNAs namely COLDAIR/COOLAIR through histone modification leads to increased flowering
2. **Nodule formation:** Enod40 (early nodulin40) regulates symbiosis between microbes and legumes for nodule organogenesis
3. **Photosensitive male sterility:** LDMAR is responsible for male fertility under long day conditions in rice and regulation of this leads to photoperiod male sterility in rice.
4. **Photomorphogenesis:** It refers to how light affects plant growth and development. The Phytochrome Interacting Factor (PIF3) protein is negatively regulated by the lncRNA known as Hidden Treasure (HID1), while photomorphogenesis is positively regulated.
5. **Phosphate uptake:** The healthy growth and development of the plants depend on phosphorus. The phosphate absorption or homeostasis process in plants is controlled by the protein PHOSPHATE2 (PHO2), which codes for the ubiquitin-conjugating E2 enzyme. One of the

known miRNAs, mir399, is in charge of suppressing the PHO2 gene, which lowers phosphate uptake. The lncRNA IPS1 (Induced by Phosphate Starvation1) is activated to prevent miR399's repression and to support the phosphate absorption pathway. The target mimic IPS1 serves as for miR399 results in negative regulation.

Conclusion:

Regulatory non-coding RNAs are widely recognized as genomic resources and as novel material of genetic engineering of crop traits. However only small portion of plant ncRNAs are explored and their functional roles in plants have been revealed and characteristics of many other regulatory non-coding RNAs still remain to be understood and in addition to this development of molecular techniques and precise manipulation is still in progress. In the near future these regulatory ncRNAs will be more frequently deployed in molecular breeding area to exploit high yielding and stress tolerant crops. Even though many challenges remain to be addressed, focusing our interests on the potency of these ncRNAs and their systematic utilization could be worthwhile in crop improvement.

References:

1. Chen, X., 2009, Small RNAs and Their Roles in Plant Development. *Annu. Rev. Cell Dev.Biol*. 35:21–44.
2. Pathak, K. and Gogoi, B., 2016, RNA interference (RNAi): Application in crop improvement: A review. *Agricultural reviews*, 37(3): 245-249.
3. Zhang, Y. and Chen, Y., 2013, Long noncoding RNAs: New regulators in plant development. Review-*Biochemical and Biophysical Research Communications* 436: 111–114.

EDIBLE PACKAGING MATERIALS

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Introduction:

Fish is one of the most-traded food commodities worldwide. Capture fisheries and aquaculture provide valuable economic and social benefits to those who work in these industries. However, post-harvest handling, processing, and storage of fish lead to food losses and waste. Post-harvest losses occur at all stages in the fish supply chain from capture to consumer. The losses can be physical, economical, or nutritional and are caused by spoilage or poor processing. So, specific requirements and preservation techniques are needed to minimize the activity of spoilage bacteria.

Packaging plays a critical role in the fish supply chain and is part of the solution to tackle food waste. Vacuum packaging (VP) and modified atmosphere packaging (MAP) are very commonly used as a supplement to ice or refrigeration to inhibit the normal spoilage flora and extend the shelf-life of fresh fish products.

Food packaging

Food packaging is an essential component of the food supply chain and is becoming a pivotal element of the final preparation process in food industries. Food packaging also plays an imperative role in society, protecting food and food products from potential damage and degradation while ensuring safety and hygiene, and actively reducing food waste.

Conventional packaging is commonly a one-time use item that is discarded upon reaching the consumer or after using the packed content. Some of the most common conventionally used materials in food packaging include paper, plastic, glass, steel, aluminium and different alloys. As such, conventional packaging poses a tremendous environmental burden despite relatively high recycling rates for some materials (over 20% recycling rate for certain paper and paperboard), while others such as various plastics are commonly recycled at low recycling rates (less than 20%). One of the main issues is the non-sustainable nature of plastics, which are commonly derived from petroleum such as polyethylene, polypropylene, and polyethylene terephthalate are widely used due to their relatively easy shape-forming properties and lower weights than other materials.

Moreover, these materials are considered as not ‘environmentally friendly’ with the majority of them being non-renewable and also non- biodegradable, which subsequently end up in the landfills or oceans. Additionally, the use of these materials for food packaging has other, secondary negative environmental impacts, such as environmental pollution via generation of CO_2 and emission of other toxicants during their incineration, reliance on non-renewable

petroleum reserves, and potential for harmful interactions between potential recycled/reused plastics and food.

Concurrently with the increased environmental concern regarding the growing rate of waste from packaging materials, current consumer demands and needs are directed towards more natural, high-quality, convenient and safer foods, posing a significant challenge to the food industry.

Smart packaging

Active and smart packaging has been used worldwide, mostly in the United States, Australia and Japan, while in Europe it was introduced after European Union (EU) legislation changes (Regulation EC, 1935/ 2004). The EU definition of active packaging states that active packaging systems are designed to "deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food". In this way, they "intend to extend the shelf-life or to maintain or improve the condition of packaged food". One of the significant emerging functions of edible packaging materials is their use as a matrix and carrier of different functional additives that can provide additional nutritive and health benefits to the packaged food. Usually, different antimicrobial and antioxidant substances, prebiotics or other nutrients can be added to edible matrices to extend shelf life and/or increase the nutritional value of the final packaged food.

Edible packaging is regarded as a sustainable and biodegradable alternative in the active food packaging field and provides food-quality optimisation compared to conventional packaging. The usefulness of edible packaging is seen in its capacity to maintain food quality, extend shelf life, reduce waste, and to contribute to the economic efficiency of packaging materials. The development and application of edible films are among the most promising fields in food science due to their versatility, potential for being made from a variety of materials, and as carriers of different active substances such as antioxidant and/or antimicrobial agents. The materials of the food packaging is derived from edible ingredients such as natural polymers that can directly be consumed by humans without any potential health risk. These materials can be transformed into different forms of films and coatings without specific differences in their material composition but rather by changes in their thicknesses. Films are generally used in the production of wraps, pouches, bags, capsules, and casings, while coatings are applied directly on the food surface. In contrast to the films, the coatings are considered an integral part of the food product, and they are typically designed not to be removed from the food item. Therefore, proper selection of edible packaging components mainly depends on the food product required to be packed, and the composition of the material that the edible packaging is developed from, including the method of processing. Moreover, the packaging should have sensory compatibility with the packed food.

Materials for edible packaging

Bio-based and biodegradable materials can be categorised into three categories based on the sources from which they originate as follows:

- Materials developed from direct biomass/natural sources (proteins, polysaccharides,

and lipids)

- Materials produced by microorganisms, usually belonging to specific types of polysaccharides
- Materials produced from bio-based monomers

Biopolymers used as edible materials are classified as Polysaccharides

- Proteins (animal- or plant-based)
- Lipids
- Composites

Characteristics of edible packaging: advantages and limitations

Edible packaging can function as a replacement and potential fortification of the layers at the outer surface of packaged fish and fishery products to prevent loss of moisture, aromas and ingredients out and between the foods, while at the same time, facilitating controlled exchange of essential gases involved in product respiration (carbon dioxide, oxygen, and ethylene).

Edible packaging can also enhance the organoleptic properties of packaged fish and fishery foods, providing various flavourings and colourings as well as tailoring surface properties (i.e. hydrophobicity, hydrophilicity). Additionally, these can serve as a carrier of functional components with potentially added health or well-being benefits. The hydrophilic nature of polysaccharides and proteins contribute to lower moisture resistance and barrier properties in comparison to lipids.

Barrier functions of edible packaging

The moisture and oil absorption, oxygen transfer, flavour and odour change, or the migration of packaging components into the food are primarily responsible for the food quality.
Edible materials are usually applied on fish and fishery products by immersion, spraying and coating or by being formed prior to a film and used as a food wrap. The difference between an edible film and coating is that coatings are applied in liquid forms, while films are obtained as a solid sheet and then applied to the food.

Conclusion and future prospective:

The emergence of new packaging technologies have enabled newly developed products to perform better than providing them with containment and physical protection. The future of edible packaging materials is very promising, and increased innovation within the food industry is both imminent and already occurring. Global consumer demands are a driving force for research and development of novel materials in order to find alternatives for fossil-based packaging materials. Their replacement with recyclable, biodegradable or edible materials, prepared from renewable and sustainable sources, are desired by consumers and the food industry alike. Several studies have incorporated known bioactive substances in edible packaging materials; the incorporation of a variety of novel functional bioactive and nutraceuticals and their controlled release from the packaging is a subject of many ongoing research studies.
Future studies should help to further develop coating technologies, and focus on optimising the formulation of packaging bio-based materials and their application technologies. Future developments of edible packaging solutions will also incorporate smart design packaging where

novel concepts such as active, intelligent, smart and sustainable solutions are integrated into one system resulting in improved safety and quality of the packed products.

References:

1. Aguirre-Joya, J. A., De Leon-Zapata, M. A., Alvarez-Perez, O. B., Torres-León, C., Nieto-Oropeza, D. E., Ventura-Sobrevilla, J. M. and Aguilar, C. N. (2018). Basic and applied concepts of edible packaging for foods. In *Food packaging and preservation* (pp. 1-61). Academic Press.
2. Barbosa, C. H., Andrade, M. A.,Vilarinho, F., Fernando, A. L., and Silva, A. S. (2021). Active edible packaging. *Encyclopedia*, *1*(2), 360-370.
3. Cuq, B., Aymard, C., CUQ, J. L., and Guilbert, S. (1995). Edible packaging films based on fish myofibrillar proteins: formulation and functional properties. *Journal of Food Science*, *60*(6), 1369-1374.
4. Janjarasskul, T., and Krochta, J. M.(2010). Edible packaging materials. *Annual review of food science and technology*, *1*, 415-448.
5. Petkoska, A.T., Daniloski, D., D'Cunha, N.M., Naumovski, N. and Broach, A. T. (2021). Edible packaging: Sustainable solutions and novel trends in food packaging. *Food Research International*, *140*, 109981.

STUDYING THE LUMINESCENCE OF Yb^{3+}/Ho^{3+} DOPED $CePo_4$ NANOPHOSPHORS THROUGH THEIR SYNTHESIS, CHARACTERIZATION, AND FABRICATION

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Abstract:

Polyol synthesis was used to create Yb^{3+}/Ho^{3+} co-doped CePO4 nanophosphors, which exhibit strong luminosity in both the UC and DC configurations. DC peaks were seen at 460, 550, 650, and 750 nm at an excitation wavelength of 300 nm. Ho^{3+}ions are seen to have a barely perceptible P-O Charge Transfer (CT) band. We found that upconversion (UC) nanophosphor $CePO_4$:Yb^{3+}/Ho^{3+} was also present. The upconversion emission spectra of the Ho^{3+} ion reveal a noticeable bright peak between 550 and 650 nm under intense 980 nm laser irradiation. The resulting nanocrystal materials are of excellent quality and measure in the tens of nm. $CePO_4$:Yb^{3+}/Ho^{3+} had a significant quantum yield at 300 nm excitation, according to the study. These results show how versatile the nanophosphor materials discussed in this method are, and they can be used to create highly efficient phosphors.

Keywords: Cerium Ion, Holmium Ion (Ho^{3+}), Photoluminescence (PL), Polyolmethod, Upconversion (UC), Ytterbium Ion (Yb^{3+}).

Introduction:

The bulk of lanthanide ion-doped materials produce visible light when excited by a near-infrared (NIR) laser. Materials based on rare earths (RE) are gaining popularity because they are easy to produce [1]. Solar cells, temperature sensors, spectral converters, and biological fields are just some of the many places you can find RE-doped nanomaterials in use. Up until recently, the protocol for mass producing up &down-conversion nanomaterials has been subpar. Rare-earth (RE) orthophosphates, due to their high thermal (up to 2200℃) and chemical stability, necessary optical properties, and limited solubility, are often cited as key hosts for the adsorption of nuclear waste [2]. Our most recent research describes the use of polyol techniques to manufacture Yb^{3+}/Ho^{3+} dual-mode converter nanomaterial $CePO_4$. As a result of this method's capacity to alter the size and shape, the luminous property following continuous wave (CW) laser stimulation can be improved, making $CePO_4$ nanoparticles a host for (DC)/(UC) luminescence [3]. The charge transfer (CT) process between O^{2-} and Ce^{3+}, which has a strong absorption at 300 nm and absorbs light from 240 to 280 nm, is responsible for this observable transition. The down-conversion is shown by the presence of sharper peaks in the emission spectrum at 460, 550, 650, and 750 nm, respectively, when UV light is used for excitation [4]. To boost the visibility of the Ho3+ ion emission in the spectrum, Yb^{3+} acts as a sensitizer. When

excited by near-IR light at around 980 nm, Ho^{3+} emits brightly. Indications from the $CePO_4$ up-conversion emission spectrum at room temperature [5]. We used a 980 nm laser to excite the material. CePO4:Ho^{3+}/Yb^{3+} nano phosphor was synthesized via a polyol-mediated method in this investigation. Excitations at 980 nm (due to Yb^{3+} absorption), 300 nm (due to indirect P-O) weak charge transfer band, (CTB), and 460 nm (due to Ho^{3+} absorption) have all been studied to get insight into its astonishing behavior [6]. The efficiency of energy transfer, both up and down, is discussed. When the synthesized nanomaterial's functional groups are activated, the particles' sensing qualities will improve, making them more useful in fields including optics, displays, cybersecurity, and microbial systems [7].

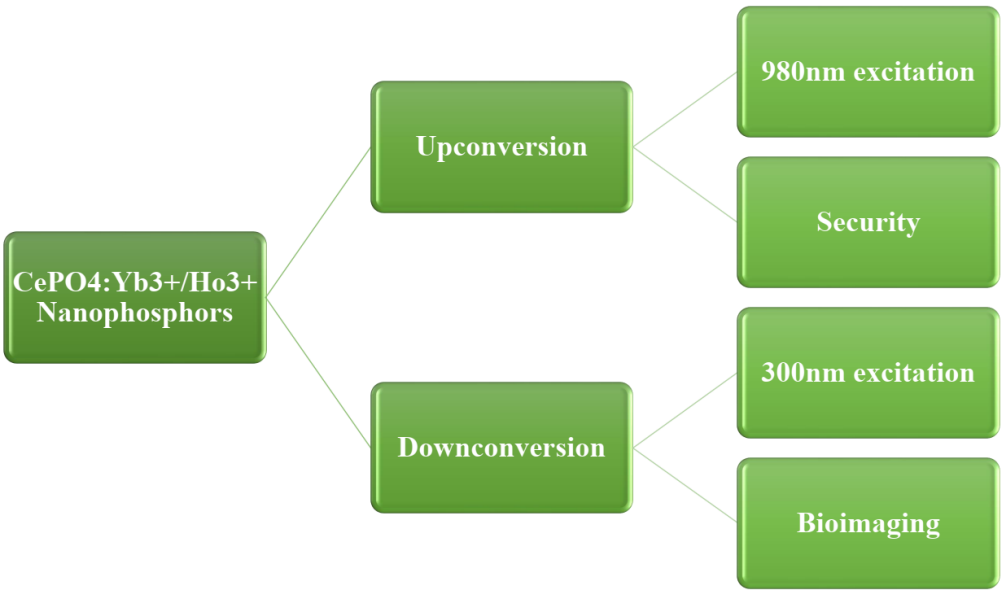

Fig. 1: Schematic depicting the steps involved in producing $CePO_4$:Ho^{3+}/Yb^{3+} Nanophosphor and the many uses it has

Experimental methods:

1. Chemical compounds and synthesis:

Reagents utilized were of the highest purity, Analytical grade reagents from Sigma-Aldrich [8]. Precursors include HCl, Ethylene glycol(EG), Sodium hydroxide, and De-ionized water, as well as cerium (III) acetate, ammonium dihydrogen phosphate, holmium (III) acetate, ytterbium (III) acetate, niobium (III) acetate, and ytterbium (IV) acetate [9].

1.1 Synthesis of CePO4: Ho3+/Yb3+ nanoparticles:

The polyol method is used to get the sample ready for analysis [10]. In Figure-1, the following are the materials used to construct samples: Glowing nanoparticles of $CePO_4$ are doped with 1% Ho^{3+} and 20 % Yb^{3+}. This product was manufactured by polyol-mediated synthesis. A typical synthesis calls for 730.76 mg of $(CH_3CO_2)_3Ce_XH_2O$, 8.7mg of $(CH_3CO_2)_3Ho_XH_2O$, and 178.48 mg of $(CH_3CO_2)_3Yb_XH_2O$ to be combined with 5 ml of concentrated HCl. Metal ions were dissolved in a crystal-clear solution [11]. The remaining HCl was neutralized by alternating additions of deionized water (10 ml) and heat (80 °C). At least five separate instances of Method 4's evaporation were carried out. A clear solution was obtained

by dissolving 298.28 mg of $(NH_4)_2HPO_4$ in 10 ml of deionized water and 2.64 g of NaOH in 10 ml of deionized water [12]. Drop by drop, the $(NH_4)_2HPO_4$ solution was added until it became clear. In a 100 ml round-bottomed flask the metal ion solution was transferred after evaporation, and 20 ml of ethylene glycol was added to the mixture before the $(NH_4)_2HPO_4$ solution was added drop by drop [13]. The mixture was then refluxed for at least 10 minutes at 75 °C. After adding the $(NH_4)_2HPO_4$ solution to the round bottom flask, the color changed from pale yellow to white after being heated for two hours at 120 °C [14]. White rain started falling after a while. Therefore, it was left to cool to room temperature. It was obtained by drying the dry powder in an infrared light oven after two washing with 10 ccs of acetone and centrifuging at 5000 rpm for five minutes. The prepared sample underwent a four-hour calcination at 900 °C. The same holds true for the percentages of Yb^{3+} (10 at. %) and Ho^{3+} (3, 5, and 7 at. %). The samples were annealed for 4 hours at 900 °C following the production of doped $CePO_4$ nanoparticles [15-17].

2. Characterization:

Equipment The typical crystal size of the sample was studied using angle dispersive X-ray diffraction at a Synchrotron [1, 18-19]. Microstructural studies and measurements of particle size and surface morphology were acquired using a scanning electron microscope. FTIR spectroscopy, a monochromator equipped with a photomultiplier tube that allowed researchers to examine UC emission, was used to analyze the vibrational structure of the manufactured materials. Diode laser light at 980 nm was utilized to stimulate the samples. Excitation of photoluminescence is the subject of research at UC (PLE) [20]. The DC-emission ranges of $CePO_4:Ho^{3+}/Yb^{3+}$ are investigated using the strong ultraviolet excitation of a Nd: YAG Laser, with an excitation WL (wavelength) of 280-300 nm [21].

Results and Discussion:

XRD sample study

In Figure-2, we show the XRD pattern of the nanophosphor material $CePO_4:Ho^{3+}$ and Yb^{3+} co-doped $CePO_4$. This material is annealable up to 900 °C. An XRD of the nanophosphor material $CePO_4$:1% Ho^{3+} and 20 % Yb^{3+} co-doped $CePO_4$ which can withstand annealing's at 900 °C and was formerly known as $CePO_4:Ho^{3+}/Yb^{3+}$ is shown in Figure-2 [22]. Diffraction patterns show prominent, sharp peaks that are continuous with the standard monoclinic phase. Dopants are assumed to be uniformly dispersed over the host lattice in the absence of an impurity peak. Two of the most intense peaks in the XRD pattern and the observed diffraction patterns are in excellent agreement with the tetragonal structure of pure $CePO_4$ [23]. Since the ionic radii of Yb^{3+} and Ho^{3+} are similar to Ce^{3+}, CN allowed these ions to be replaced into the $CePO_4$ lattice in place of Ce^{3+}. The nanophosphor has a monoclinic structure due to the presence of nine O^{2-} ions around the Ce^{3+} ion, resulting in a Pentagonal Interpenetrating Tetrahedral Polyhedron (PITP) [24].

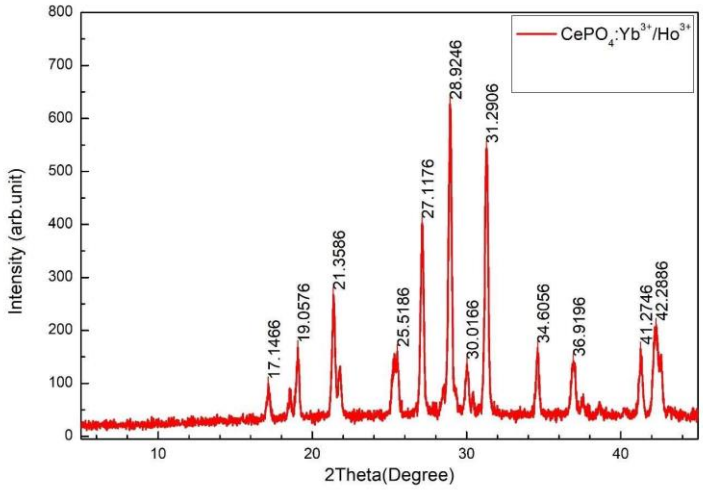

Fig. 2: The X-ray diffraction (XRD) pattern of $CePO_4:Ho^{3+}/Yb^{3+}$ samples

SEM study

An SEM image of $CePO_4:Ho^{3+}/Yb^{3+}$ is displayed in Figure -3. Nanophosphor was annealed at 900 ℃. This demonstrates the atypical shapes of nanoparticles mostly sponge-shaped particles with some spherical ones mixed in cones, cuboids, and spherical Shapes [25]. The average diameter of spherical particles is 50 nm. As seen in Figure 3, Ce, O, Yb, Ho, and P each have their own unique basic compositional images [26].

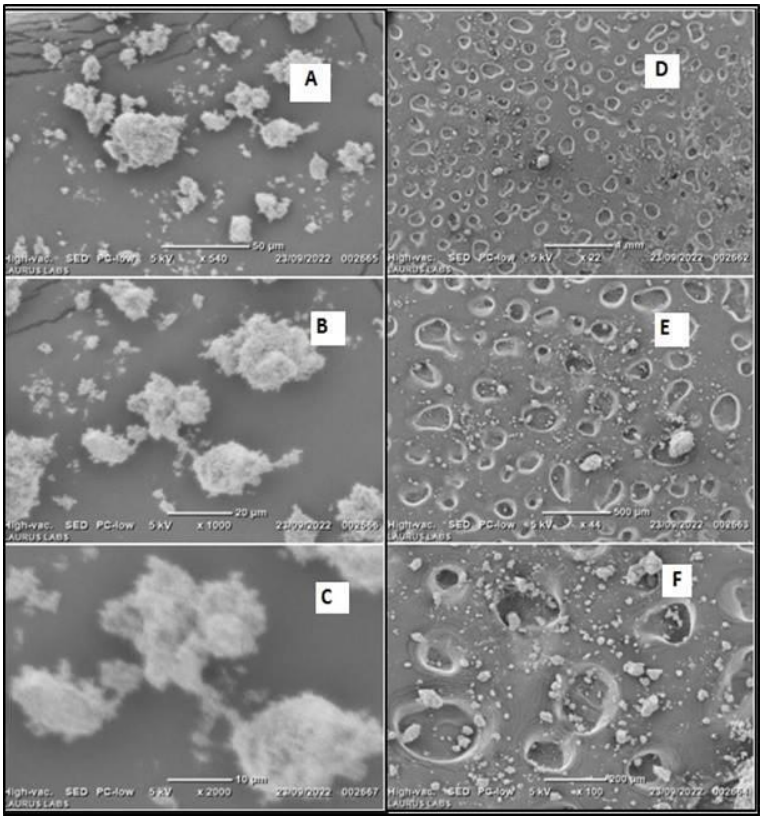

Fig. 3: SEM images of $CePO_4:Ho^{3+}/Yb^{3+}$

FTIR

The vibrational structure of the synthesized materials was analyzed using FTIR spectroscopy [27] with a resolution of 1 cm^{-1}. The topics of our conversation are depicted in Figure 4.

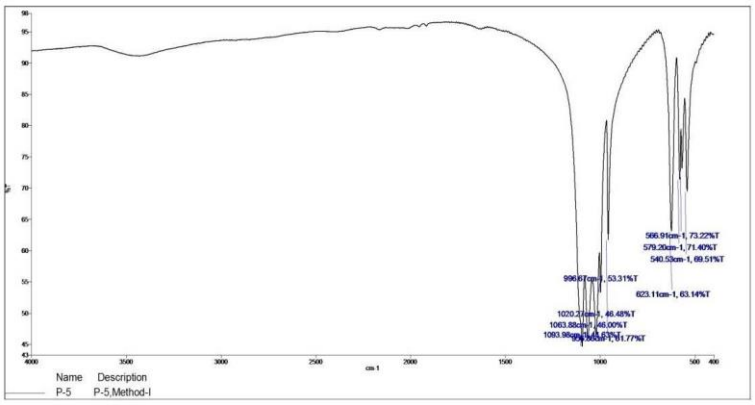

Fig. 4: FTIR of $CePO_4:Ho^{3+}/Yb^{3+}$

Study of photoluminescence of $CePO_4:Ho^{3+}$, Yb^{3+}

Up-Conversion study

Up-conversion luminescence, an optical phenomenon that emits high-energy photons while absorbing low-energy photons, has its origins in anti-Stokes luminescence (multiphoton) [28].

Depending on the concentrations of the host and co-dopant, Ho^{3+} can emit either green or red light. $CePO_4$ (Ho^{3+}, Yb^{3+}) emission (DC) and ultraviolet (UC) spectra are shown in Figures 5 and 6, respectively. $CePO_4$:0.01Ho^{3+}/0.2Yb^{3+} nanophosphor material was developed by combining Ho^{3+} doped $CePO_4$ (Ho^{3+} = 1 at. %) and Yb^{3+} doped $CePO_4$ (Yb^{3+} = 20 at. %). The optimal nanophosphor material for UC and DC luminescence testing is the subject of ongoing investigation. $CePO_4:Ho^{3+}/Yb^{3+}$ (1% Ho^{3+} and 20 % Yb^{3+}) up-conversion emission spectra at several laser strengths beyond 980 nm of excitation are displayed in Figure-5. The green color emission bands by 550 nm and the red color emission depending on the relative concentrations of the host and co-dopant bands at 650 nm (R = red) are the result of electronic transitions of the Ho^{3+} ion at $^5F_4$, $^5S_2 \rightarrow {}^5I_8$, $^5F_5 \rightarrow {}^5I_8$, and 650 nm, respectively [29]. At 980 nm excitation Yb^{3+} ions have a larger absorption fraction than Ho^{3+}, making them effective sensitizers. Excitation at 980 nm causes an absorption coefficient of 11.6 × 10^{-20} cm^2 for Yb^{3+}. The composition of a single photon is depicted in Figure 5. The $CePO_4$ excitation spectrum displays the P-O CTB, the peak at 460 nm, Ho^{3+}emission = 550 nm, and the emission spectrum displays the P-O and Ho^{3+}peaks at excitation = 300 and 460 nm, respectively [30].

Down-Conversion

This process involves first discharging higher-energy absorbed light (Exec), and then emitting lower-energy radiative light (Eem). As the Stokes shift takes place, this is mentioned. In Figure 6, we see the $CePO_4:Ho^{3+}/Yb^{3+}$ (1 at. % Ho) DC Emission Spectrum after UV Excitation at 300 nm. Green (550 nm), red (650 nm), and NIR (750 nm) emission bands are seen for the

Ho^{3+} ion, while the 5F4and5S2 5I8 and $^5F_5 \rightarrow {}^5I_8$ ET'S are seen [31]. The emission spectra are tracked using four distinct excitation wavelengths: 260, 290, 300, and 460 nm. The peaks of the Ho^{3+} emission are shown for each excitation. The emission intensity of Ho3+ is lower when it is stimulated directly at 460 nm ($^5I_8 \rightarrow {}^5G_6$) as contrasted to when it is stimulated indirectly at 300 nm. This is because of the small absorption cross section4 for Ho^{3+} f-f transitions. The broad emission band at 460 nm, with maxima at PO and Ho^{3+}, is seen when excitation occurs at 300 nm. Ho^{3+} emission peaks are shown for several excitations [32]. The emission intensity of Ho^{3+} is lower when it is directly excited at 460 nm ($^5I_8 \rightarrow {}^5G_6$) as opposed to indirectly excited at 300 nm. This is due to the fact that Ho^{3+} transitions have a relatively low absorption cross section. For excitation at 300 nm, we see Ho^{3+} peaks and the broad 460 nm emission band typically associated with PO43-. A large absorption cross section at 300 nm is caused by the P-O CTB's allowed transition. The radiative rate of Ho^{3+} increases when more and more exciting photons from P-O are de-excited, transferring their energy to Ho^{3+}. This is the so-called electron transfer resonance (ET) from PO to Ho^{3+}. The measurement of 550 nm emission from $CePO_4$:Ho^{3+}/Yb^{3+} is shown in Figure. From 260 to 360 nm, there is a broad peak with its maximum point at 300 nm. This has to do with the P-O CTB change that is legal [33]. Ho^{3+} (550-580 nm) was found to cause narrow, low-intensity peaks at 460 nm. $CePO_4$ is doped with or higher concentrations of Ho3+ at the specified concentration of Yb3+ to display the concentration-dependent luminescence (20 at. %). $CePO_4$:Ho^{3+},Yb^{3+} (x atomic % = 1, y atomic % = 20) exhibits Ho3+ emission peaks at 300 nm and 460 nm upon stimulation. The light output decreases by 1% for every 1% rise in Ho3+ ion concentration (Figure-6). The effect of quenching concentration is crucial [34-35].

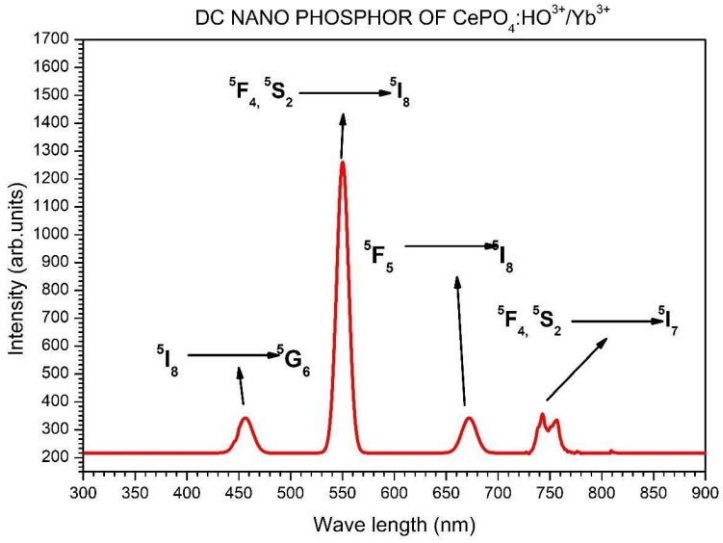

Fig. 6: $CePO_4$ excitation spectrum is comprised of 300 nm and 460 nm photons due to the presence of Ho^{3+} and Yb^{3+} ions (1% Ho)

Table 1: Wavelength Vs Intensity peaks for up-conversion nanophosphors

Wavelength (nm)	Intensity (arb. Units)
520.77	3182.51
524.31	2763.19
530.78	3334.11
543.72	1150.43
52.53	1767.13
655.90	356.12
669.25	332.76

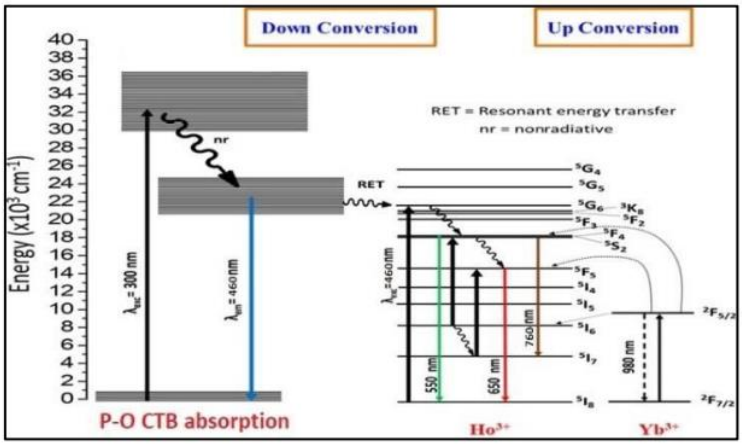

Fig. 7: $CePO_4$:Ho^{3+}/Yb^{3+}nanophosphor energy level diagram for DC&UC

Conclusions:

In conclusion, a polyolmediated method was used to successfully produce $CePO_4$:0.01Ho^{3+}/0.2Yb^{3+} nanophosphor material. Evaporation of water, removal of organic debris, and enhancement of crystallinity We annealed the sample at 900 ℃ for 4 hours. The monoclinic structural phase, with space group was identified by XRD. $CePO_4$:0.01Ho^{3+}/0.2Yb^{3+} under 980 nm illumination generates strong up-converting green ($^5F_4$, $^5S_2 \rightarrow {}^5I_8$) and red ($^5F_5 \rightarrow {}^4I_8$) color bands. Excitation occurs at 300 nm, and typical peaks for $^5F_4$, $^5S_2$, $^5I_7$, and Ho^{3+} can be seen at 550 nm, 650 nm, 750 nm, and 750 nm, respectively. Lower conversion rates are shown in Figure -7, indicating that the charge transfer band from the ligand to the metal is the primary cause of the broad emission band (P-O). According to the research conducted at UC, the green and red bands are the result of two-photon absorption.

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References:

1. Sinha, I., Verma, A., Shrivastava, A. (2023). Synthesis of Polymer Nanocomposites Based on Nano Alumina: Recent Development. *European Chemical Bulletin*, 12 (Special Issue 4), 7905-7913.

2. Gizer, S. G., Bhethanabotla, V. R., Ayyala, R. S., & Sahiner, N. (2023). Low-Pressure Plasma Treated Polycarbonate and Polymethyl Methacrylate (PMMA) Sheets with Different Surface Patterns to Change Their Surface Properties. *Surfaces and Interfaces*, 102646.
3. García-García, J., González-Hernández, J., Mendoza-Alvarez, J. G., Cruz, E. L., & Contreras-Puente, G. (1990). Photoluminescence characterization of the surface layer of chemically etched CdTe. *Journal of applied physics*, *67*(8), 3810-3814.
4. Singh, S., Diwakar, A. K., Kashyap, P., & Verma, A. (2022). Synthesis, Characterization & Luminescence Properties of Rare Earth Nano Phosphors Doped Eu2+ &Gd3+. *Journal of Optoelectronics Laser*, *41*(6), 238-242.
5. Hollingsworth, R. E., & Sites, J. R. (1982). Photoluminescence dead layer in p-type InP. *Journal of Applied Physics*, *53*(7), 5357-5358.
6. Olson, J. M., Ahrenkiel, R. K., Dunlavy, D. J., Keyes, B., & Kibbler, A. E. (1989). Ultralow recombination velocity at Ga0. 5In0. 5P/GaAs heterointerfaces. *Applied physics letters*, *55*(12), 1208-1210.
7. Verma, A., Diwakar, A. K., Richhariya, T., Singh, A., & Chaware, L. (2022). Aluminum Oxide Used Between Molybdenum Trioxide and Poly (3, 4-Ethylene Dioxy Thiophene) Polystyrene Sulfonate In Organic Solar Cells By Indium Tin Oxide Free Structures. *Journal of Optoelectronics Laser*, *41*(6), 230-233.
8. Komiya, S., Yamaguchi, A., & Umebu, I. (1986). Characterization of radiative efficiency in double heterostructures of InGaAsP/InP by photoluminescence intensity analysis. *Solid-state electronics*, *29*(2), 235-240.
9. Mettler, K. (1977). Photoluminescence as a tool for the study of the electronic surface properties of gallium arsenide. *Applied physics*, *12*, 75-82.
10. Verma, A., Shrivastava, S., & Diwakar, A. K. (2022). The Synthesis of Zinc Sulfide for Use in Solar Cells by Sol-Gel Nanomaterials. *Recent Trends of Innovations in Chemical and Biological*, *4*, 69.
11. Müllenborn, M., & Haegel, N. M. (1993). Recombination model for heterostructure interfaces. *Journal of applied physics*, *74*(9), 5748-5753.
12. Gfroerer, T. H., Cornell, E. A., & Wanlass, M. W. (1998). Efficient directional spontaneous emission from an InGaAs/InP heterostructure with an integral parabolic reflector. *Journal of applied physics*, *84*(9), 5360-5362.
13. Wei, D., Yang, X., Liu, Y., & Seo, H. J. (2023). Surface reconstruction via Eu3+-coating and recrystallization to improve photochemical properties of BiLa2O4. 5 particles. *Surfaces and Interfaces*, *36*, 102538.
14. Verma, A. K., Goswami, P., Patel, R. P., Das, S. C., & Verma, A. (2020). Futuristic energy source of CTB (Cs2TiBr6) thin films based lead-free perovskite solar cells: synthesis and characterization. *Solid State Technology*, *63*(6), 13008-13011.

15. Deveaud, B., Regreny, A., Emery, J. Y., & Chomette, A. (1986). Photoluminescence study of interface defects in high-quality GaAs-GaAlAs superlattices. *Journal of applied physics*, *59*(5), 1633-1640.
16. Kashyap, P., Diwakar, A. K., Singh, S., & Verma, A. (2022). Gd3+ Co-Doping in Al2MgSiO4: Eu2+ Photoluminescence Properties of Eu2+ and Gd3+ Phosphors Presence. *Journal of Optoelectronics Laser*, *41*(6), 243-247.
17. Gang, R., Xia, Y., Xu, L., Zhang, L., Ju, S., Wang, Z., & Koppala, S. (2022). Size controlled Ag decorated TiO2 plasmonic photocatalysts for tetracycline degradation under visible light. *Surfaces and Interfaces*, *31*, 102018.
18. Verma, A., Diwakar, A. K., Patel, R. P., & Goswami, P. (2021, September). Characterization CH3NH3PbI3/TiO2 nano-based new generation heterojunction organometallic perovskite solar cell using thin-film technology. In *AIP Conference Proceedings* (Vol. 2369, No. 1, p. 020006). AIP Publishing LLC.
19. Saitoh, T., Iwadate, H. I. H., & Hasegawa, H. H. H. (1991). In situ surface state spectroscopy by photoluminescence and surface current transport for compound semiconductors. *Japanese journal of applied physics*, *30*(12S), 3750.
20. Liu, W., et. al. (2022). The improvement properties of InGaAs/InGaAsP multiple quantum wells using the GaAs insertion layer. *Thin Solid Films*, *756*, 139363.
21. Verma, A., Diwakar, A. K., & Patel, R. P. (2019). Synthesis and characterization of high-performance solar cell. *International Journal of Scientific Research in Physics and Applied Sciences*, *7*(2), 24-26.
22. Foad, M. A., et. al. (1991). High-resolution dry etching of zinc telluride: characterization of etched surfaces by x-ray photoelectron spectroscopy, photoluminescence and Raman scattering. *Semiconductor Science and Technology*, *6*(9A), A115.
23. Verma, A., Diwakar, A. K., & Patel, R. P. (2020, March). Characterization of Photovoltaic Property of a CH3NH3Sn1-xGexI3 Lead-Free Perovskite Solar Cell. In *IOP Conference Series: Materials Science and Engineering* (Vol. 798, No. 1, p. 012024). IOP Publishing.
24. Kashyap, P., Diwakar, A. K., Verma, A. (2022). Photosensitive Behavior Studies of the Ba3CaSi2O8:Eu3+ and NaCeSiO4:Eu3+ Luminescence Material Synthesis. *International Journal of Advanced Research in Science, Communication and Technology (IJARSCT)*, 2 (1), 160-165.
25. Verma, A. (2023). Review of Nanomaterials' Current Function in Pollution Control. *Recent Trends of Innovations in Chemical and Biological*, *5*, 34.
26. Shrivastava, S. & Verma, A. (2023). Nano Chemistry and Their Application. *Recent Trends of Innovations in Chemical and Biological*, *5*, 67.
27. Krishna, D. N. G., & Philip, J. (2022). Review on surface-characterization applications of X-ray photoelectron spectroscopy (XPS): Recent developments and challenges. *Applied Surface Science Advances*, *12*, 100332.
28. Ko, J. S., et. al. (2022). Blue-shifted and strongly-enhanced light emission in transition-metal dichalcogenide twisted heterobilayers. *npj 2D Materials and Applications*, *6*(1), 36.

29. Verma, A. (2016). Study of Temperature Dependent Dialectical Properties of One System Ceramics using BNT-BZ. *International Journal of Science and Research (IJSR)*, 5(11), 963-966.
30. Singh, S., Diwakar, A. K., Kashyap, P., Verma, A. (2022). *International Journal of All Research Education & Scientific Methods (IJARESM)*, 10(5), 2914-17.
31. Zhang, F., Castaneda, J. F., Gfroerer, T. H., Friedman, D., Zhang, Y. H., Wanlass, M. W., & Zhang, Y. (2022). An all optical approach for comprehensive in-operando analysis of radiative and nonradiative recombination processes in GaAs double heterostructures. *Light: Science & Applications*, *11*(1), 137.
32. Vignaud, D., Wallart, X., Mollot, F., Sermage, B. (1998). Photolu- minescence Study of the Interface in Type II InAlAs – InP Heterostructures. *J. Appl. Phys.*, 84, 2138 – 2145.
33. Kumar, S., Kumar, Verma, A. (2023). A Comprehensive Analysis of the Factors Influencing the Stability of Perovskite Solar Cells. *GIS Science Journal*. 10 (4) 1851-58.
34. Sandya , P., Verma, A. (2023). Optical and Physical Properties of Rice and its By Products: A Detailed Analysis. *Journal of University of Shanghai for Science and Technology*. 25(4), 133-146.
35. Sahu, G., Dewangan, K., Johan, S., Verma, A. (2023 May). Simulating the Performance of AlxGa1-xAs/InP/Ge MJSC Under Variation of SI and Temperature. *European Chemical Bulletin*, 12 (Special Issue 4), 7914-7923.

GENERATIVE ARTIFICIAL INTELLIGENCE TOOL – ChatGPT – AN OVERVIEW

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Generative AI - Introduction

Generative AI is an artificial intelligence which describes algorithm (such as ChatGPT) that can generate content including images, text, video, audio, code, and synthetic data. To create a new content, Generative AI learn patterns from existing data and generate knowledge. Rapid advancements can write engaging text and create realistic images. Recent breakthroughs in the field have the potential to drastically change the way to approach the content creation.

Generative AI systems fall under the broad category of machine learning, the ChatGPT system. It allows computers to generate all sorts of new and exciting content, from music and art to entire virtual worlds.

How does Generative AI works?

Generative AI models use neural networks to identify the patterns and structures within existing data to generate new and original content.[1]

The generative AI model is the ability to leverage different learning approaches, including unsupervised or semi-supervised learning for training. This has given organizations the ability to more easily and quickly leverage a large amount of unlabeled data to create foundation models. As the name suggests, foundation models can be used as a base for AI systems that can perform multiple tasks.

ChatGPT-An overview

The GPT stands for generative pretrained transformer It is one of the hot topic in AI world now. It's a free chatbot that can generate an answer to almost any question it's asked. Developed by OpenAI, and released for testing to the general public in November 2022, it's already considered the best AI chatbot ever. And it's popular too. Starry-eyed fans posted examples of the chatbot producing computer code, poems, college-level essays, and even decent jokes. Others, among the wide range of people who earn their living by creating content, from advertising copywriters to tenured professors, are quaking in their boots.

Presently many have fear to use ChatGPT (and AI and machine learning more broadly) machine learning clearly has the potential for good. In the years since its wide deployment, machine learning has demonstrated impact in a number of industries, accomplishing things like high-resolution weather forecasts and medical imaging analysis. A 2022 McKinsey survey shows that AI adoption has more than doubled over the past five years, and investment in AI is increasing apace. It's clear that generative AI tools like ChatGPT and DALL-E (a tool for AI-generated art) have the potential to change the range of jobs.

How ChatGPT works - the model behind the Bot

ChatGPT is a large language model that uses deep learning techniques to generate human-like text. The outputs from it can be in-distinguishable from human generated content. This architecture uses a transformer neural network to process and generate text. The model is pre-trained on a massive dataset of text, such as articles, books and websites. It understands the patterns and structure of natural language. When given a starting point or a prompt, the model generates text by using the pre-trained knowledge and continues the given input in a coherent and natural way.

The transformer architecture used in chatGPT consists of multiple layers; each consists of two sub layers; a multi-head-self attention mechanism and a feed-forward neural network. The self-attention mechanism allows the model to weigh the importance of different parts of the input when making predictions. And the feed forward neural network helps the model to understand the context of input and make predictions.

Large language models for ChatGPT

ChatGPT is an extrapolation of a class of machine learning Natural Language Processing models known as Large Language Model (LLMs)[2] . LLMs digest huge quantities of text data and infer relationships between words within the text. These models have grown advancements in computational power. LLMs increase their capability as the size of their input datasets and parameter space increase.

The most basic training of language models involves predicting a word in a sequence of words. Most commonly, this is observed as either next-token-prediction or masked-language-modeling.

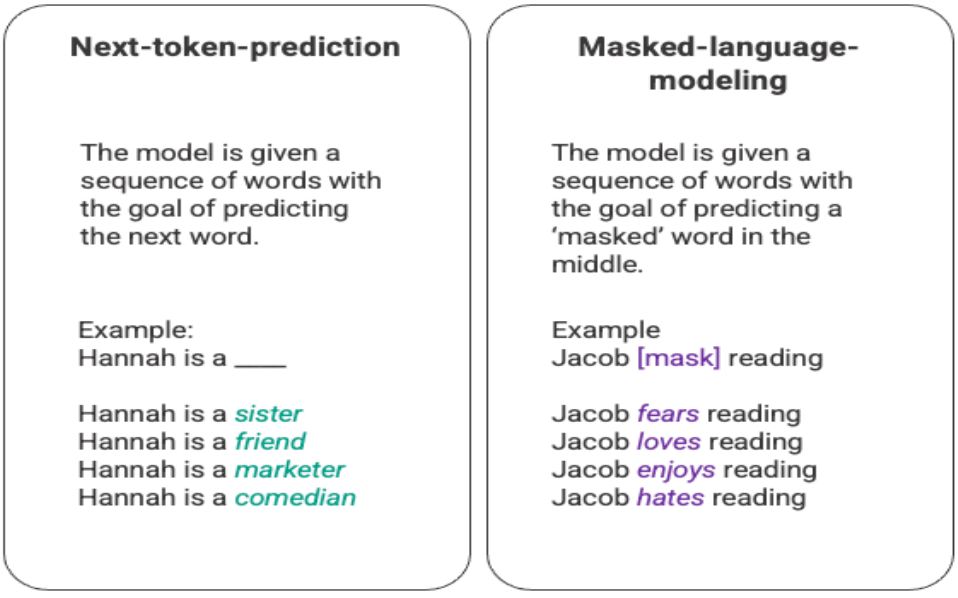

Fig. 1: Arbitrary example of next-token-prediction and masked-language-modeling generated by the author

In this basic sequencing technique, often deployed through a Long-Short-Term-Memory (LSTM) model, the model is filling in the blank with the most statistically probable word given the surrounding context.

There are two major limitations with this sequential modeling structure.[3]

1. The model is unable to value some of the surrounding words more than others. In the above example, while ‘reading’ may most often associate with ‘hates’, in the database ‘Jacob’ may be such an avid reader that the model should give more weight to ‘Jacob’ than to ‘reading’ and choose ‘love’ instead of ‘hates’.
2. The input data is processed individually and sequentially rather than as a whole corpus. This means that when an LSTM is trained, the window of context is fixed, extending only beyond an individual input for several steps in the sequence. This limits the complexity of the relationships between words and the meanings that can be derived.

When to use Generative AI tool

a) Creating marketing content

Generative AI can create marketing content, including text, images, videos, and audio. If you ever get stuck finding the right words, AI can become a handy assistant. For example, AI can create a a photo caption, blog post, social media copy, an email or product descriptions. It helps us to create photorealistic art and graphics to include in your marketing campaigns.

b) Customer service and technical support chatbots

This type of artificial intelligence can be used for customer support Chatbots, whether that’s on your website or via SMS text support. This can speed up response time to customer inquiries and free up reps to address more complex issues.

c) Writing email responses

Sales reps can also benefit from using generative AI to write email responses. If your reps are tired of writing messages in bulk, generative AI can create custom messages with specific tones — all in seconds. Further, AI can help pull data from your CRM to ensure personalized messaging.

d) Product demonstrations

Generative AI can be particularly useful for product demonstrations as well. For example, if you want to create a demo video, you can use generative AI tools to make it for you.

AI can also make suggestions to help you improve the user experience. You can have AI write you a script or answer any questions you have during the post-production process.

Generative AI tools

Now we will look into some generative AI tools.

a) **Content assistant tools** from HubSpot [4]can help you craft copy of any length in seconds. You can easily switch between manual and AI content creation to write posts, landing pages, marketing emails, and more.

Content assistant can help you throughout the writing process. The tool can generate ideas for blog posts and create an outline to help guide you.

b) **ChatSpot** is a conversational CRM bot that you can connect to HubSpot. With chat-based commands, you can interact with your CRM data. You can also pull insights by entering a text prompt or send emails.

It has the ability to write long-form content and used to generate drafts for your blog.

c) **Writesonic** is one of the best generative AI tools for creating any type of creative copy. This tool can help you create SEO-optimized content for your ads, blogs, emails, and website 10 times faster.

d) **Jasper** can help to craft emails, blog posts and social messaging. You can even prompt the AI to make art. Let's say you want to write a blog post. Just open a new document in Jasper's interface. You can input the topic, a content brief, your desired tone, and relevant keywords. Text appears once you press generate. If you keep clicking "generate," AI will base additional text on what's already been written in the document.

Jasper uses a similar process when generating art. You can describe your ideal image, the style of the piece, and the artistic medium.

e) **Synthesia** is one of the best options for AI-driven video content. The platform uses AI to create realistic AI avatars with voiceover capabilities. You can customize your avatars' appearances, voices, and languages. Then, all you need to do is upload your script. Soon, you'll have a life-like video without hours spent on production. With, Synthesia you can easily create both training and product marketing videos.

f) **Copy.ai** If you have writer's block or are just tired of rephrasing the same message for different tweets, Copy.ai can help. This platform can help you write long-form content and social media messages optimized for different platforms.

Start by specifying the platform you're writing for. Then, give the AI context for your post. That could be a topic and keyword or, for social media, a section of a report. Then, Copy.ai can generate text. If you're creating social copy, Copy.ai will generate multiple options for you to use. You can choose which works best or schedule them all.

g) **ChatGPT** is a conversational artificial intelligence that can help with a variety of tasks. You can use ChatGPT to compose emails and create any type of marketing copy.

Just type a command into the platform's interface, and you'll get a response. You can ask ChatGPT to help you research different topics, create new copy, or paraphrase existing work. This paper is describing the activity of chatGPT in detail.

h**) Rephrase.AI** is another generative AI platform that focuses on providing personalized videos for your marketing needs. This tool can convert plain text, such as your blogs, into professional-quality videos in minutes.The company emphasizes repurposing old content to improve the customer journey and your marketing campaigns.

i) **Soundraw** is highly accessible and can quickly create high-quality music for a variety of marketing applications. You can use this tool to create a theme song for your company's podcast or background music for an explainer video. It allows you to sort by mood.

Advantages of Chat GPT

Chat GPT is a hot topic in natural language processing tool and in the marketing community. The benefits of Chat GPT include its cost-effectiveness, ability to automate mundane tasks, and providing accurate and detailed responses for customer service departments. It is open source and available for anyone to use, making it a cost-effective solution for businesses.

1. One of the main benefits of Chat GPT is its ability to generate responses quickly.
2. Using Chat GPT can be incredibly beneficial for tasks that require a quick turnaround. You can complete your tasks in a fraction of the time it would have taken you manually.
3. It can also be used to generate ideas and content and can automate mundane tasks such as data entry or categorization, freeing up your team's time for more important work. Chatbots powered by this technology can understand customer questions accurately and provide detailed answers in almost real time. This makes them ideal for customer service departments, reducing response times and providing more accurate responses than traditional call centres.
4. It can provide feedback on your writing skills and suggest ways to improve.
5. ChatGPT can help you come up with ideas for new business or products.
6. We can create our own Chatbot with the right tools can talk to other people.

Disadvantages of Generative AI - ChatGPT

The potential drawbacks such as the risk of plagiarism and the need for fine-tuning must also be considered. Additionally, ethical and responsible usage is crucial to avoid negatively impacting SEO or violating Google's guidelines.

However, there are some drawbacks to using Chat GPT:

1. There is the risk of plagiarism, which is unacceptable at all costs.
2. While Chat GPT can generate content quickly and accurately on simple topics, it struggles with more complex ideas or issues.
3. Users must consider that Chat GPT is still learning and is unable to produce perfect results.
4. Although it can be advantageous regarding its learning capabilities, it also means that the tool may only sometimes generate the most accurate or appropriate responses. With the lack of reliability in content, it is evident that this could lead to errors or misunderstandings, which may negatively impact the user experience.
5. It requires its user's significant training and fine-tuning to get the best results. It can become more time-consuming and require higher technical expertise, which may be a challenge for some users.
6. ChatGPT is only as good as the data it is trained on and that might be biased in the content generated by ChatGPT & seek out additional sources of information to verify any claims made by ChatGPT.
7. Finally, while Chat GPT is open source and available to everyone, its requirement for additional resources, such as computing power or storage space, to run effectively may create a barrier for entry-level users.

Conclusion:

Generative AI may be new on the scene, but it's not going anywhere anytime soon. While it can be particularly useful for marketing teams, it's important to be aware of the limitations of the technology.

Find the tools to help your marketing team save time and optimize resources to make the most of how AI can improve its productivity.

References:

1. https://www.nvidia.com/en-us/glossary/data-science/generative-ai/
2. https://towardsdatascience.com/how-chatgpt-works-the-models-behind-the-bot-1ce5fca96286
3. https://www.sayvee.com/chat-gpt-pros-and-cons/
4. https://blog.hubspot.com/marketing/generative-ai#when-is-using-gen-ai

THE EFFECT OF SOLVENT/SOLVENT-MIXTURE ON THE MORPHOLOGY OF BARIUM CARBONATE AND STRONTIUM CARBONATE NANOPARTICLES

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Abstract:

Barium carbonate and strontium carbonate has been widely use in various technological applications. Therefore controlled synthesis of barium carbonate and strontium carbonate with desired morphology affects their properties significantly. In this work, barium carbonate and strontium carbonate were prepared by controlled precipitation in different solvent/solvent mixture having different dielectric constants. Barium carbonate and strontium carbonate samples were obtained by reacting strontium nitrate/barium nitrate with sodium carbonate in aqueous medium/methanol-water mixture separately. Barium carbonate and strontium carbonate crystallized in witherite ($BaCO_3$) and $SrCO_3$ (strontionite) in both aqueous medium/methanol-water mixture separately. X-ray powder diffraction, infrared spectral data confirms the phases obtained for barium carbonate and strontium carbonate. While scanning electron micrographs show rod like morphology for barium carbonate in aqueous medium/methanol-water mixture, while strontium carbonate exhibits rod like morphology in aqueous medium and irregular sphericular sheets in water-methanol mixture like morphology. This clearly demonstrates the role of solvent/solvent mixture on morphological control of strontium carbonate.

Keywords: Barium carbonate, strontium carbonate, crystal structure; precipitation condition; morphology.

Introduction:

Barium carbonate is a naturally occurring mineral which can exist in three polymorphic modifications i.e orthorhombic, hexagonal and cubic system (Lander, 1949; Earnest, 1989). Of which, orthorhombic phase of barium carbonate (witherite) is thermodynamically stable and most easily obtained during the preparation. Barium carbonate been widely used by material scientists due to its application as carbon dioxide and humidity sensors, luminescent material, as catalyst, additives in polymeric composites, ceramics and optical glass (Zhang *et al.,* 2011; Clarkson, 2013; Tao *et al.,* 2016; Kim *et al.,* 2017; Gu *et al.,* 2017; Shahid *et al.,* 2018;). Barium carbonate is used as precursors in magnetic and ferroelectric materials (Kaszuwara *et al.,* 2008). Due to its wide applications, there are several methods adopted for the synthesis of barium carbonate both in micrometer and nanometer dimensions (Gutmann *et al.,* 1993; Chen *et al.,* 2007, 2013; Ischenko *et al.,* 2007; Thongtem *et al.,* 2010). Of which, most common methods adopted are sol-gel, precipitation, hydrothermal process and precipitation in presence of additives (Teicher *et al.,* 1955; Wenjie *et al.,* 2012; Wang *et al.,* 2011; Zhang *et al.,* 2008). Morphology of the sample plays an important role in deciding the property of barium carbonate.

Strontium carbonate is one of the most widely studied biomaterial crystallized in srontianite phase and is a structural analogue of calcium carbonate (Li *et al.*, 2012). Strontium carbonate has also been used as a luminescent material, adsorbent, sensor and catalyst (Shi *et al.*, 2002; Wang *et al.*, 2005; Zhu *et al.*, 2012; Liao *et al.*, 2014). Hence strontium carbonate with different types of morphologies has been prepared by varying experimental conditions (Rautaray *et al.*, 2004; Li *et al.*, 2012; Han *et al.*, 2014; Ranjbar *et al.*, 2015; Divya *et al.*, 2019; Xu *et al.*, 2019). In this article, we have investigated the effect of dielectric constant of the solvent on the morphology of barium carbonate and strontium carbonate. There is no change in the morphology of barium carbonate with change in the nature of the solvent while in case of strontium carbonate we get irregular sphericular sheet like morphology when methanol-water was used as solvent and rod like morphology when water was used.

Experimental:

Sodium hydrogen carbonate, barium chloride, strontium nitrate and methanol reagents were procured from SD Fine chemicals (analytical grade) and used as received.

Preparative procedure was divided into two parts: Barium carbonate/strontium carbonate ($BaCO_3$/$SrCO_3$) was prepared by reacting barium chloride/strontium nitrate solution with a mixture of sodium hydrogen carbonate at room temperature in the following solvents.

Part-A (water was used as solvent): About 100 mL of barium chloride solution/strontium nitrate solution (0.1M) was added dropwise to separate beakers containing 100 mL of sodium hydrogen carbonate solution (0.1 M) at room temperature. White precipitate formed in the mother liquor was aged for 21h.

Part-B (water-methanol mixture as solvent): About 100 mL of barium chloride solution/strontium nitrate solution (0.1M) was added dropwise to separate beakers containing sodium hydrogen carbonate solution (0.1 M) containing 70 mL water and 30 mL methanol. White precipitate formed in the mother liquor was aged for 21h.

The samples obtained in Part-A and Part-B were filtered and dried at 65-80 °C.

Characterization:

The barium carbonate/strontium carbonate samples were characterized using powder X-ray diffraction, Infrared spectroscopy and scanning electron microscopy. The pXRD patterns were recorded using Bruker D8 advanced diffractometer in the 2θ range of 15-50° (Cu Kα radiation-λ = 1.5418 Å, 4° min^{-1} scan rate with step size: 0.05°). The Infrared spectra were recorded using Bruker alpha FTIR spectrometer (ATR mode) in the range of 4000-400 cm^{-1} with 4 cm^{-1} resolution. The morphology of the samples was recorded using scanning electron microscopy (Jeol/EO JSM-6390 model).

Results and Discussion:

The experimental conditions can affect the phase formation of barium carbonate/strontium carbonate and its morphology. To study the crystal growth of barium carbonate/strontium carbonate, precipitations were carried out in aqueous medium and methanol-water mixture separately. Figure 1 shows the PXRD patterns of the barium carbonate obtained by the addition of barium chloride to i) sodium hydrogen carbonate solution in aqueous medium

and ii) sodium hydrogen carbonate solution containing water-methanol mixture respectively. The peak positions in the diffraction patterns of barium carbonate obtained in both the conditions can be indexed to orthorhombic phase (wihterite) (Code-00-005-0378) with cell dimensions a = 5.314 Å; b = 9.040 Å; c= 6.430 Å; α = 90°; β= 90.42°; γ = 90°.

Figure 2 shows the PXRD patterns of samples obtained by the addition of strontium nitrate to i) sodium hydrogen carbonate solution and ii) sodium hydrogen carbonate solution containing water-methanol mixture respectively.

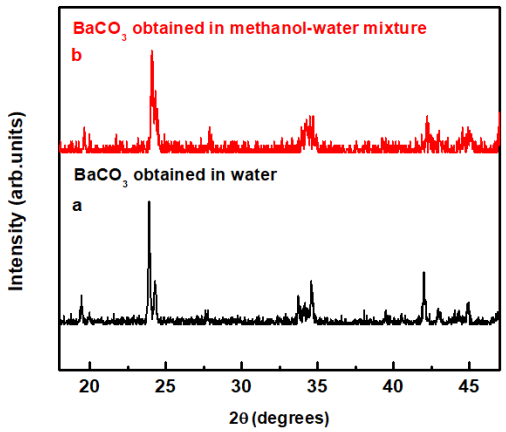

Fig. 1: XRD patterns of barium carbonate obtained on addition of barium chloride to sodium hydrogen carbonate: i in aqueous medium, ii water-methanol mixture

The peak positions in the PXRD patterns of the strontium carbonate samples obtained in the above method matches with orthorhombic phase of strontianite with lattice parameters a = 5.1039 Å; b = 8.4022 Å; c= 6.022 Å; α = 90°; β= 90°; γ = 90° (space group Pmcn) and the reflections also matches to that of $SrCO_3$ (JCPDS card number: 05-418).

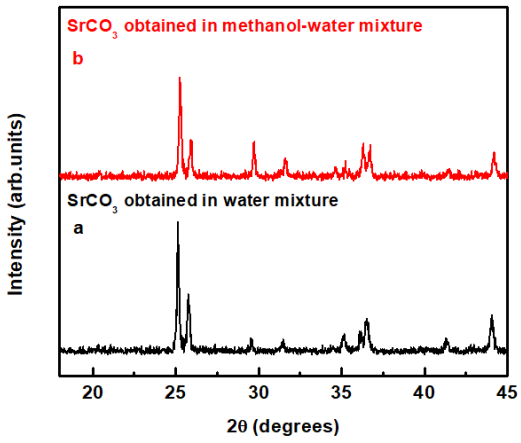

Fig. 2: XRD patterns of strontium carbonate obtained on addition of strontium nitrate to sodium hydrogen carbonate: i in aqueous medium, ii water-methanol mixture

Figure 3 shows the infrared (IR) spectra of barium carbonate obtained on addition of barium chloride solution to i) sodium hydrogen carbonate solution in aqueous medium and ii) sodium hydrogen carbonate solution containing water-methanol mixture respectively. The vibrational peaks in the mid-IR region arise due to carbonate ion. The distortion of carbonate ion leads to splitting of peaks in the infrared spectra. Infrared spectra of barium carbonate obtained at

both the conditions (in aqueous medium/water-methanol mixture) shows carbonate ion bending vibrations at i) 1414±2 cm^{-1} (assigned to C-O asymmetric stretching mode) while the weak band at 1059 cm^{-1} (symmetric C-O stretching vibration). The peaks at 856, 693 cm^{-1} are carbonate in plane and out of plane bending modes in barium carbonate (Figure 3).

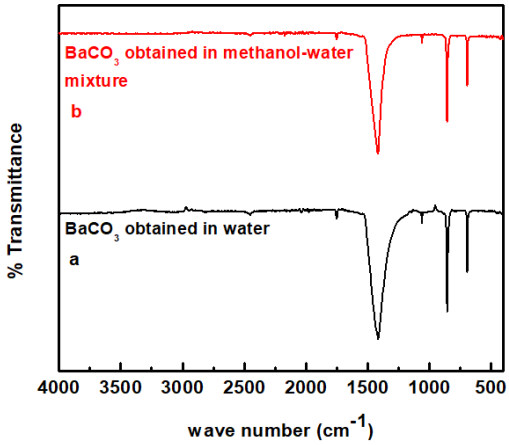

Fig. 3: Infrared spectra of barium carbonate obtained on addition of barium chloride to sodium hydrogen carbonate: i in aqueous medium, ii water-methanol mixture

Figure 4 shows the infrared (IR) spectra of strontium carbonate obtained on addition of strontium nitrate solution to i) sodium hydrogen carbonate solution in aqueous medium and ii) sodium hydrogen carbonate solution containing water-methanol mixture exhibit similar peak positions. Both the samples exhibit sharp peaks at 1444 cm^{-1} assigned to C-O asymmetric stretching mode, 1070 cm^{-1} to symmetric C-O stretching vibration, 859 cm^{-1} (ν_2 mode) and 698 cm^{-1} arises due to out of plane bending modes respectively and matches with the reported data of $SrCO_3$ (Figure 4) (Chansan and Norwitz, 1971).

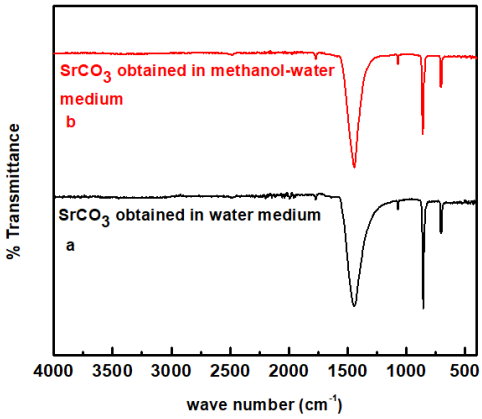

Fig. 4: Infrared spectra of strontium carbonate obtained on addition of strontium nitrate to sodium hydrogen carbonate: i in aqueous medium, ii water-methanol mixture

Morphologies of the witherite phase of barium carbonate samples obtained i) in aqueous medium and ii) in water-methanol mixture are shown in Figure 5. Barium carbonate samples exhibit rod like morphologies with the particle size in the range of 240 to 360 nm (rod diameter) in both the conditions (aqueous medium/water-methanol mixture).

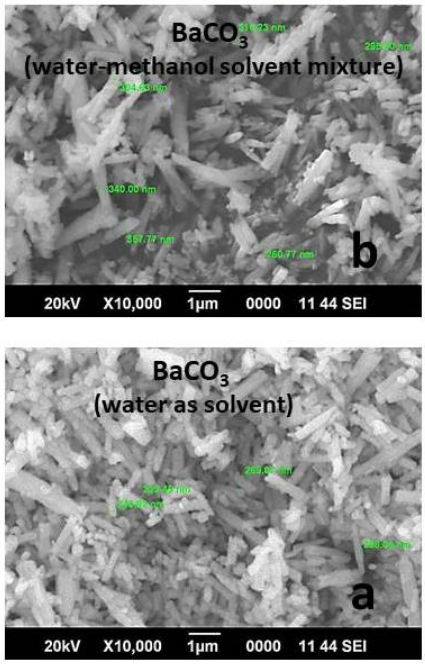

Fig. 5: Scanning electron micrographs of barium carbonate obtained on addition of barium chloride to hydrogen sodium carbonate: i in aqueous medium, ii water-methanol mixture

Figure 6 shows the scanning electron micrographs of strontium carbonate samples obtained in i) aqueous medium and ii) in water-methanol mixture. Barium carbonate obtained in aqueous medium display irregular sphericular sheet like morphology with particle size in 1μm and rod like morphology with 540-600 nm size particles in water-methanol mixture. When methanol-water mixture was used for precipitation by lowering the dielectric constant value and viscosity, crystal growth rate was significantly altered only in case of strontium carbonate.

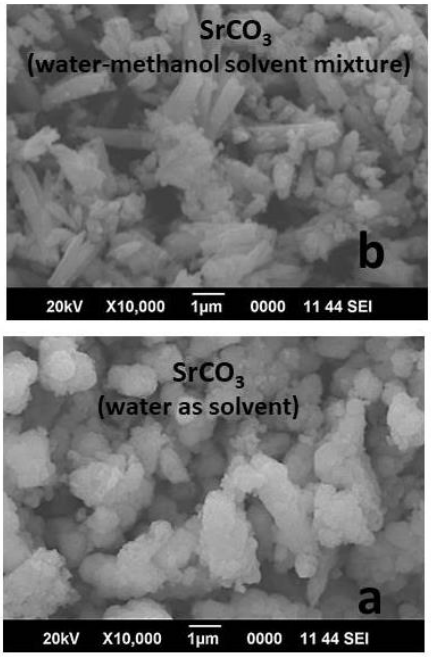

Fig. 6: Scanning electron micrographs of strontium carbonate obtained on addition of strontium nitrate to sodium hydrogen carbonate:
(i) in aqueous medium, (ii) water-methanol mixture

Conclusion:

The nature of solvent and its dielectric constant can significantly affect the morphology of the samples. Even in absence of additives, we can get different morphologies in case of strontium carbonate by altering the solvents and their dielectric constants. Nanometer size particles are obtained in case of barium carbonate and both nano and micrometer size particles were obtained for strontium carbonate samples indicating the importance of solvent during the precipitation process.

Acknowledgement:

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References:

1. Chasan DE, Norwitz G (1971): Infrared determination of barium, strontium, and calcium carbonates, singly and together, by the Pellet technique, Applied Spectroscopy, 25:226-228.
2. Chen L, Shen Y, Xie A, Zhu J, Wu Z, Yang L (2007): Nanosized barium carbonate particles stabilized by cetyltrimethylammonium bromide at the water/hexamethylene interface, Crystal Research and Technology, 42:886–889.
3. Chen L, Jiang J, Bao Z, Pan J, Xu W, Zhou L, Wu Z, Chen X (2013): Synthesis of barium and strontium carbonate crystals with unusual morphologies using an organic additive, Russian Journal of Physical Chemistry, A, 87:2239–2245.
4. Clarkson TW, (2001): in Handbook of Pesticide toxicology, edited by R. I. Krieger, W. C. Krieger, chapter 61, Inorganic and Organometal Pesticides, 2:1357-1428.
5. Divya A, Mathavan T, Harish S, Archana J, Franklin Benial AM, Hayakawad Y, Navaneethan M (2019): Synthesis and characterization of branchlet-like $SrCO_3$ nanorods using triethylamine as a capping agent by wet chemical method, Applied Surface Science, 487:1271-1278.
6. Earnest CML (1989): Calorimetric heat of transition assignments by microcomputer-based differential thermal analysis: Part II. The γ-β (orthorhombic to hexagonal) transition of barium carbonate, Thermochimica Acta, 137:365–71.
7. Gu J, Bian Z, Yin B, Jin C, Liu X, Gao Y, Wu J, Tang S, Gao F, Zhao Y (2017): Simultaneous structure and luminescence property control of barium carbonate nanocrystals through small amount of lanthanide doping, Science Bulletin, 62:1239–1244.
8. Gutmann B, Chalup A (1993): Barium carbonate, American Ceramic Society Bulletin, 72:83–84.
9. Han Y, Nishimura T, Kato T (2014): Biomineralization-inspired approach to the development of hybrid materials: preparation of patterned polymer/strontium carbonate thin films using thermoresponsive polymer brush matrices, Polymer Journal, 46:499–504.

10. Ischenko V, Woltersdorf J, Pippel E, Koferstein R, Abicht H-P (2007): Formation of metastable calcite-type barium carbonate during low-temperature decomposition of (Ba,Ti)-precursor complexes, Solid State Sciences, 93:303-309.
11. Kaszuwara W, Witkowski A, Leonowicz M, Pawlik P., Paszula J (2008): Effect of milling medium on the structure and magnetic properties of mechanically alloyed barium ferrite, Reviews on Advanced Materials Science, 18:497-500.
12. Kim DY, Kang H, Choi N-.J, Park K.H, Lee H.K (2017): A carbon dioxide gas sensor based on cobalt oxide containing barium carbonate, Sensors and Actuators B, 248:987–992.
13. Lander JJ (1949): Polymorphism and anion rotational disorder in the alkaline earth carbonates, Journal of Chemical Physics, 17:892-900.
14. Li L, Lin R, Tong Z, Feng Q (2012): Facile synthesis of $SrCO_3$ nanostructures in methanol/water solution without additives, Nanoscale Research Letters, 7:1-5.
15. Li, J-M, Wei D-P, Hu Y-B, Fanga J, Xuab Z-A (2014): Synthesis of ultrafine green-emitting $BaCO_3$ nanowires with 18.5 nm-diameter by CO_2 vapor-assisted electrospinning, Crystal Engineering Communications, 16:964–968.
16. Liao F, Zhao L, Zhai C, Zhang Z, Ma X (2014): Morphology and photoluminescence properties of $SrCO_3$ prepared by a simple solution method. Materials Letters, 122:331-333.
17. Ranjbar M, Ghasempour H (2015): Synthesis and characterization of strontium carbonate nano structures via simple and fast microwave approach, Nano Science and Nanotechnology: An Indian Journal, 9:119-122.
18. Rautaray D, Sanyal A, Adyanthaya S.D, Ahmad A, Murali Sastry (2004): Biological synthesis of strontium carbonate crystals using the fungus fusarium oxysporum, Langmuir, 20: 6827-6833.
19. Shahid T, Arfan M, Zeb A, BiBi T, Khan T.M (2018): Preparation and physical properties of functional barium carbonate nanostructures by a facile composite-hydroxide-mediated route, Nanomaterials and Nanotechnology, 8:1–8.
20. Shi J, Li J, Zhu Y, Wei F, Zhang X (2002): Nanosized $SrCO_3$-based chemiluminescence sensor for ethanol, Analytica Chimica Acta, 466:69-78.
21. Tao H, Kyle SB, Changrong X (2016): Barium carbonate nanoparticles as synergistic catalysts for the oxygen reduction reaction on $La_{0.6}Sr_{0.4}Co_{0.2}Fe_{0.8}O_{3-\delta}$ solid-oxide fuel cell cathodes, Chem ElectroChem, 3:805–813.
22. Teicher H (1955): Precipitation of barium carbonate, Analytical Chemistry, 27:1416-1418.
23. Thongtem T, Tipcompor N, Phuruangrat A, Thongtem, S (2010): Characterization of $SrCO_3$ and $BaCO_3$ nanoparticles synthesized by sonochemical method, Materials Letters, 64:510–512.
24. Wang L-N, Huo J-C, Liu S-X, Lei Y-L (2011): A new route to the synthesis of barium carbonate crystals by the induction of bacillus pasteurii, Chinese Journal of Structural Chemistry, 30:738–742.

25. Wang L, Zhu Y (2005): Effects of Nanostructure on Catalytic Degradation of Ethanol on $SrCO_3$ Catalysts. Journal of Physical Chemistry B, 109:5118-5123.
26. Wenjie Z, Chunhua C, Jiaping L (2012): Polymer micelle-directed growth of $BaCO_3$ spiral nanobelts, Chemical Communications, 48:8544–8546.
27. Xu Y, Zhong X, Li Y, Liu J (2019): Morphology-controllable self-assembly of strontium carbonate ($SrCO_3$) crystals under the action of different regulators, Journal of Materials Science and Electronics, 30:21150–21159.
28. Zhang H, Hu C, Zhang M, Yang R, Zheng C (2011): Synthesis of $BaCO_3$ nanowires and their humidity sensitive property, Journal of Nanoscience and Nanotechnology, 11:10706–10709.
29. Zhang HY, Hong JM, Ni YH (2008): One-step preparation of a novel $SrCO_3$/g-C_3N_4 nano-composite and its application in selective adsorption of crystal violet, Crystal Engineering Communications, 10:1031–1036.
30. Zhu W, Liang Z, Liu X, Zhang H, Zheng Y, Piao X-L, Zhang Q (2012): Soft-template self assembly of hierarchical mesoporous $SrCO_3$ by low-temperature hydrothermal route and their application as adsorbents for methylene blue and heavy metal ions. Powder Technology, 226: 165-172.

ROLES OF NANOPARTICLES IN PLANT DISEASE MANAGEMENT

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Abstract:

Each year, 20%–40% of crops are lost due to plant pests and pathogens. Existing plant disease management relies predominantly on toxic pesticides that are potentially harmful to humans and the environment. Nanotechnology can offer advantages to pesticides, like reducing toxicity, improving the shelf-life, and increasing the solubility of poorly water-soluble pesticides, all of which could have positive environmental impacts. Nanotechnology is one of the most fascinating and rapidly advancing sciences and possess potential to revolutionize many disciplines of science, technology, medicine and agriculture. Conversion of macro materials in to Nano size particles (1-100 nm) gives birth to new characteristics and the material behaves differently. Nanoparticles can be produced by different methods, chemical and biological, the former is commercially used. Nanomaterials can be potentially used in the crop protection, especially in the plant disease management. Nanoparticles may act upon pathogens in a way similar to chemical pesticides or the nanomaterials can be used as carrier of active ingredients of pesticides, host defense inducing chemicals, etc. to the target pathogens. Because of ultra-small size, nanoparticles may hit/target virus particles and may open a new field of virus control in plants.

Keywords: Nanoparticles, virus, biosynthesis, Nano pesticide, disease management

Introduction:

Plant pests and pathogens cause significant reductions in crop production, with estimated global losses of 20%–40% per year (Flood, 2010). Current pest management relies heavily on the application of pesticides, such as insecticides, fungicides, and herbicides. In spite of many advantages, like high availability, fast action, and reliability, pesticides have harmful side effects towards non-target organisms, the resurgence of the pest population, and the development of resistance (Stephenson 2003). Furthermore, it is estimated that 90% of applied pesticides are lost during or after application (Ghormade, 2011). As a result, there is an increased motivation to develop cost-efficient, high-performing pesticides, that are less harmful to the environment.

The term nanotechnology was first coined by Taniguchi (1974). Nanotechnology has led to the development of new concepts and agricultural products with immense potential to manage the aforementioned problems. Nanotechnology has substantially advanced in medicine and pharmacology, but has received comparatively less interest for agricultural applications (Sinha 2017, Balaure 2017). The use of nanotechnology in agriculture is currently being explored in

plant hormone delivery, seed germination, water management, transfer of target genes, Nano barcoding, Nano sensors, and controlled release of agrichemicals. The use of nanoparticles to protect plants can occur via two different mechanisms: (a) nanoparticles themselves providing crop protection, or (b) nanoparticles as carriers for existing pesticides or other actives, such as double-stranded RNA (dsRNA), and can be applied by spray application or drenching/soaking onto seeds, foliar tissue, or roots. Nanoparticles, as carriers, can provide several benefits, like (i) enhanced shelf-life, (ii) improved solubility of poorly water-soluble pesticides, (iii) reduced toxicity, and (iv) boosting site-specific uptake into the target pest (Hayles 2017). Another possible Nano carrier benefit includes an increase in the efficacy of the activity and stability of the Nano pesticides under environmental pressures (UV and rain), significantly reducing the number of applications, thereby decreasing toxicity and reducing their costs.

Nanotechnology science that largely deals with synthesis and application of Nano size particles (1 -1 00 nm or 1.0 10-9 m) of any material. When a material is reduced to Nano size, it acts differently and expresses some new properties completely lacking in its macro scale form. The nanoparticles (NPs) have a high surface to volume ratio that increases their reactivity and possible biochemical activity (Dubchak et al., 2010). For example, when 1 g gold is converted into Nano scale, the particles may cover an area of 100 km^2. Gold nanoparticles (2.5 nm) melt at much lower temperatures (300°C) than a gold slab (1064°C). Absorption of solar radiation is much higher in materials composed of nanoparticles than its thin film. The gold nanoparticles show toxic effect on bacteria, Salmonella typhimurium, in which the macro gold did not exhibit (Wang *et al.,* 2011). Similarly, the silver nanoparticles have anti-bacterial and anti-fungal properties while silver in macro form does not do this (Sofi *et al.,* 2012). Nanoparticles are of different shapes and many times smaller than bacterial cells. The nanoparticle cannot be imaged by the optical microscopes. The particle size is even smaller than a virus particle like influenza virus (80-120 nm diameter) and tomato mosaic virus (300 nm length and 10-18 nm diameter). The nanoparticles appear as point of scattering light under high resolution. The nanoparticles move under Brownian motion and their speed varies strongly with the particle size. Nanoparticles may be spherical, polyhedral rod shaped, etc. Nanotechnology has a wide scope of application in medicine, industry and agriculture and can revolutionize the entire society, if exploited properly. In agriculture, nanotechnology has potential scope for use in the natural resource exploitation and conservation, and production and protection of the crops and livestock. Nanotechnology has two major aspects, the first aspect is synthesis of Nano-size materials and second, the use or application of Nano materials for the desired objectives.

Synthesis of nanoparticles

The nanoparticles are synthesized by chemical, physical and biological methods. The property of the nanoparticles and efficacy of synthesis vary with procedure of synthesis. The chemical methods have been found to synthesize the nanoparticles more efficiently than other methods.

Chemical synthesis of nanoparticles: The commercial synthesis of nanoparticles is largely done by chemical methods. There are different chemical methods to synthesize the nanoparticles,

however, choice of the methods may vary with the material. Some of the important chemical methods are reduction method, colloidal method, sonochemical method etc.

Chemical reduction method: In 1857, Michael Faraday for the first time reported a systematic study of the synthesis of colloidal gold using chemical reduction route. The chemical reduction of copper salts is the easiest, simplest and the most commonly used method to synthesize copper nanoparticles. In fact, the production of Nano sized metal copper particles with good control of morphologies and sizes can be achieved with the reduction method. In the chemical reduction methods, a copper salt is reduced by a reducing agent such as sodium borohydride ascorbate isopropyl alcohol with Cetyl Trimethyl Ammonium Bromide (CTAB) as well as glucose. Chemical reduction of copper salts using ascorbic acid (Vitamin C) is a new and green approach in which ascorbic acid is used both as the reduction and capping agent (Umer *et al.*, 2012).

Micro emulsion/colloidal method: Hoar and Schulman (1943) observed that an appropriate amount of water, oil, surfactant and an alcohol or amine-based co-surfactant produced clear and homogeneous solutions called micromoles ion. Micro emulsion is a good technique for the synthesis of nanoparticles in which two immiscible fluids such as water in oil (W/O) or oil in water (O/W) or water in supercritical carbon dioxide (W/Sc. CO_2) become a thermodynamically stable dispersion with the aid of a surfactant. A typical emulsion is a single phase of three components, water, oil and a surfactant. Normally, oil and water are immiscible but with the addition of a surfactant, the oil and water become miscible because the surfactant is able to bridge the interfacial tension between the two fluids. Micro emulsion consists of surfactant aggregates that are in the ranges of 1-100 nm. The micro emulsion is said to be oil in water (O/W), if water is the bulk fluid and oil is in less quantity with small amounts of surfactant. Similarly, the system is said to be water in oil (W/O), if oil is the bulk fluid and water is present in less quantity. The product of oil in water and surfactant (O/W) is called micelles which is an aggregate form to reduce free energy (Umer *et al.*, 2012). Hydrophobic surfactants in nanoscale oil and micelles point towards the center of aggregate, whereas the hydrophobic head groups toward water, the bulk solvent. The water in oil micro emulsion carries oil or organic solvent as bulk. The system is thermodynamically stable and called reverse micelles. Purely metallic nanoparticles (Cu, Ag, Co, Al), oxides (TiO SiO,), metal sulphides (CdS, ZnS) and various other nanomaterials are prepared using this technique. The pioneering demonstrated synthesis of copper and silver nanoparticles using reverse micelle system. The use of salts and the presence of anions within reverse mice lies have been shown to alter the physical properties of the water environment and surfactant layer resulting in variations in the size and shape of the reverse micelles and metallic particles synthesized.

Biosynthesis of nanoparticles: Soil microorganisms and plants (extracts) are important bio agents and possess great potential and scope in the synthesis of nanoparticles. Development of an efficient and eco-friendly processes of synthesis of nanoparticles is an important aspect of bio nanotechnology (Khan *et al.*, 2009). One of the important options to achieve this objective is to use ‘natural bio resources’ such as microorganisms and plant based materials to produce Nano size particles of the matter.

Microbial synthesis of nanoparticle: Soil microorganisms constitute one of the vast and strong natural factories to harness beneficial effects through biotechnology and nanotechnology (Gurunathan *et al.,* 2009). Numerous microorganisms have been found to produce nanoparticles in the substrate (Khan and Anwer, 2011; El-Rafie *et al.,* 2012). Several researchers have shown that macro matter can be converted into nanomaterial with the use of microorganisms. reported synthesis of silver nanoparticles (Ag NPs) through reduction of Ag+ ions by the culture supernatant of E. coli. The synthesized silver nanoparticles were purified by using sucrose density gradient centrifugation. The purified sample was further characterized by UV-vis spectra, fluorescence spectroscopy and TEM. The purified solution yielded the maximum absorbance peak at 420 nm and the TEM characterization showed a uniform distribution of nanoparticles of an average size of 50 nm. (Manonmani and Juliet, 2011) worked on extracellular biosynthesis of "silver nanoparticles" using Escherichia coli and characterization of nanoparticles by UV- visible spectroscopy, FTIR and SEM of nanoparticles against different human pathogenic bacteria in food. This technique is used to combine function of Surface Enhanced Resonance Raman Scattering (SERRS) and silver nanoparticles to produce antigen-antibody interaction onto biological products. used fungus Fusarium solani for biosynthesis of silver nanoparticles. They also examined the factors affecting the biomass concentration, pH of the reaction medium, AgNO3 concentration and the ratio of AgNO3 to biomass concentration on the production of AgNPs. Optimum conditions for biosynthesis of AgNPs could be attained using biomass of F. solani (10 g/100 mL), AgNO (0.078 g/100 mL), pH, 12, temperature, 25°C and duration, 24 h that yielded stabilized concentration of 2000 ppm Ag NPs with a mean diameter range of 8-15 nm.

The biosynthesis of Ag and Au nanoparticles (NPs) was investigated by (Mourato *et al.,* 2011) using an extremophiles yeast strain isolated from acid mine drainage in Portugal. A successful route for the metal NPs synthesis was obtained using the yeast biomass that yielded Ag NPs (20 nm) and Au NPs (30-100 nm). Using the bacterium Bacillus licheniformis, the biosynthesis of silver nanoparticles of 50 nm size has been achieved. Similarly, extracellular biosynthesis of silver nanoparticles was achieved by Aspergillus niger. Geobacter sulfurreducens reduced Ag(I) as insoluble AgCl or Ag+ ions, via a mechanism involving c-type cytochromes, precipitating extracellular nanoscale silver. isolated silver nanoparticle from culture of gram positive bacteria Morganella morganii. A good quantity of gold nanoparticles was obtained with the use of extremophilic bacteria, Thermomonos pora sp. have reported synthesis of gold nanoparticle with the help of mesophilic bacterium Shewanella, using H, as an electron donor. The bacterium Rhodo pseudomonas capsulate produced gold nanoparticles of 10-20 nm size. Bacterial cell supernatants of Pseudomonas aeruginosa have been found to reduce the gold ions for extracellular biosynthesis of gold nanoparticles. S. cerevisiae has been found to produce gold nanoparticles in the size range of 2-10 nm. Biosynthesis of nanoparticles using fungi such as *F. oxysporum, Collitotrichum sp. Trichothecium sp., Trichoderma asperellum, T. viride, Phaenerochaete chryosporium, F. solani, F. semitectum, Aspergillus fumigatus, Coriolus*

versicolor, Aspergillus niger, Phoma glomerata, Penicillium brevicompactum, Cladosporium cladosporioidis, Penicillium fellutanum and *Volvariella volvacea* has been extensively studied.

Commonly used Nanoparticles for plant disease management

The nanoparticles of metalloids, nonmetals, metal oxides and carbon nanoparticles have antifungal and antibacterial activity. Some of these nanoparticles also have nutritional benefits on plants and also increase host resistance to disease. The nanoparticles CuO, Sio2 can enhance the defence products in plants. The nanoparticle which are commonly used as carries for fungicides, insecticides, herbicides are as fallowing Silver Nanoparticle Silver can disinfect almost 650 microbes and also nontoxic to humans but controls the metabolism function inside the microbes It could be applied for controlling of many plant pathogens in a safer way compared to synthetic fungicides Nano silver reduces different plant diseases caused by spore producing fungal pathogens. The silver nanoparticles are applied before the penetration and colonization of fungal spore within the plant tissues. The small size of the active ingredient (1-5 nm) of silver effectively controls diseases like powdery mildew.

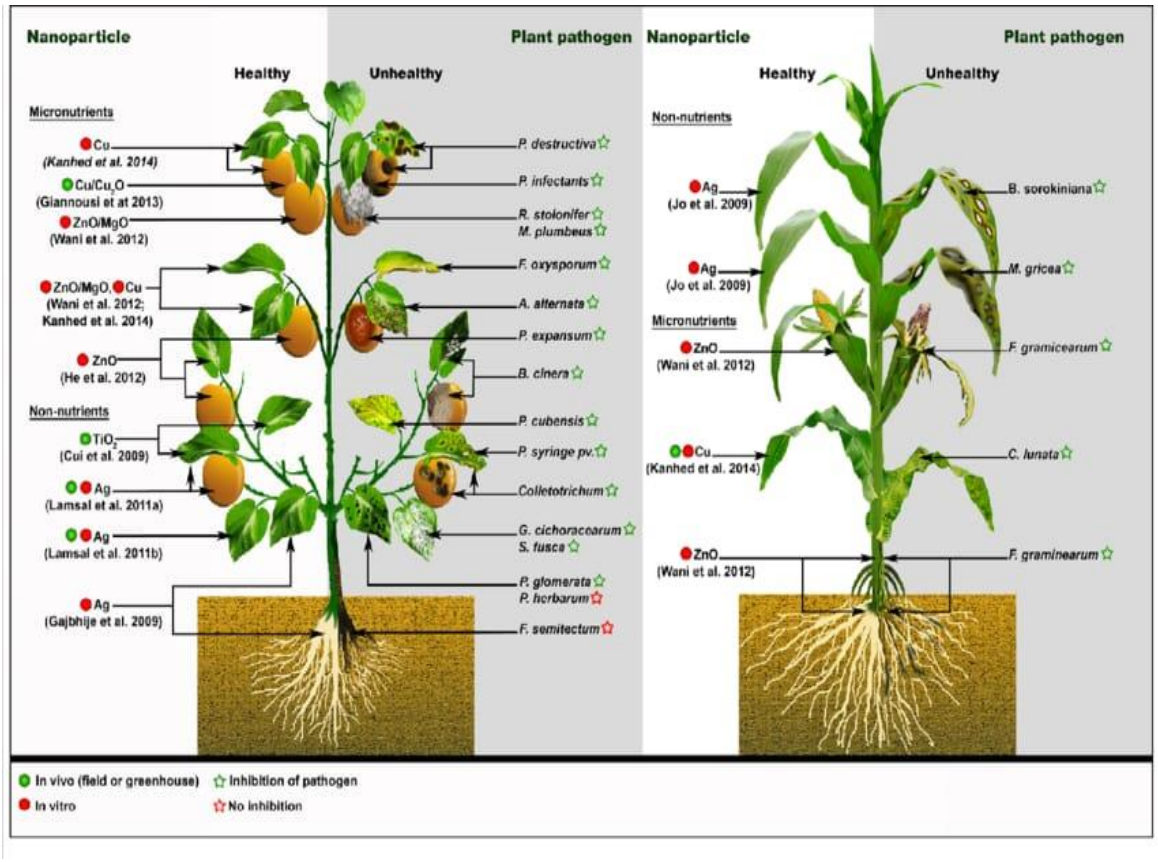

Fig. 1: Nanoparticle studies with micronutrient and non-nutrient on diseases of citrus and maize

A. Silica nanoparticle: Silica is known to be observed into plants to increase the disease resistance and stress resistance. Silica nanoparticles can be easily synthesized with controlled size and shape, making them as delivery vehicles, it promotes the physiological activity and growth of plants and also induces the diseases and stress resistance in plants. Mesoporous silica nanoparticles can be used for targeted delivery of DNA and chemicals. The shell structure of

porous hallow silica nanoparticles protect the active molecule inside the nanoparticle against degradation by UV light. The silica coating causes the plant to receive the particles through cell wall of the cell, where the genes are put in and stimulated in an accurate and controlled manner with no toxic side effects. This method has been used to successfully introducing DNA into plants such as corn and tobacco. Silica-silver nanoparticles reportedly have antifungal activity against *Botrytis cinerea, Rhizoctonoa solani*, *Collectotrichum gloeosporioides*.

B. Chitosan nanoparticle: Chitosan nanoparticles have hydrophobic properties due to this property it is low soluble in aqueous solution. As a result, chitosan is commonly mixed with organic and inorganics to improve solubility. The chitosan inhibition mode against fungi is defined by these three mechanism (i) it is proposed that chitosan can penetrate the cell wall of fungus and bind to its DNA and hinder mRNA synthesis and in turn, impact the production of vital enzymes and protein. (ii) chitosan chelates with metal ions, which are suggested as a possible antimicrobial action mode (iii) The positively charged chitosan interact with negative charge of phospholipid components of fungi membrane, which alter cell permeability of plasma membrane and lead to leakage of cellular components, which subsequently leads to death of cell. Chitosan nanoparticle exhibited antifungal activity in vitro and could protect the finger millet plants from blast disease caused by *Pyricularia grisea*. Chitosan nanoparticles were reported to be effective against plant pathogenic fungi and bacteria effecting tomatoes. The growth inhibitory effects were maximum in *F. oxysporum* fallowed by *P. capsici*, *Xanthomonas compestris* pv. *Vesicatoria* as well as *Erwnia carotovora was* inhibited.

C. Copper nanoparticles: Due to broad spectrum antimicrobial properties copperbased compounds has been used for centuries to manage plant pathogens. The first metal-based fungicides used in plant disease management comprised of copper and coppercontaining compounds. Usage of copper oxychloride and copper hydroxide, Bordeaux mixture etc. to control bacterial blight in pomegranate continues even today. Bordeaux mixture that is composed of lime, copper sulphate and water, which was used to control grapevine downy mildew disease caused by *Plasmopora viticola* an oomycete pathogen. It was discovered that nanoparticle can be effective in controlling bacterial disease, namely leaf spot of mung and bacterial leaf blight in rice. Copper nanoparticles at a concentration of 200 mg/L were inhibitory to *Pseudomonas srringae*, whereas the particle were not biocidal against Rhizobium spp. and *Trichoderma harzianumin* comparison to copper oxychloride. This study demonstrated that Nano copper could potentially be used in agriculture because of evidence of the non-biocidal effect.

D. Gold nanoparticles: DNA-gold nanoparticle probes hold promise as a new generation of biosensors in the detection of pathogenic microorganisms. In this technique, the gold nanoparticle oligonucleotide probes are hybridized with the complementary DNA, which stabilizes gold nanoparticles against aggregation (retaining the native pink color of the colloidal gold). In the absence of complementary DNA, the solution turns purple because the aggregation of gold nanoparticles leads to the shift in absorbance peak toward a longer wavelength. Gold nanoparticles feature agglomeration related to color production that was used in the detection of pesticide. Developing a color signal facilitated easy visual detection when gold nanoparticles

marked antibodies bound to the pesticide residues. The gold nanoparticle-based dipstick technique suited the detection of numerous toxins in environmental and food samples and can be used for rapid examination of pesticides on the site. Gold nanoparticle-based optical immune sensors have been developed for detection of Kernel bunt disease in wheat.

E. Zinc nanoparticles: In agriculture ZnO is primarily used as a micronutrient fertilizer however, its antimicrobial properties are well known. Mechanism of action of Nano-ZnO derived from zinc nitrate on important pathogen Aspergillus fumigates demonstrated that it made cell wall deformity by hydroxyl and superoxide radicals mediated in fungal and finally led to death due to high energy transfer. ZnO nanoparticles (ZnO NPs) were also reported to be effective against two postharvest pathogenic fungi (*Botrytis cinerea* and *Penicillium expansum*), thus contributing in agriculture and food safety application. ZnO nanoparticles prevented the development of conidiophores and conidia of *P. expansum*, which eventually lead to death of fungal hyphae.

Effect of nanoparticles on the pathogens/ microorganisms: Since, physio chemical properties of Nano forms from its macroform vary greatly, it becomes important to examine the effect of NPs on microorganisms to harness the benefit of this technology in the plant protection especially against phyto-pathogens. Nanoparticles because of ultra-small size, even smaller than a virus particle and high reactivity, may affect the activity of microorganisms. The silver has been generally found non injurious to microorganisms. However, silver NPs inhibited the colonization of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*. The highest antimicrobial activity of silver nanoparticles (30 mm) synthesized by *Solanum tricobatum* and *Oeirnum tenutflorum* leaf extracts was found against *S. aureus* and *E. coli*, respectively. The information so far available on this aspect has shown that the nanoparticles have definite effect on the colonization of bacteria and fungi. However, these effects are suppressive as well as stimulatory and hence cannot be generalized.

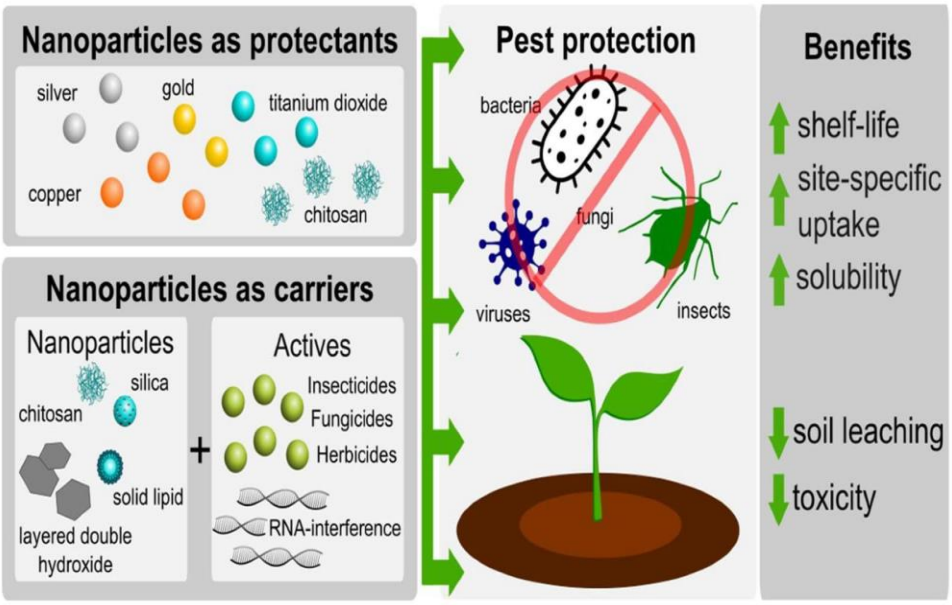

Fig. 2: Nanoparticles

a. Effect of nanoparticles on bacteria: Antibacterial activity of zinc nanoparticles against *P. aeruginosa* has been reported by Jayaseelan *et al.* (2012). The maximum zone of inhibition in the colonization of the bacteria (22+1 .8 mm) was recorded at 25 ng mL-1ZnO NPs. The study showed that the ZnO NPs proved to be a novel antimicrobial material. The antibacterial activity of the synthesized Ag NPs/PVP (hybrid materials based on polyvinylpyrrolidone with silver nanoparticles) against three different groups of Bacteria-*Staphylococcus aureus* (gram positive bacteria), *E. coli* (gram-negative bacteria), *P. aeruginosa* (nonferment gram-negative bacteria), as well as against spores of *Bacillus subtilis* has been studied (Bryaskova *et al.*, 2011). The antibacterial activities of CuO NPs have also been reported against S. aureus, Bacillus subtilis, *P. aeruginosa* and *E. coli* silver nanoparticles showed high antimicrobial and bactericidal activity against gram positive bacteria such as *E. coli*, *P. aureginosa* and *S. aureus* which are highly methicillin resistant strains. The antibacterial activity of nanoparticles was found to be dependent of NPs concentration, physiology, metabolism, intracellular selective permeability of membranes and the type of microbial cell.

b. Effect of nanoparticles on plant pathogenic fungi: The nanoparticles have also been found suppressive to fungi (Singh *et al.*, 2013) reported that among Nano forms of 15 micronutrients, $CuSo_4$, and $Na_2B_4SO_7$ were found most effective in controlling rust disease of field peas. Microelements such as manganese and zinc also suppressed the damping off and charcoal rot diseases in sunflower. The Ag NPs/PVP were tested for fungicidal activity against different yeasts and molds such as *Candida albicans, C. krusei, C. tropicalis, C. glabrata* and *Aspergillus brasiliensis*. The hybrid materials showed strong antifungal effects against the tested microbes (Bryaskova *et al.*, 2011). Fungicidal effect of zinc oxide nanoparticles (ZnO, NPs) against two postharvest pathogenic fungi, *Botrytis cinerea* and *Penicillium expansum*, have been reported. Traditional microbiological plating, Scanning Electron Microscopy (SEM) and Raman spectroscopy were used to study antifungal activities of ZnO, NPs and to characterize the changes in morphology and cellular compositions of fungal hyphae. The ZnO, NPs (70+15 nm) at the concentrations greater than 3 Mmol L-1 significantly inhibited the growth of *B. cinerea* and *P. expansum*, the later fungus was found more sensitive to the treatments. The NP treatments caused deformation in the hyphae of *B. cinerea* and prevented the development of conidiophores and conidia in. expansum which eventually led to the death of fungal hyphae. examined the effect of silver nanoparticles on plant pathogenic fungi, *Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, B. cinerea* and *Curvularia lunata* and found that 15 mg L -1concentration of NPs greatly inhibited the activity of all the tested pathogens. The zinc nanoparticles (25 mg mL-1) suppressed the colonization of A. jaw (Jayaseelan *et al.*, 2012).

Application of nanoparticles in management of plant diseases

The nanoparticles are used to protect plants in two different mechanisms:

A. **Nanoparticle act as carrier for pesticides can be applied by spray application:**

Nanoparticle act as carrier can provide several benefits (a) increases shelf life of pesticides (b) it increases the solubility of poorly water soluble pesticides (c) increases the site specific uptake into target pest (d) reduces toxicity.

B. **Nanoparticle themselves providing protection to crop:** Nanoparticles can also be directly applied to plant seed, foliage, roots for protection against pest and pathogen. Metal nanoparticles such as silver, copper, zinc oxide and titanium oxide have been intensively researched for their antibacterial and antifungal properties.

Silver nanoparticles have shown antifungal inhibition of *Alternaria alternata, Sclerotinia sclerotiorum, Rhizoctina solanai* (Krishnaraj *et al.*, 2012). When poly-dispersed gold nanoparticles introduced into plant through mechanical abrasive was seen to melt and dissolve the Barley yellow mosaic virus particles conferring resistance to plant Perusal of the literature and critical analysis of the relevant information have revealed that nanotechnology has potential prospects of use and application in the detection, diagnosis and management of plant diseases. The limited studies so far conducted on this aspect are sufficient enough to warrant potential future use of nanotechnology in plant disease management. The researches have shown that direct application of nanoparticles significantly suppressed the plant pathogenic fungi and bacteria tested. Nanomaterials, nanotubes and microcapsules can efficiently carry higher concentration of active ingredients of pesticides, host resistance inducing chemicals, polyamine synthesis inhibitors, etc. and would regulate the release of chemicals from the Nano carriers as per requirement. Enzyme based biosensors coated with nanoparticles of Au, Ag, Co, Ti, etc., may greatly help in the precise and quick diagnosis of plant infection and also residue detection of pesticides. Despite of tremendous application scope of nanotechnology in plant disease management, there are certain dements, risk and apprehensions in the use of NPs in agriculture which are required to be worked out on priority and before the commercial use of nanotechnology in agriculture. Foremost is the phytotoxic behavior of the nanomaterials which needs to be thoroughly understood and ascertained at different plant growth stages such as seed germination, seedling growth, fruit setting and maturity. Effect of seed coating with NPs on the seed germination, root development and plant growth/ productivity will be of immediate concern.

Conclusion:

Nanotechnology has potential prospects of use and application in the detection, diagnosis and management of plant diseases. Nanoparticles significantly suppressed the plant pathogenic fungi and bacteria. Nanomaterials, nanotubes and microcapsules can efficiently carry higher concentration of active ingredients of pesticides, host resistance inducing chemicals, polyamine synthesis inhibitors, etc. and would regulate the release of chemicals from the Nano carriers as per requirement. Enzyme based biosensors coated with nanoparticles of Au, Ag, Co, Ti, etc., may greatly help in the precise and quick diagnosis of plant infection and also residue detection of pesticides. Foremost is the phytotoxic behavior of the nanomaterials which needs to be

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References:

Azam, A., Ahmed, A.S., Oves, M., Khan, M.S and Memic, A. 2012. Size-dependent antimicrobial properties of CuO nanoparticles against Gram- positive and-negative bacterial strains. International journal of Nano medicine. 3527-3535.

Balaure, P.C., Gudovan, D., Gudovan, I. 2017. Nano pesticides: A new paradigm in crop protection. New Pestic. Soil Sens. 2017, 129–192.

Bryaskova, R., Pencheva, D., Nikolov, S. and Kantardjiev T. 2011. Synthesis and comparative study on the antimicrobial activity of hybrid materials based on silver nanoparticles (AgNps) stabilized by polyvinylpyrrolidone (PVP). Journal of Chemical biology. 4: 185-191.

Dubchak, S., Ogar, A., Mietelski, J.W. and Turnau, K. 2010. Influence of silver and titanium nanoparticles on *arbuscular mycorrhiza* colonization and accumulation of radiocaesium in *Helianthus annuus*. Spanish Journal of Agricultural Research, (1), 103-108.

El-Rafie, M.H., Shaheen, T.I., Mohamed, A.A., and Hebeish, A. 2012. Bio-synthesis and applications of silver nanoparticles onto cotton fabrics. Carbohydrate. Polymers., 90 (2): 915-920.

Flood, J. 2010 The importance of plant health to food security. Food Security. 2, 215–231.

Ghormade, V., Deshpande, M.V., Paknikar, K.M. 2011 Perspectives for Nano-biotechnology enabled protection and nutrition of plants. Biotechnology Advances. 29(6): 792–803.

Gurunathan, S., Kalishwaralal, K., Vaidyanathan, R., Venkataraman, D. and Pandian S.R.K 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. Colloids Surfaces. B:Biointerfaces,74(4): 328-335.

Hayles, J., Johnson, L., Worthley, C., Losic, D. 2017 Nano pesticides: A review of current research and perspectives. New Pesticides and soil Sensors. 193–225.

Hoar, T.P. and J.H. Schulman. 1943. Transparent water-in-oil dispersions: The Oleopathic Hydro- micellea, Nature 152:102-103.

Jayaseelan, C. Rahuman, A.A. Kirthi, A., Marimuthu, V. S. and Santhoshkumar, T. 2012 Novel microbial route to synthesize ZnO nanoparticles using *Aeromonas hydrophila* and their activity against pathogenic bacteria and fungi. Spectrochimica Acta part A: Molecular and Biomolecular Spectroscopy. 90:78-84.

Khan, M.R. and Anwer M.A. 2011. Fungal bio inoculants for Plant Disease Management. Microbes and Microbial Technology, Agricultural and Environmental Applications. 447-488.

Khan, M.R., Altaf, S., Mohidin, F.A., Khan, U. and Anwer, A. 2009. Biological Control of Plant Nematodes with Phosphate Solubilizing Microorganisms. Phosphate Solubilizing Microbes Crop Improvemen.395-426.

Khan, M.R., Altaf, S., Mohidin, F.A., Khan, U. and Anwer, A. 2009. Biological Control of Plant Nematodes with Phosphate Solubilizing Microorganisms. Phosphate Solubilizing Microbes Crop Improvemen.395-426.

Khan, M.R., Altaf, S., Mohidin, F.A., Khan, U. and Komarneni, S. 2003. Nano phase materials by hydrothermal. Microwave-hydrothermal and microwave- solvothermal methods. Current science. 85: 1730-1734.

Krishnaraj, C., Ramachandran, R., Mohan, K. and Kalaichelvan, P.T. 2012. Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. Spectrochimica. Acta Part A: Molecular and. Biomolecular Spectroscopy. 93: 95-99.

Manonmani, V. and Juliet, V. 2011. Biosynthesis of Ag nanoparticles for the detection of pathogenic bacteria. Proceedings of the 2nd International Conference on Innovation, Management and Service, September 16-18, 2011, Singapore, 307- 311.

Mourato, A., Gadanho, M., Lino A.R. and Tenreiro, R. 2011. Biosynthesis of crystalline silver and gold nanoparticles by extremophiles yeasts. Bioinorganic chemistry and applications :8. (10)

Singh, D., Kumar, A., Singh, A.K. and Tripathi, H.S. 2013. Induction of resistance in field pea against rust disease through various chemicals/ micronutrients and their impact on growth and yield. Plant Pathology Journal.12: 36-49.

Sinha, K., Ghosh, J., and Sil, P.C. 2017 New pesticides: A cutting-edge view of contributions from nanotechnology for the development of sustainable agricultural pest control in New Pesticides and Soil Sensors. Academic Press:47-79.

Sofi, W., Gowri, M., Shruthllaya, M., Rayala, s. and Venkatraman, G. 2012. Silver nanoparticles as an antibacterial agent for endodontic infections. BMC Infectious Disease.12(1) 1-1.

Stephenson, G.R. 2003 Pesticide Use and World Food Production: Risks and Benefits; ACS Publications: Washington, DC, USA

Taniguchi, N., 1974. On the basic concept of Nano- technology. Proceedings of the International Conference on Production Engineering, August 26- 29, Tokyo, pp: 18-23.

Umer, A., Naveed, S., Ramzan, N. and Rafique, M.S. 2012. Selection of a suitable method for the synthesis of copper nanoparticles. Brief Rep. Rev: 1-18.

Wang, S., Lawson, R., Ray, P.C. and Yu, H. 2011.Toxic effects of gold nanoparticles on *Salmonella typhimurium* bacteria. Toxicology and Industrial health, 27(6): 547-554.

VARIOUS METHODS OF PREPARATION OF NANOPARTICLES

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Abstract:

A nanotechnology that measures, manipulate constituents or structures with a critical dimension between 1nm – 100nm, and also whose application exploit properties distinct from bulk macroscopic systems from which they arise. Novel physical properties and new technologies both in sample preparation and device manufacture induce on account of the development of nanoscience. Various research fields like chemistry, material science, engineering and physics of mechanical and electrical are involved in this research. Synthesis of nanoparticles that are important part of nanomaterial. Materials scientists and engineers have made significant developments in the improvement of methods of synthesis of nanomaterial solid. In this chapter various methods of preparing nanoparticles they are discussing including physical and chemical,

Keywords: Nanoparticle; Preparation methods, Application

Introduction:

Man in search of knowledge is imagining and developing the physical world and its elements. In 1984, German scientist Gleiter et al. successfully synthesized iron nanoparticles for the first time by inert gas agglomeration. For decade, research on the preparation and application of nanoparticles has produced successful results. The basic constituent particles of nanomaterial are of the order of nanometres in size and are particles in the intersection of atomic clusters and macroscopic objects. Nanoparticles are particles which lie in dimension between 1 –100 nm. They consist of micro molecular materials in which the active ingredients like biological active material is dissolved, entrapped, encapsulated, absorbed or attached. They are many preparation methods for nanomaterial show in figure 1. Classification of nanomaterials in based on origin, dimension and structure. The most commonly adopted method of classification is based on their dimension. On the basis of dimensions, they are classified as follows (as shown in figure 2).

- **0-dimensional nanomaterial (0-D):** In Such materials have all three length scales along X-axis, Y-axis, and Z-axis in the nanoscopic range. Recently, 0-D such as uniform particle array and heterogeneous particle array, core shell quantum dots have been studied in LEDs, single electron transisters and solar cell. Example of quantum dots.
- **1-dimensional nanomaterials (1-D):** In such materials, one of the dimensions is out of the nanoscopic range. Have been extensively studied because both of their functional properties and highly controllable morphology. Examples of nanotubes and nanowires.

- **2-dimensional nanomaterials (2-D)**: These materials, any two dimensions are out of the nanoscopic range. Interesting for investigation and developing novel applications in sensor, photo catalysts, nano containers, and nanoreactor. Examples of nanofilms, nanolayers and nanosheets.
- **3-dimensional nanomaterials (3-D):** In these materials, all three dimensions are out of the nanoscopic range.

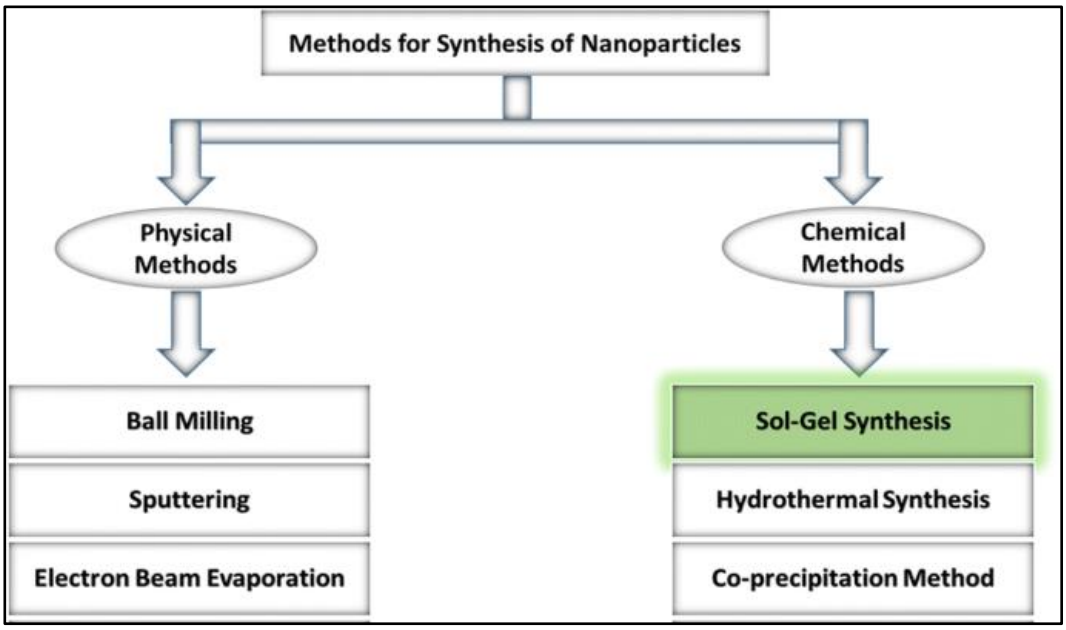
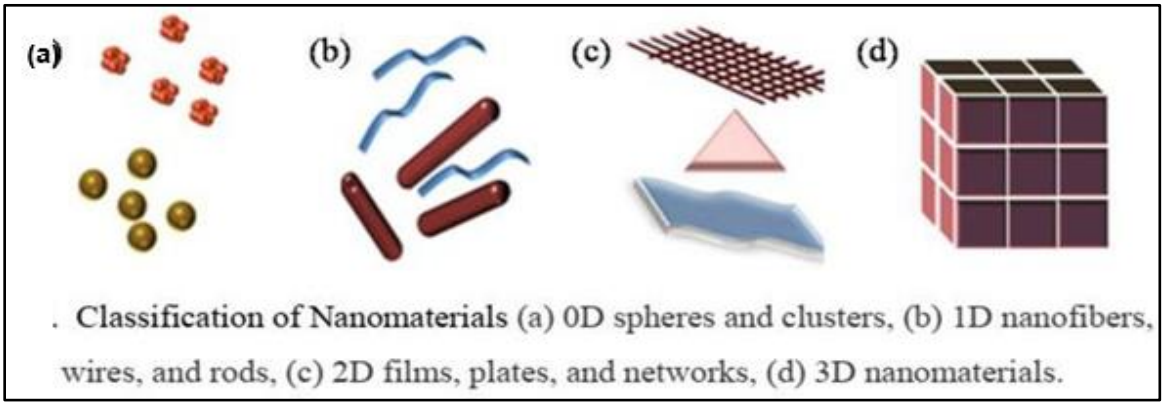


Fig. 1: Synthesis method of nanoparticles



. Classification of Nanomaterials (a) 0D spheres and clusters, (b) 1D nanofibers, wires, and rods, (c) 2D films, plates, and networks, (d) 3D nanomaterials.

Fig. 2: **Classification of nanomaterials**

Synthesis of nanomaterial

All the synthesis techniques are divided into two categories based on the phase of starting materials.

1. **Top-Down approach** - In this technique, the starting material is solid state.
2. **Bottom-Up approach** – All the Bottom-up technique, the starting material is either gaseous state or liquid state of matter.

- **Physical methods:**

1. Ball Milling:

Ball milling is a method of production of nanomaterial. A ball mill works on the principle of impact and attrition. Size reduction is done by impact as the ball drop from near the top of the shell.

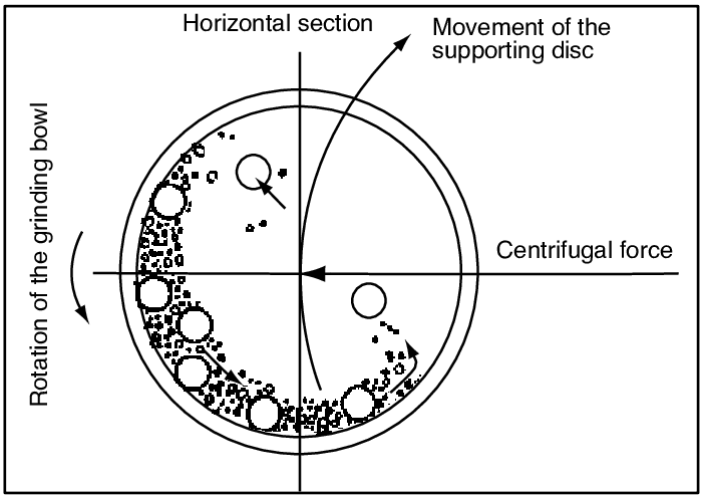


Fig. 3: Rotation of milling ball

A ball mill is a type of grinder used to grind materials into extremely fine powder. During the ball milling process, the collision between the tiny rigid balls in a concealed container will generate localized high pressure. Typically stainless steel, flint pebbles and ceramic are used. Ball milling of the nanoparticles with the base fluid is also one of the traditional methods for nanofluid preparation (1). Temperature, size and number of the balls, nature of the ball, and rotation speed this is major parameter for ball milling. Ball mills rotate around a horizontal axis, partially filled with the material to be ground plus the grinding medium as shown in figure 3.

Advantages:

1) Low cost of installation
2) Low cost of production
3) It is suitable for materials of all degree of hardness.
4) The grinding medium is cheap

2. Sputtering:

The sputtering process is a physical vapour deposition (PVD) process that deposits materials onto a particular surface by ejecting atoms from that material and condenses ejected atoms onto the surface when a high vacuum environment is maintained. Physical coating process involve 3 steps as below.

1) **Vaporization -** Vaporization of the material is done from a solid or strong source assisted by high temperature and vacuum.

2) **Transportation –** Transportation of the vapour is carried out in the vacuum towards the substrate surface. For this vacuum of the different ranges is used which depends on the mean free path required in the system.

3) **Condensation –** Condensation of the vapours is done onto the substrate to generate thin films. The chemical composition of the deposited film depends on the type of source material.

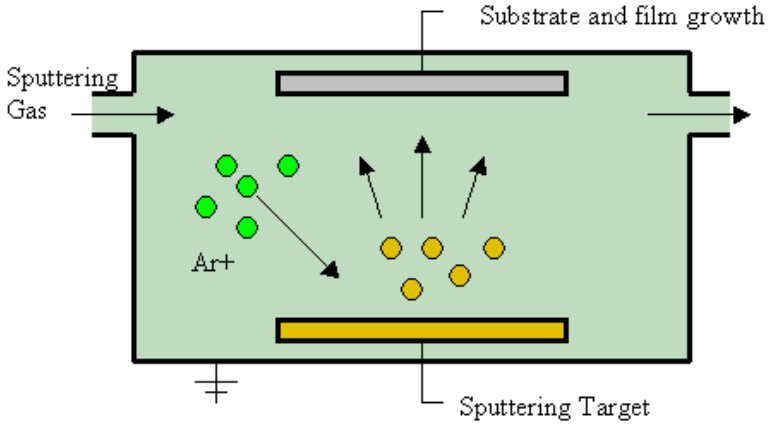


Fig. 4: Sputter deposition

PVD is formation of film at atomic and molecular level and thus it is used for high melting point and low vapour pressure materials. It is a collective set of processes used to deposit thin layers of material typically in the range of few microns to some micrometres. Sputtering is plasma assisted technique that creates a vapour from the source through bombarded with the accelerated gaseous ions (plasma). The gas used is generally Argon. Sputtering works on the basis of momentum principle, formed by the collision of the atoms and molecules. Plasma glow, ion accelerator or radioactive emitting is used to evaporate material shown in fig 4.

Advantages:

1) High film deposition rates can be achieved in PVD.
2) It gives high strength and durability
3) It provides high adhesion and hardness
4) It has less tendency for unintentional heating of the substrate surface.

3. Electron beam evaporation:

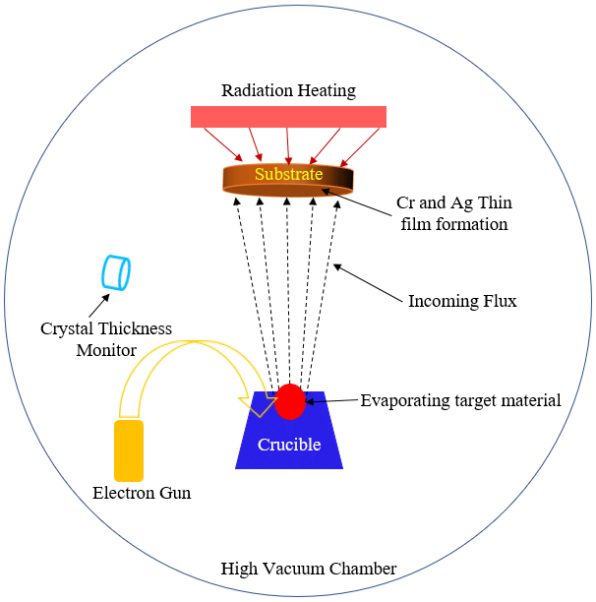


Fig. 5: Electron beam evaporation

The beam of electrons are emitted in a way that it will heat and vaporize the material to be deposited. Once the material changes its state of being a liquid to a vapour, it is able to condense itself on the substrate. Electron beam evaporation is obtainable on a variety of PVD platforms and for several applications like metallization, dielectric coating, optical coatings, and Josephson junctions.

Advantages:

The main advantage of electron beam evaporation is the ability to rotate several source materials into the path of the electron so that multiple thin films can be deposited consecutively without breaking vacuum.

- **Chemical methods:**

Sol Gel

The sol-gel method (shown in fig. 6) is one of the most important chemical methods for material preparation. Involves the evolution of inorganic networks through the formation of a colloidal suspension (sol) and gelation of the sol to form a network in a continuous liquid phase (gel). It provides a new way to synthesize inorganic ceramics, glass and nanomaterials at room temperature and pressure. The main step of preparing the nanoparticles by using sol-gel method is to select the metal compound to be prepared, then dissolve the metal compound in a suitable solvent and solidify it by a sol-gel process, and finally obtain a nanoparticle by low temperature treatment. Application of this method is protective coatings, thin film and fibres, nanoscale powder and opto-mechanical.

Advantages:

1) Mono sized nanoparticles are produce.
2) Cheap and low temperature operation very thin of metals oxide can be obtained.
3) This method is useful for the synthesis of glasses, glass ceramic at lower temperature

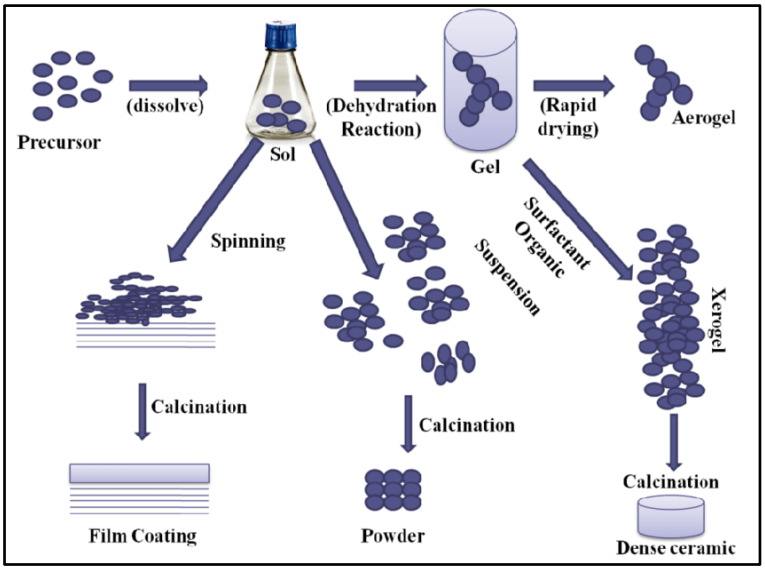


Fig. 6: Sol-Gel method

Hydrothermal

The hydrothermal synthesis can be defined as a method of synthesis of single crystals that depends on the solubility of minerals in hot water under high pressure. It employs the combination of heat and water as a media to convert unutilized resources in various shapes and characteristics into uniform product. It is a functional way to active reaction via dissolution. It involves heating of raw slurry at 300^0-350^0C in at pressure to maintain water phase. It is a thermo chemical conversion technology.

The reactants are dissolved in water or another solvent in closed vessel. Conventional microwave oven. Commercially tons of zeolites daily. Hydrothermal synthesis can produce nanomaterials which are not stable at high temperatures. Nanomaterials with high vapour pressures can be produced by the hydrothermal method with minimum loss of materials.

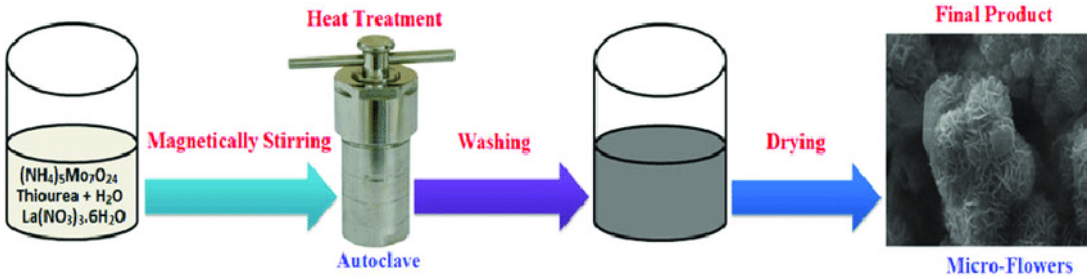


Fig. 7 : Schematic diagram of hydrothermal synthesis process for 3D flower

Advantages:

1) Simple procedure
2) Low cost
3) Suitable for mass production of hap powder
4) The method of choice for commercial production.

Co-Precipitation:

Coprecipitation is the occurrence in which soluble compounds are removed from solution during precipitation formation. In chemistry area coprecipitation is of the substances normally soluble under the conditions employed. Analogously, in medicine, coprecipitation is specifically the precipitation of an unbound “antigen along with an antigen-antibody complex”. There are four type of coprecipitation methods.

1) Surface Adsorption
2) Mixed crystal formation
3) Occlusion, and
4) Mechanical Entrapment

Surface adsorption and Mixed-crystal formation are equilibrium processes. Occlusion and Mechanical Entrapment arise from kinetic of crystal growth,

Surface adsorption:

It is a common source of coprecipitation that is likely to cause significant contamination of precipitates with large specific surface areas. Coagulation of a colloid does not significantly

decrease the amount of adsorption. The net effect of surface adsorption is therefore the carrying down of an otherwise soluble compound as a surface contaminant.

Mixed crystal formation

In this formation, one of the ions in the crystal lattice of a solid is replaced by an ion of another element.

Occlusion, and mechanical entrapment

When a crystals is growing rapidly during precipitate formation, foreign ions in the counter ion layer may become trapped within the growing crystal. Occlusion in which a compound is trapped within a pocket formed during rapid crystal growth. Mechanical entrapment occurs when crystals lie close together during growth.

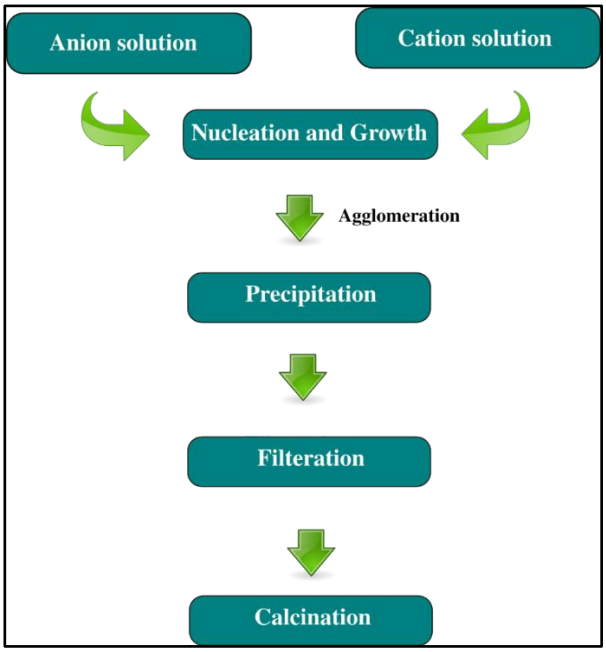


Fig. 8: Coprecipitation Method

Advantages:

1) Simple procedure
2) High yield
3) Low cost
4) High product purity

Application of nanomaterial:

Nanomaterials can be used in different applications such as in photovoltaic cells, drug, electrical device like (transistor, basic switches), military applications, chemical industry, Food production industry like (bakery product), paints, catalysts, and sunscreens. Few nanoparticles do not have an effect on the environment, while others have an effect on it.

Conclusion:

Nanomaterials have distinct properties that are different from bulk materials and individual molecules – quantum size effect, surface effect and macroscopic quantum orbital effect due to the special physical, optical, structural and chemical properties of nanomaterials. In this review described the details about nanoparticles synthesis by various method and its

applications in the food processing, ceramic, medicine, packaging, biology and optics. We observed that basically so many researcher used wet chemical method because this methods is very easily prepare nanoparticles and low cost.

References:

1. Bharat Bhanvase, Divya Barai, Nanofluids for Heat and Mass Transfer, Fundamentals, Sustainable Manufacturing and Application, 2021, 69-97
2. B. Rodr´ıguez, Angew. Chem., Int. Ed., 2006, 45, 6924
3. Guan-Wu Wang, J. Org. Chem., 2008, 73, 7088
4. G.Kaupp, Top. Curr. Chem., 2005, 254, 95
5. M. Reza Naimi-Jamal, Eur. J. Org. Chem. 2009, 3567–3572
6. Fatin Fatihah Binti Zahari, EEN3016 Processing And Fabrication Technology Trimester, 2011/12, 2
7. Khalid Nadeem Riaz, Nadeem Yousaf, Muhammad Bilal Tahir, Zainab Israr, Tahir Iqbal, International journal of Energy Research, 2018
8. S. Kandasamy and R. Sorna Prema, Journal of Chemical and Pharmaceutical Research, 2015, 7(3), 278-285
9. Hadeel J. Imran1, Kadhim A. Hubeatir , Kadhim A. Aadim , Dhuha Sh. Abd, Journal of Physics: Conference Series, 2021, 1818, 012127

CRISPR/CAS9 TECHNOLOGY AND THEIR APPLICATIONS

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Genome editing otherwise called as genome editing, gene editing and genome engineering is a method the DNA is inserted, modified, deleted and replaced in a genome of living organism. This mechanism mainly focused to the targets the insertions to a site-specific location (Saurabh and Bak, 2021).

In Genome editing technology involved various field including biotechnology to improve food production, Disease curing in Human and as well as animal, involved in pest control and generation of bio-medicine using animal as a model. Growing population of human is a biggest challenge for the future in human survive due to the reasons of increasing demand for food requirements. (UN, 2017).

Genome editing technology TALENs and ZFN are powerful tool (Gaj *et al.,* 2017) Genome editing technology involves methods of site-specific endonucleases methods including Transcription Activator Like Effector Nucleases (TALENs), Zinc finger nucleases and CRISPR-cas system which make a double stranded break in the specific sites of host genome. After the double bond formation, the endogenous DNA repaired by non-homologous end joining (NHEJ) or Homologous recombination (HR) (Tahir *et al.*, 2017).

The NHEJ method of repair which create a small deletion and repaired by the HR method which create a specific change in a desired region. In CRISPR – Cas method make an alternation in target site, for example, changes DNA sequence by nucleotide base editing or altering the chromatin to cause a heritable change (Zafar *et al.*, 2020). CRISPR -Cas based system which target site by an RNA, and this system site-specific and programmable. This system is more efficient, easy to design, cost-effective, robust and popular than the TALENs and ZFN genome editing system (Zhang *et al.*, 2020).

Due to the increasing the population of Human and some changes occurring in our climate which causing a heavy pressure on global food security. United Nations (UN) states that, the world population increase and demand for food will rise to 8.5 billion and 11.6 billion tons by 2023 (UN, 2019).

CRISPR, or Clustered Regularly Interspaced Short Palindromic Repeats, is an integral part of a bacterial defense system (Horizon Discovery, 2016). Now, CRISPR -Cas used in various field to edit a genome in efficient level includes human, animal and plant genomes (Shan and Cong, 2013) and also involved in animal domestication, food science and pharmacology (Kaboli *et al.*, 2018).

In this chapter, we discussed how genome editing tools involved in various field including Agricultural for crop improvement and using animal as a model for a treating a various

human disease with the help of ZFNs, TALENs and most importantly using CRISPR/Cas9 method for gene editing, modification.

Genome editing tool

ZFN (Zinc Finger Nuclease)

The zinc-finger protein with site-specific binding properties to DNA was discovered primarily in 1985 as part of transcription factor IIIa in *Xenopus* oocytes (Diakun *et al.,* 1996) ZFNs are assembled by fusing a non-sequence-specific cleavage domain to a site-specific DNA-binding domain that is loaded on the zinc finger (Kim, 1996). The chimeric nucleases, ZFNs, were developed in 2001 (Bibikova *et al.,* 2001) and designed to target and disrupt precise DNA sequences (Qomi *et al.,* 2019).

The specificity of zinc finger domain of cys2 Hos2 Zinc fingers are derived from the zinc finger domains with homologous DNA sequences. Individual Cys 2 His 2 contain 30 amino acid and two anti-parallel beta sheets of alpha helix (Beerli, 2002). Cys 2_His 2 ZF is most adaptable DNA recognition domain and common type of DNA-binding motif in eukaryotic transcriptions factors (Schopfer, 2000). Each zinc finger recognizes 3bp of DNA and produces base specific interaction in alpha helix major groove of DNA (Buck and Fairall, 2012).

The motif combined with genetically engineered enzyme FokI type II restriction endonuclease derived from *Flavobacterium okeanokoites* and identify the target site. Two zinc finger modules are bind to the DNA in sites that oppose every one with the FokI in the middle, which creates a homodimer complex. After the formation of homodimer, the nuclease breaks both the DNA strands and insertions of mutation is occurred (Adli, 2018). The target sit will change the residues in single zinc finger and alters the DNA recognition and zinc finger can be altered recognize many numbers of different DNA triplets (Carroll, 2017).

After the clevation of DNA by ZFN is achieved in eukaryotic cells, Double stranded Breaks at a specific site of genome is formed and creating the endogenous of Non-Homologous End Joining or HDR systems (Kim, 1996).

The target sequence are recognition and specification of ZFNs are identified by the methods of the amino acid sequence of each finger, the total number of fingers and the binding of nuclease domain (Beumer *et al.,* 2008) using ZFN method, recently architectural diversification was improved and targeting accuracy based on the “selection-based methods” (Paschon *et al.,* 2019).

TALENs (Transcription Activator Like Effector Nucleases)

TALENS are another genome editing tool which have a better efficiency and specificity than the ZFNs. Similar to the ZFNs, TALENs are non-specific DNA cleavage domain fused to an altered site- specific DNA binding domain and forms a Double Stranded Breaks (DSBs). This DNA binding domain contain a highly conserved repeat sequence from the transcription activator -like effector (TALE), these are the protein discovered by *Xanthomonas* bacteria which alters the genes in host plant cells (Boch and Bogdanove 2010).

Like ZFNs, TALENs also contain a FokI is fused to DNA -binding domain to create site specific Double Stranded Breaks and influenced DNA recognition and create a TALEN induced

target genomic modification. The cleaved strand of target site of DNA, The Fok I cleavage must be dimerized. Similar of ZFN, TALENs which bind an opposing of a DNA locus of binding site (Li *et al.,* 2011) Compare to the ZFN, TALENs support the different genomic modification such as nucleases (Mussolino *et al.,* 2011). transcriptional activators (Zhang *et al.,* 2011) and site-specific recombinases (Mercer *et al.,* 2012). the major difference between the ZFN and TALENs are related to the number of nucleotides are recognized by the protein domain 3bp vs 1 bp which making a more site- specific and low likely to cause an off-target cleavage. target cleavage (Khan, 2019).

The simpler cipher codes which give a better simplicity in design than triplet-confined zinc-finger proteins, these are the major hurdles for cloning repeat TALE array is design for large scale of identical repeats sequences. For this limitation some policy which facilitate the fast assemble of custom TALE arrays, such as Golden Gate molecular cloning (Cermak*et al.,* 2011) high-throughput solid phase assembly (Reyon and Briggs 2012) and connection-independent cloning techniques (Schmid *et al.,* 2013).

CRISPR/Cas9 (Clustered regularly interspersed short palindromic repeats)

CRISPR/Csa9 discovered in *E. coli* in the year of 1987 and later identified in many other species (Ishino *et al.,* 1987). the CRISPR/Cas9 complex was evolved as a primitive acquired immune system of some bacterial and their group of archaea species to defense against the foreign DNA of bacteriophage Humphrey and Kasinski, 2015) by inducing RNA-guided DNA cleavage (Pourcel and Bolotin 2005).

Commonly the CRISPR/Cas9 systems are divided into two types based on their variation in structure of Cas genes and their arrangement (Jinek *et al.,* 2012).

Class -I CRISPR/Cas9 system contains multiprotein effector complexes

Class -II CRISPR/Cas9 system contains only single effector protein

Together of all totally six-CRISPR/Cas types and contain 29 subtypes have been reported (Makarova *et al.,* 2015)

Oftenly used subtype of CRISPR system is the CRISPR/Cas 9 system of type-II these depends on the single cas protein of Streptococcus pyogenes which target particular DNA sequences and an effective gene editing tool (Jiang *et al.,* 2013) CRISPR/cas 9 system contain two components these are single-stranded guide RNA and a Cas9 endonucleases.

Single-stranded guide RNA (sg RNA) contains 20bp sequence for the complement of the target DNA site in a sequence specific manner and followed by a short DNA sequence of upstream and essential for the compatibility with the Cas 9 protein used these are called as “protospacer adjacent motif” of an NAG (Sternberg and Deveau 2014). The sgRNA bind to the specific site of DNA sequence by Watson and crick base pairing and cas 9 which cleaves the DNA and generate a Double stranded break (Gasiunas *et al.,* 2012). These double stranded breaks are repaired by NHEJ or HDR. The targeted sites are modified including the small deletions or insertions can be made (Ran *et al.,* 2013).

RNA guided system, CRISPR/Cas 9 is one of the most suitable methods for gene editing technologies compared to the ZFNs and TALENs and having a significant advantage (Cox *et al.,* 2015)

Another advantage of using CRISPR/Cas 9 important to simultaneously making a multiple locus editing, and these technologies are easy, more efficient genome editing tool compared to other methods. Now, CRISPR/Cas9 is most important in biological research.
Commonly 3 methods are developed for gene editing with CRISPR/Cas9 system, these are

- The plasmid based CRISPR/Cas9

  The plasmid which encodes a Cas9 protein and sgRNA **(**Cong and mali 2013) which assembles cas9 gene and sgRNA into the similar plasmid in in vitro method. This method is longer lasting and prevents the multiple transfections with the help of expression of the Cas9 and sgRNA **(**Doudna *et al.,* 2014). The plasmid needs the introduction of nucleus of target cells, which is main challenge in this system
- Direct intracellular delivery of Cas9 mRNA and sg RNA

  This method having a largest drawback which are poor stability of m RNA, and their results in transient expression of m RNA and having a gene modification in short duration.
- Direct delivery of Cas 9 protein and sgRNA (Biagioni *et al.,* 2018).

  Which have a several advantage, for example, rapid action, most stable and low amount of antigenicity.

The editing of DNA that are irreversible and permanent change of genome information, and this editing mechanism also having a risk and some ethical problems. For example, some types of neuron cells are difficulty to modify DNA using CRISPR/Cas9 method of gene editing, this method having a limited application in gene therapy for some nervous system diseases. Some scientists (Abudayyeh and Merkle 2019) proposed that the gene editing method only for RNA.

During DNA transcription, RNA is responsible for the production of downstream proteins. With the use of CRISPR technology, RNA mutation is modified briefly, and not only avoids the irreversible modification of the genome and also repair protein function in almost all cells to treat a many disease (Tucker and Homma 2013).

The genetically modified crops are modified by New Plant Breeding Technologies (NPBTs) to speed up the improved characters in plants. But now there is variety of methods are involved for the modification such as CRISPR/Cas9 and TALEN (Ezezika *et al.,* 2012) NPBTs not only involved in the crop modification also involved in speed breeding (Ghosh and Watson, 2018) next-generation genotyping and phenotyping (Barabaschi *et al.,* 2016).

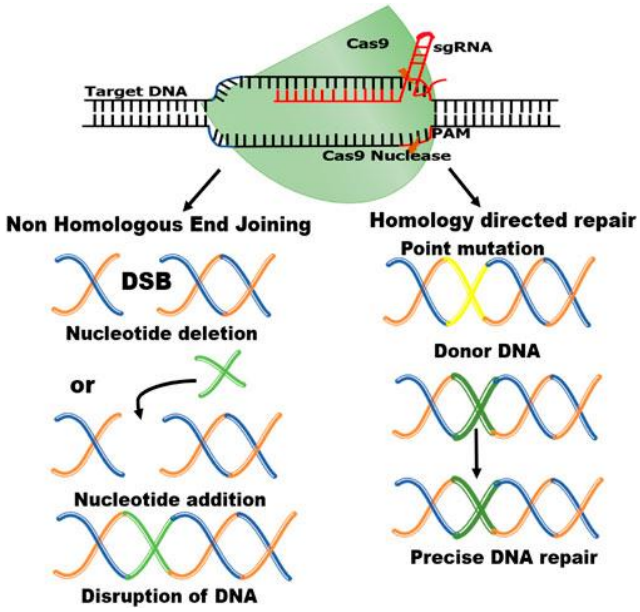

Fig.1: Mechanism of CRISPR/Cas9

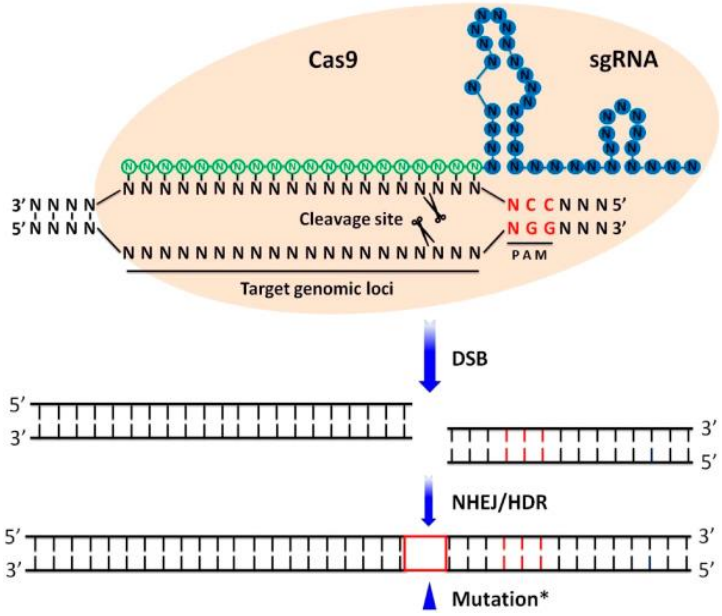

Fig. 2: Double strand break formation and Repaired by NHEJ/HDR joining method

CRISPR-CAS 9 application in Plant biotechnology

In Plant biotechnology (PBT), consists of variety of scientific methods and techniques used to indicate, identify, modulate and manipulate plant genes to make a difference in specific character and desired products in plants 2018. By using the PBT methods beneficial crops are analysed and amplified, at mean time undesirable plants are eliminated. For example, the allergens crops are eliminated by PBT methods including Rice and Soy-beans (Fuchs and Azevedo, 2018).

Now various methods are available for genetic modification of plants such as emerging of sequence -specific Nucleases (SSNs), Transcriptional activator like effector nucleases

(TALENs), Zinc Finger nuclease (ZFNs) and recently more developed in clustered regularly interspaced short palindromic repeats (CRISPR/ CRISPR associated protein 9 (cas9) these all methods are permit the less sporadic and accurate gene modification in plants. (Baltes and Sun 2016).

CRISPR-CAS 9 technology is one of the most important and impression methods to implements to enhance the quality of the agricultural products. Here some crops are genetically modified by CRISPR genome editing tool such as Rice and Soybean.

Rice

Rice is an important and major food source in the global population (Fukagawa and Ziska, 2019). Miao et al 2013. Successfully manipulated using CRISPR-CAS 9 technology. Who demonstrated that the possibility of applying the system for targeted mutation in rice. Many attempt to determine the functions and activities of individual genes and observe the function of the effect of gene modifications in rice crops to apply the method in practically.

For example, studied by Guo et al 2020 who used CRISPR/CAS 9 determine both induce over expression and knockout the OsProDH gene in rice. That gene encodes the enzyme of mitochondrial enzyme of proline dehydrogenase this is responsible for the degradation of proline amino acid in rice. Proline plays an important role in protecting plants from the various stresses for example, biotic and abiotic stress by inducing various physiological responses of plants and by scavenging reactive oxygen species (ROS).

Hayat *et al.,* 2012. In mutation of OsProDH in rice resulted in the abundance of proline, which turn to lower levels of ROS. So, the higher manipulation of OsProDH gene and subsequently the proline metabolism, higher thermotolerance could be conferred onto the rice. (Guo *et al.,* 2020).

Soybean

CRISPR/CAS-9 based mutagenesis successfully performed in many plant species for example, Soybean conducted by Jacobs et al. (2015). In this plant modification based on gene knockout was performed on the green fluorescent protein (GFP) gene. This knowledge which has a large effort in applying in CRISPR/CAS 9 gene editing in soybean.

CRISPR/Cas-9 to induce mutation in a target specific site of E1 gene in control the flowering of soybean and shorten the E1 protein prevent the inhibition of the GmFT2a/5a gene, increase its activity and allowed to earlier flowering time under long day conditions. This changes which allowed to the evolution of the photo insensitive soybean variant, which is important of soybean in more parallel (Han *et al*.,2019).

Using CRISPR/Cas 9 genome editing tool many varieties of plants are successfully edited and increase the crops in quantity for example in Maize (Liu *et al.,* 2020) wheat (Hayta *et al.,* 2019) and apples (Pompili *et al.,* 2020) in more efficiency (Haque and Adhikari 2020).

Reason for increasing the yield of crops, recently CRISPR/Cas 9 was very effective phytoene desaturase gene in muskmelon plant of cmPDS gene in using knocking out method. This is the first method to apply the CRISPR/Cas 9 genomes editing on the species (Hooghvorst *et al.,* 2019)

The same PDS gene was knocked out in albino phenotype in CRISPR/Cas9 genome editing in watermelon and apples in successfully (Nishitani and Tian, 2017).

CRISPR/Cas9 application in animal as a model

Animal models are impossible to understanding the disease pathogenesis and developing therapeutic agents and their treatments (Gongile, 2014) genetically modified model of organism for example, nematodes, fruit flies, zebra fish and rodents have provided variety of experimental data and advanced to understanding the human biology and disease. (Bendixen and Prabhakaran *et al.,* 2012) study of animal models in translation mechanism are more reality. Large animal groups are share more similar in human such as the characters are physiology, size of organ, anatomy and metabolism.

For example, non-human primates (NHPs), dogs and pigs having a similarity in those characteristics (Chan *et al.,* 2013) these similarities which make an important in organ xenotransplantation and model for human disease including neurodegenerative disease and cardiovascular disease. Identification of these mutation for human disease, the site-specific modifications of these type of gene in large animal group which provide an important model for pathology studies, discover if drug and also development in medicine research of regeneration.

But using pigs and dogs as animal model which have a very slow and because of lacking of embryonic stem cells and most low efficiency in homologous recombination (HR) and also need a time-consuming program to make a biallelic genetically modified animals

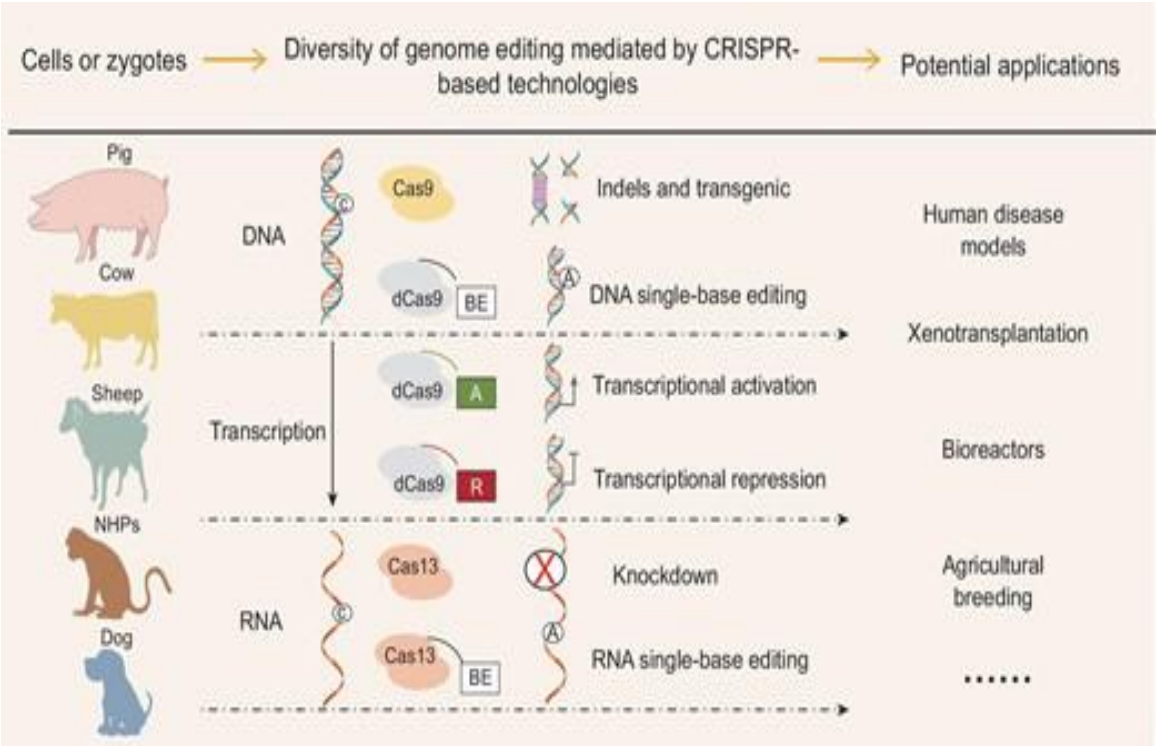

Fig. 3: The various group of animals are used as a model for gene editing tool of CRISPR/Cas9 technology to edit the DNA, RNA, knockout, insertion, deletion of specific genes and involved in transcription regulation. The animals are monkey, dog, pig and, cow and sheep as model and involved in human disease modelling, xenotransplantation organs and livestock breeding etc…

Pigs

Pigs as used in animal model in recent decades because which having a reproduction and easy handling advantages and also having some ethical concerns compared to the non-human primates. Pigs also having a sexual maturity in 5-8 months, and short gestation period 114 days and offspring rate also high 10-12 piglets/litter and suitable for species for preclinical experiment. In gene editing tool method of knockout and have been regularly developed since 2002. (Dai *et al.,* 2002).

In pigs, the nuclease mediated gene editing method of ZFNs were first applied to pigs with knockout and the transgenic Enhanced Green Fluorescent Protein (EGEP) and PPARy genes proving gene editing in pigs. (Yang and Whyte *et al.,* 2011).

Also, development of TALENs and CRISPR/Cas9 these method efficiency, high throughput and crucial role in modification of gene was recently developed and many models are generated. For example, TALENs were used to generate a Low-density Lipoprotein Receptor (LDLR) monoallelic and biallelic mutant was observed and pigs as a model for important in hypercholesterolemia (Carlson *et al.,* 2012).

Chinese bama miniatue pigs was used as animal model and study the how NPC1L1 influences cardiovascular and metabolic disease. Using zygote co-injection of Cas9 m RNA and sgRNA which is used to delete NPC1LI (Niemann-Pick C1-Like1) which produces biallelic mutant in pigs (Wang *et al., 2015*). various disease studies are studied in pigs as a model animal using gene knockout methods. human atherosclerosis, apolipoprotein E and Low-Density lipoprotein (LDL-C) total cholesterol (TC) and apolipoprotein B in serum (Huang *et al.,* 2017).

He *et al.,* 2015make a PKD1 (polycystic kidney disease1) using ZFNs gene knocking out method in monoallelic pigs after the resulted pigs revealed a renal cyst at 6 moths in step by step in their growth and which is a good model for studying the disease of grenal cystogenesis.

Using pig as a model created a three pig lines using TALENs and CRISPR/cas9

- DJ1 knockout,
- PARK2/PINK1 double knockout
- Parkin/DJ-1/ PINK1 triple knockout.

This type of model pigs used as a model for therapeutic development and pathological studies (Wang and Yao *et al.,* 2016).

Pig also good model animal in skin disease. Because the structure of skin and their thickness, hair follicle content, pigmentation, lipid and collagen components, dermal blood and dermal-epidermal surface are all the traits are similar to the structure of human skin (Liu and Summerfield *et al.,* 2015). these similarities are very easy to studying the human skin disease and many gene associated with pigmentation are modified by nuclease-mediated genetic tool and making a model for disease. Tyrosine (TYR) was mutated with CRISPR/Cas9 in biallelically, albinism was predicted in mutant pigs, for example, loss in the skin, hair and eyes (Zhou *et al.,* 2015).

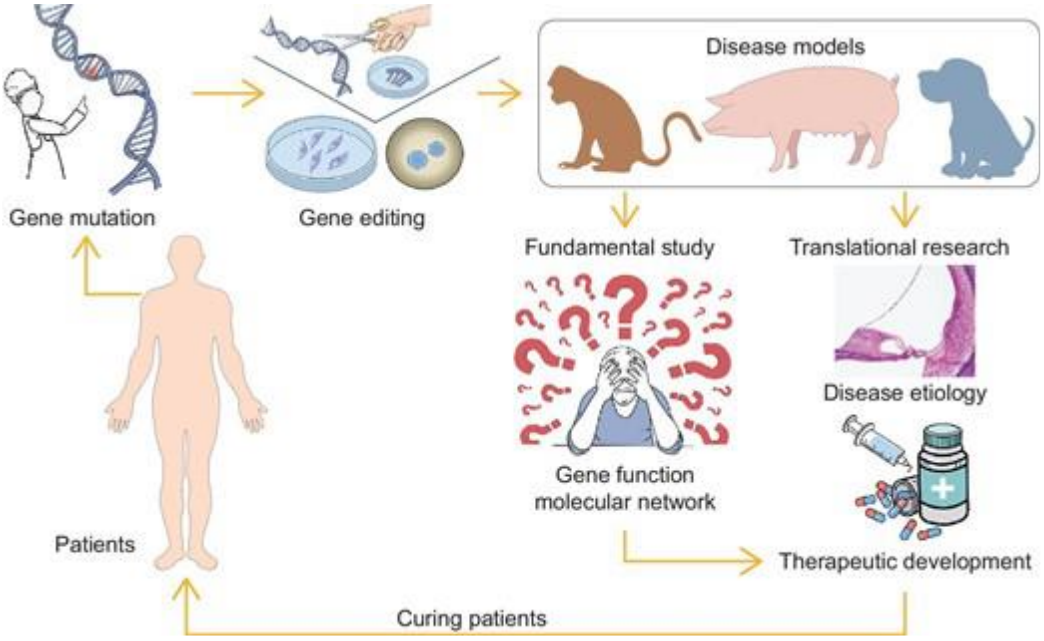

Fig. 4: Using Non-Human Primates (NHPs), pigs and dogs are as model animal for nuclease mediated genome editing and introducing a specific mutation in orthologous gene in animals. These large model animals which make a huge understanding the mechanism of disease and finally, development to treat or cure human disease

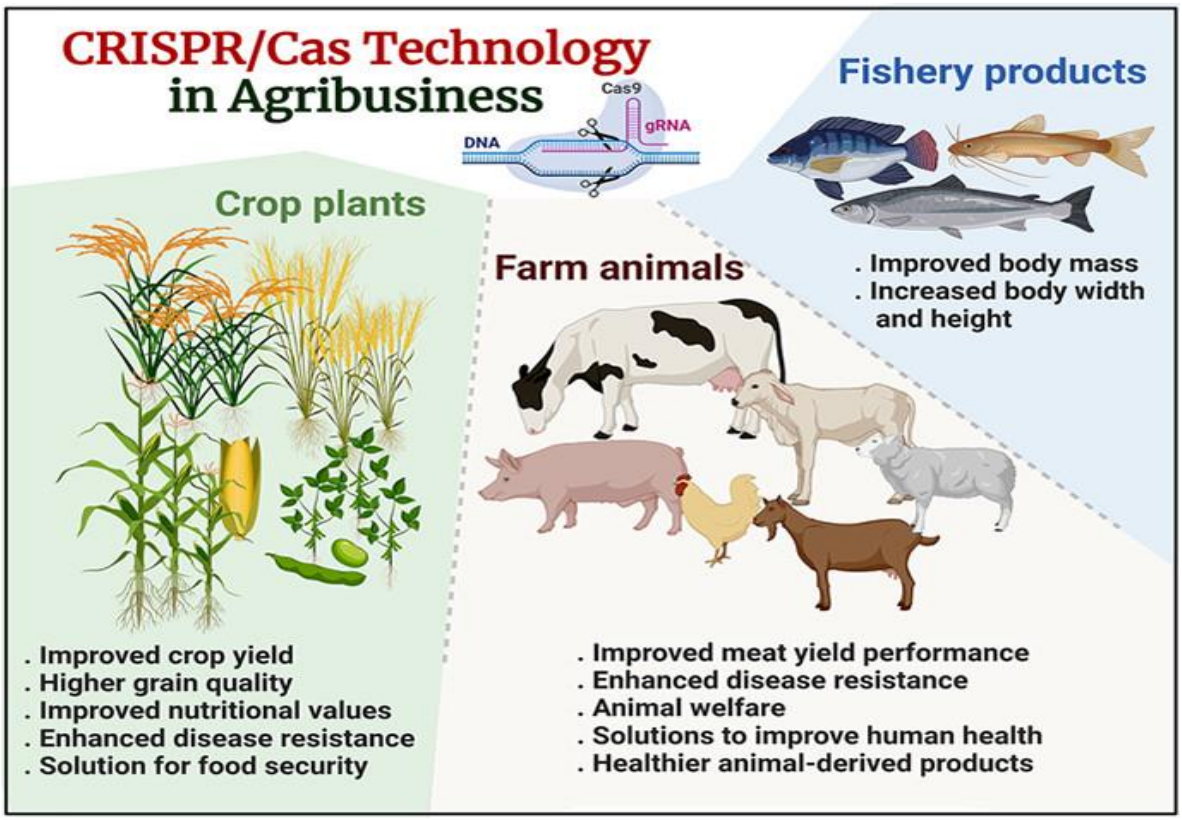

Fig. 5: CRISPR/Cas technology in Agribusiness

Dogs

Dogs like Non-human primates and pigs which share many metabolic, physiological and anatomical characters with humans occurring of hereditary disease in dogs and also contributed the genes and genetic associated with the disease of DMA (Kornegay and Mata 2018).

Zou *et al.,* making a MSTN (myostatin) autologous embryo transfer using the Cas9 and sgRNA combined and biallelic knockout dogs through zygote injection methods. The resulting dogs revealed a double-muscle phenotype of the thighs at 4 months. Conclusion of this method capability of generating dog as a model for biomedical research (Zou *et al.,* 2015).

In the same way used in many diseases for example, atherosclerotic cardiovascular disease model incorporated an ApoE biallelic mutation in using gene editing tool (Feng *et al.,* 2018) dog as a model animal for suggesting the clinical purpose. In a DMD dog model the dystrophin gene expression was successfully restored by CRISPR/Cas9 gene editing components in systemic delivery. The dogs show an improved muscle histology, result declared that many applications of gene editing in the treatment with DMD (Amoasii *et al.,* 2018).

Conclusion:

Conclusion of this chapter, using CRISPR/Cas9 technology is now a days huge application in various field including agriculture for crop improvement, using animal as model to treat a several diseases in human and improved the growth in fish also. In recently, making a CRISPR edited salad was introduced in May 16, 2023(Emily mullin) Gene editing tool of CRISPR/Cas was used to edit a mustard green to reduce the bitter compared to the original plant. This is the first vegetable in CRISPR edited Food in the US market. In future many things are evolved due to the CRISPR technology.

References:

1. Abudayyeh, O.O. *et al*. “RNA targeting with CRISPR-Cas13”. *Nature.* 2017; 550: 280–284.
2. Adhikari, P., Poudel, M. “CRISPR-Cas9 in agriculture: Approaches, applications, future perspectives, and associated challenges”. Malays. J. Halal Res.2020; 3(1): 6–16.
3. Amoasii, L., Hildyard, J.C.W. and Li, H., *et al*. “Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science 2018; 362: 86–91.
4. Bak, R.O., Gomez, O., NataliaP. and Matthew, H."Gene Editingon Centre Stage". Trends inGenetics. 2018; 34 (8):600–611.
5. Baltes, N.J., Gil-Humanes, J., Cermak, T., Atkins, P.A. and Voytas, D.F. “DNA replicons for plant genome engineering”. *Plant Cell*.2014; 26(1): 151–163.
6. Barabaschi, D., *et al*. “Next generation breeding”. Plant Sci. 2016;242: 3–13.
7. Barh, D., Azevedo, V. “Omics technologies and bio-engineering. Volume 2: towards improving quality of life”. London: Elsevier.2018.
8. Beerli, R. R. And Barbas, C. F.” Engineering polydactyl zinc-finger transcription factors”. Nat. Biotechnol. 2002; 20: 135–141.
9. Beerli, R. R., Schopfer, U., Dreier, B. And Barbas, C. F. “Chemically regulated zinc finger transcription factors”. J. Biol. Chem. 2000; 275: 32617–32627.

10. Bendixen, E., Danielsen, M. and Larsen, K. *et al.* "Advances in porcine genomics and proteomics–a toolbox for developing the pig as a model organism for molecular biomedical research". Brief Funct Genomics. 2010; 9: 208–19.
11. Beumer, K. J. *et al*. "Efficient gene targeting in *Drosophila* by direct embryo injection with zinc-finger nucleases". Proc. Natl Acad. Sci. USA 2008; 105: 19821–19826.
12. Biagioni, A. *et al*. "Delivery systems of CRISPR/Cas9-based cancer gene therapy". J. Biol. Eng.2018; 1: 33.
13. Boch, J. *et al*. "Breaking the code of DNA binding specificity of TAL-type III effectors". *Science*. 2009; 326: 1509–1512s.
14. Bogdanove, A. J., Schornack, S. And Lahaye, T. "TAL effectors: finding plant genes for disease and defense". *Curr. Opin. Plant Biol.* 2010; 13: 394–401.
15. Bolotin, A., Quinquis, B., Sorokin, A. and Ehrlich, S.D. "Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin". Microbiology.2005;151 (8): 2551–2561.
16. Bolotin, A., Quinquis, B., Sorokin, A. and Ehrlich, S.D. "Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin". Microbiology 2005; 151: 2551–2561.
17. Briggs, A.W. *et al*. "Iterative capped assembly: rapid and scalable synthesis of repeat-module DNA such as TAL effectors from individual monomers". *Nucleic Acids Res.*2012*;* 40: e117.
18. Buck-Koehntop, B.A. *et al*. "Molecular basis for recognition of methylated and specific DNA sequences by the zinc finger protein Kaiso". *Proc. Natl Acad. Sci. USA* 2012; 109: 15229–15234.
19. Carlson, D.F., Tan, W. and Lillico, S.G., *et al*. "Efficient TALEN-mediated gene knockout in livestock". Proc Natl Acad Sci *USA.* 2012; 109: 17382–7.
20. Cermak, T. *et al*. "Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting". Nucleic Acids Res. 2011; 39: 82.
21. Chan, A. "Progress and prospects for genetic modification of nonhuman primate models in biomedical research". ILAR J .2013; 54: 211–23.
22. Cong, L. *et al*. "Multiplex genome engineering using CRISPR/Cas systems". *Science.*2013; 339: 819–823.
23. Cong, L. *et al*. "Multiplex genome engineering using CRISPR/Cas systems". Science. 2013; 339: 819–823.
24. Cong, L.*et al*. "Multiplex genome engineering using CRISPR/Cas systems". Science .2013; 339, 819-823.
25. Cox, D. B., Platt, R.J. and Zhang, F. "Therapeutic genome editing": prospects and challenges. Nat. Med. 2015; 21: 121–131.
26. Dai, Y., Vaught, T.D., and Boone, J., *et al*. "Targeted disruption of the alpha1,3-galactosyltransferase gene in cloned pigs". Nat Biotechnol. 2002; 20: 251–5.

27. Deveau, H. *et al.* "Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus"*. J. Bacteriol.2008; 190: 1390–1400.
28. Diakun, G. P., Fairall, L. and Klug, A. "EXAFS study of the zinc-binding sites in the protein transcription factor IIIA". Nature 1986; 324: 698–699.
29. Doudna, J.A. and Charpentier, E. "Genome editing". The new frontier of genome engineering with CRISPR-Cas9. Science. 2014; 346:1258096.
30. Endo, M., Mikami, M., and Toki, S. "Biallelic gene targeting in rice", Plant Physiol.2016; 170(2): 667–677.
31. Ezezika, O.C., *et al*. "Factors influencing ag biotech adoption and development in sub-Saharan Africa". Nat Biotechnol.2012;30(1):38–40.
32. Fairall, L., Schwabe, J.W., Chapman, L., Finch, J.T. and Rhodes, D. "The crystal structure of a two zinc-finger peptide reveals an extension to the rules for zinc-finger/DNA recognition". *Nature* 1993; 366: 483–487.
33. Fauser, F., Schiml, S. and Puchta, H. "Both CRISPR/Casbased nucleases and nickases can be used efficiently for genome engineering in *Arabidopsis thaliana*". *Plant J*. 2014;79(2): 348–359.
34. Feng, C., Wang, X., and Shi, H., *et al*. "Generation of ApoE deficient dogs via combination of embryo injection of CRISPR/Cas9 with somatic cell nuclear transfer". *J Genet Genomics.* 2018; 45: 47–50.
35. Fuchs, R.L., Mackey, M.A. "Genetically modified foods" in *Encyclopedia of food sciences and nutrition,* Second Edition, Caballero B. Elsevier Science. 2003; 2876–2882.
36. Fukagawa, N.K., Ziska, L.H. "Rice: importance for global nutrition". J. Nutr. Sci. Vitaminol. 65(Supplement): S2–S3. 2019.
37. Gaj, T., Gersbach, C.A. and Barbas, C.F. "ZFN, TALEN and CRISPR/Cas-based methods for genome engineering". Trends Bio technol. 2013; 31 (7): 397– 405.
38. Gasiunas, G., Barrangou, R., Horvath, P. and Siksnys, V. "Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria". Proc. Natl Acad. Sci. USA. 2012; 109: E2579–E2586.
39. Ghosh, S., et al. "Speed breeding in growth chambers and glasshouses for crop breeding and model plant research". Nat Protoc.2018;13(12):2944–63.
40. Guo, M., Zhang, X., Liu, J., Hou, L., Liu, H., and Zhao, X. "OsProDH negatively regulates thermotolerance in rice by modulating proline metabolism and reactive oxygen species scavenging". Rice. 2020; 13(1): 1–5.
41. Han, J., Guo, B., Guo, Y., Zhang, B., Wang, X., and Qiu, L.J. "Creation of early flowering germplasm of soybean by CRISPR/Cas9 technology". *Front. Plant Sci*. 2019; 10: 1446.
42. Haque, E., Taniguchi, H., Hassan, M.M., Bhowmik, P., Karim, M.R., Smiech, M., Zhao, K., Rahman M., and Islam, T. "Application of CRISPR/Cas9 genome editing technology for the improvement of crops cultivated in tropical climates: Recent progress, prospects, and challenges". *Front. Plant Sci*.2018; 9: 617. 1

43. Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A. "Role of proline under changing environments: A review". *Plant Signal. Behav*. 2012;7(11): 1456–1466.
44. He, J., Li, Q. and Fang, S, *et al*. "PKD1 mono-allelic knockout is sufficient to trigger renal cystogenesis in a mini-pig model". Int J Biol Sci .2015; 11:361–9.
45. Homma, K. *et al*. "Developing rods transplanted into the degenerating retina of Crx-knockout mice exhibit neural activity similar to native photoreceptors". Stem Cells (Dayton, OH) .2013; 31: 1149–1159.
46. Hooghvorst, I., Lopez-Cristoffanini, C. and Nogues, S. "Efficient knockout of phytoene desaturase gene using CRISPR/Cas9 in melon". Sci. Rep.2019; 9(1): 1–7.
47. Horizon Discovery.2016. CRISP/CRISPR Cas9.https://www.horizondiscovery.com/gene-editing/CRISPR.
48. Huang, L., Hu, Z. and Xiao, H., *et al.* "CRISPR/Cas9-mediated ApoE and LDLR-/- double gene knockout in pigs elevates serum LDL-C and TC levels". Onco target .2017; 8: 37751–60.
49. Ishino, Y., Shinagawa, H., Makino, K., Amemura, M. and Nakata, A. "Nucleotide sequence of the iap gene", "Responsible for alkaline phosphatase isozyme conversion in Escherichia coli", and "Identification of the gene product". J. Bacteriol. 1987; 169: 5429–5433.
50. Jansen, R., Embden, J.D.A.V., Gaastra, W. and Schouls, L.M. "Identification of genes that are associated with DNA repeats in prokaryotes". *Mol.* Microbiol. 2002;43: 1565– 1575.
51. Jiang, W., Bikard, D., Cox, D., Zhang, F. and Marraffini, L. A. "RNA-guided editing of bacterial genomes using CRISPR-Cas systems". Nat. Biotechnol. 2013; 31: 233–239.
52. Jinek, M. *et al*. "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity". Science. 2012; 337: 816–821.
53. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. "A programmable dual- RNA-guided DNA endonuclease in adaptive bacterial immunity". Science.2012; 337 (6096): 816–821.
54. Kaboli, S. and babazada, H. "CRISPR mediated genome engineering and its application in industry". Curr. issuesmol.biol. 208; 26, 81-92.
55. Kaboli, S. and Babazada, H. "CRISPR mediated genome engineering and its application in industry". Curr. Issues Mol. *Biol.* 2018;26: 81–92.
56. Kim, Y. G., Cha, J. And Chandrasegaran, S. "Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain". Proc. Natl Acad. Sci. USA 1996; 93: 1156–1160.
57. Kim, Y. G., Cha, J. and Chandrasegaran, S. "Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain". Proc. Natl Acad. Sci. USA 1996; 93: 1156–1160.
58. Kornegay, J.N. "The golden retriever model of Duchenne muscular dystrophy". Skelet Muscle 2017; 7: 9.
59. Li, T. *et al*. "TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain". Nucleic Acids Res. 2011; 39: 359–372.

60. Li, T., Liu, B., Chen, C.Y., and Yang, B. "TALEN-mediated homologous recombination produces site-directed DNA base change and herbicide-resistant rice". J. Genet. Genomics.2016; 43(5): 297–305.
61. Liu, H.J., Jian, L., Xu, J., Zhang, Q., Zhang, M., Jin, M., Peng, Y., Yan, J., Han, B. and Liu ,J. *et al*. "High-throughput CRISPR/Cas9 mutagenesis streamlines trait gene identification in maize*". Plant Cell.* 2020;32(5):
62. Liu, Y., Chen., J.Y., and Shang, H.T. *et al*. "Light microscopic, electron microscopic", and "Immunohistochemical comparison of Bama minipig (Sus scrofa domestica) and human skin". Comp Med .2010; 60: 142–8.
63. Makarova, K. S. *et al.* "Evolution and classification of the CRISPR-Cas systems". Nat. Rev. Microbiol.2011; 9:467–477.
64. Makarova, K.S. *et al*. "An updated evolutionary classification of CRISPR-Cas systems". Nat. Rev. Microbiol. 2015; 13: 722–736.
65. Mali, P. *et al.* "RNA-guided human genome engineering via Cas9*". Science.* 2013; 339:823–826.
66. Mata Lopez, S., Hammond, J.J. and Rigsby, M.B., *et al*. "A novel canine model for Duchenne muscular dystrophy (DMD): single nucleotide deletion in DMD gene exon 20". Skelet Muscle. 2018; 8: 16.
67. Mc Gonigle, P. and Ruggeri, B. "Animal models of human disease: challenges in enabling translation". Biochem Pharmacol. 2014; 87: 162–71.
68. Mercer, A. C., Gaj, T., Fuller, R. P. and Barbas, C. F. "Chimeric TALE recombinases with programmable DNA sequence specificity". Nucleic Acids Res. 2012; 40: 11163–11172.
69. Merkle, T. *et al*. Precise RNA editing by recruiting endogenous ADARs with antisense oligonucleotides. Nat. Biotechnol. 2019; 37: 133–138.
70. Miao, J., Guo, D., Zhang, J., Huang, Q., Qin, G., Zhang, X., Wan, J., Gu, H., and Qu, L.J. "Targeted mutagenesis in rice using CRISPR-Cas system". Cell Res. 2013;23(10): 1233–1236.
71. Mussolino, C. *et al.* "A novel TALE nuclease scaffold enables high genome editing activity in combination with low toxicity". Nucleic Acids Res. 2011; 39: 9283–9293.
72. Nguyen, K.L., *et al.* "Next-generation sequencing accelerates crop gene discovery". Trends Plant Sci. 2019;24(3):263–74.
73. Nishitani, C., Hirai, N., Komo,S., Wada, M., Okada, K., Osakabe, K., Yamamoto ,T. and Osakabe, Y. " Efficient genome editing in apple using a CRISPR/Cas9 system". Sci. Rep. 2016; 6: 31481.
74. Paschon, D. E. *et al.* "Diversifying the structure of zinc finger nucleases for high-precision genome editing". *Nat. Commun.* 2019; 10: 1133.
75. Pompili, V., Dalla Costa, L., Piazza, S., Pindo, M. and Malnoy, M. "Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system". Plant Biotechnol. J. 2020; 18(3): 845–858.

76. Pourcel, C., Salvignol, G. and Vergnaud, G. "CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA", and "Provide additional tools for evolutionary studies". Microbiology 2005; 151: 653–663.
77. Prabhakar, S. "Translational research challenges: finding the right animal models". J Investing Med. 2012; 60: 1141–6.
78. Ran, F.A. *et al*. "Genome engineering using the CRISPR-Cas9 system". Nat. Protoc. 2013; 8(2): 2281–2308.
79. Reyon, D. *et al.* "FLASH assembly of TALENs for high-throughput genome editing". Nat. Biotechnol. 2012; 30: 460–465.
80. Saurabh, S S" Genome Editing: Revolutionizing the Crop Improvement". Plant Molecular Biology Reporte. 2021;39 (4): 752–772.
81. Schmid-Burgk, J.L., Schmidt, T., Kaiser, V., Honing, K. and Hornung, V. "A ligation-independent cloning technique for high-throughput assembly of transcription activator–like effector genes". Nat. Biotechnol. 2013; 31: 76–81.
82. Shan, Q. *et al*. "Targeted genome modification of crop plants using CRISPR-Cas system". Nat. bio technol. 2013; 31. 686-688.
83. Sternberg, S.H., Redding, S., Jinek, M., Greene, E.C. and Doudna, J. A. "DNA interrogation by the CRISPR RNA-guided endonuclease Cas9". Nature. 2014; 507: 62–67.
84. Sternberg, S.H., Richter, H., Charpentier, E. and Qimron, U. "Adaptation in CRISPR-Cas systems". Mol. Cell. 2016*;* 61: 797–808.
85. Summerfield, A., Meurens, F. and Ricklin, M.E. "The immunology of the porcine skin and its value as a model for human skin". *Mol Immunol.* 2015; 66: 14–21.
86. Sun, Y., Li, J. and Xia, L. (2016a) "Precise genome modification via sequence-specific nucleases-mediated gene targeting for crop improvement". Front. Plant Sci. 2016a; 7: 1928.
87. Sun, Y., Zhang, X., Wu, C., He, Y., Ma, Y., Hou, H., Guo, X., Du, W., Zhao, Y., and Xia, L. (2016b) "Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase". Mol. Plant. 2016b; 9(4):628–631. 10.
88. Tahir, T., Ali, Q., Rashid, M. and Malik, A. "The journey of CRISPR-Cas9 from bacterial defense mechanism to a gene editing tool in both animals and plants Biol". Clin. Sci. Res. J.2020.
89. Tia, S., Jiang,L., Gao, Q., Zhang, J., Zong ,M., Zhang ,H., Ren ,Y., Guo, S., Gong ,G. and Liu, F., et al.. "Efficient CRISPR/Cas9-based gene knockout in watermelon". Plant Cell Rep. 2017;36(3): 399–406.
90. Tsai, K. L., Clark, L.A. and Murphy, K.E. "Understanding hereditary diseases using the dog and human as companion model systems". Mamm Genome .2007;18: 444–51.
91. UN World population prospects: The 2017 revision, key findings and advance tables Affairs Do EaS, United Nations, New York", 2017.
92. United Nations. Goal 2: Zero Hunger, 2019.
93. Wang, X., Cao, C. and Huang, J., *et al*. "One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system". Sci Rep .2016; 6: 20620.

94. Wang, Y., Du, Y. and Shen, B., *et al*. "Efficient generation of gene-modified pigs via injection of zygote with Cas9/sgRNA". *Sci Rep* .2015; 5: 8256.
95. Watson, A., *et al*. "Speed breeding is a powerful tool to accelerate crop research and breeding". Nat Plants. 2018;4(1):23–9.
96. Whyte, J.J., Zhao, J., and Wells, K.D., *et al.* "Gene targeting with zinc finger nucleases to produce cloned eGFP knockout pigs". Mol Reprod *Dev.* 2011;78: 2.
97. Yang, D., Yang, H. and Li, W., *et al*. "Generation of PPARγ mono-allelic knockout pigs via zinc-finger nucleases and nuclear transfer cloning". Cell Res. 2011; 21: 979–82.
98. Yao, J., Huang, J. and Hai, T., *et al.* "Efficient bi-allelic gene knockout and site-specific knock-in mediated by TALENs in pigs". Sci Rep .2015; 4: 6926.
99. Zafar, K., Sedeek, K. E., M., Rao, G. S., Khan, M. Z., Amin, I., Kamel, R., Mukhtar, Z. Zafar, M., Mansoor, S., and Mahfouz, M.M. "Genome Editing Technologies for Rice Improvement: Progress, Prospects, and Safety Concerns Fron" t. Genome Ed, 2020.
100. Zaidi, S.S., *et al. "*New plant breeding technologies for food security Science". 2019;363(6434):1390–1.
101. Zhang, F. *et al*. "Efficient construction of sequence-specific TAL effectors for modulating mammalian transcription". Nat. Biotechnol. 2011;29: 149–153.
102. Zhang, Y., Pribil, M., Palmgren, M. and Gao, C. "A CRISPR way for accelerating improvement of food crops Nat". Food. 2020;1(4),200-205.
103. Zhou, X., Xin, J. and Fan, N., *et al*. "Generation of CRISPR/Cas9-mediated gene-targeted pigs via somatic cell nuclear transfer". Cell Mol Life Sci .2015; 72:1175–84.
104. Zou, Q., Wang, X., and Liu, Y., *et al*. "Generation of gene-target dogs using CRISPR/Cas9 system". J Mol Cell Biol. 2015; 7: 580–3.

EXPLORING THE LINK BETWEEN CIRCADIAN RHYTHM, DISRUPTION AND DIABETES MELLITUS

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Abstract:

Circadian rhythms are endogenous physiological processes that adhere to a 24-hour cycle. These are regulated by a central biological clock located in the Suprachiasmatic nucleus of the hypothalamus, along with a series of clocks in peripheral tissues throughout the body, including liver, muscle, and adipose tissue. These rhythmic processes are essential for maintaining normal physiological functions, including glucose metabolism, cholesterol biosynthesis, fatty acid oxidation, and energy expenditure. Disruption of circadian rhythms has been linked to various health problems, including obesity, cardiovascular disease, and diabetes mellitus.

Diabetes mellitus is a complex metabolic disorder characterised by high blood glucose levels leading to serious micro & macrovascular complications resulting in the risk of developing cardiovascular diseases. Diabetes mellitus is estimated to have caused 2 million deaths worldwide. Managing diabetes involves a multifaceted approach, as there is no single-line treatment modality. Along with medical interventions, a healthy diet, regular exercise and proper sleep are vital. The management of diabetes mellitus is closely related to a balanced circadian rhythm. This review aims to explore the impact of circadian rhythm and its disturbances on Diabetes mellitus

Introduction:

Circadian rhythm

Circadian rhythms are internal, autonomous biological processes that occur in a roughly 24-hour cycle and are found in most living organisms, from bacteria to humans. These rhythms play a fundamental role in regulating a wide range of physiological and behavioural processes, such as sleep-wake cycles, hormone secretion, metabolism, and immune function [Farhud, D., & Aryan, Z. (2018)]. The study of circadian rhythms has implications for numerous fields, including chronobiology, sleep medicine, pharmacology, and neuroscience.

Mechanism underlying circadian rhythm

Circadian rhythms are generated by a complex network of molecular and cellular processes synchronized with the external world using environmental cues referred to as zeitgebers. These cues include exposure to light, temperature, and eating habits. The mechanism underlying circadian rhythms is tightly regulated by a master biological clock located in the

suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives input from specialized intrinsically photoreceptive retinal ganglion cell (ipRGC) expressing the photopigment melanopsin that responds to light, together with the retinal rod and cone photoreceptors send signals via the retinohypothalamic tract, which synchronizes the biological clock with environmental cues such as the light-dark cycle [Chen *et al.* (2011)]. In turn, the SCN sends signals to peripheral tissues and organs, which express a set of clock genes that drive circadian rhythms in a tissue-specific manner.

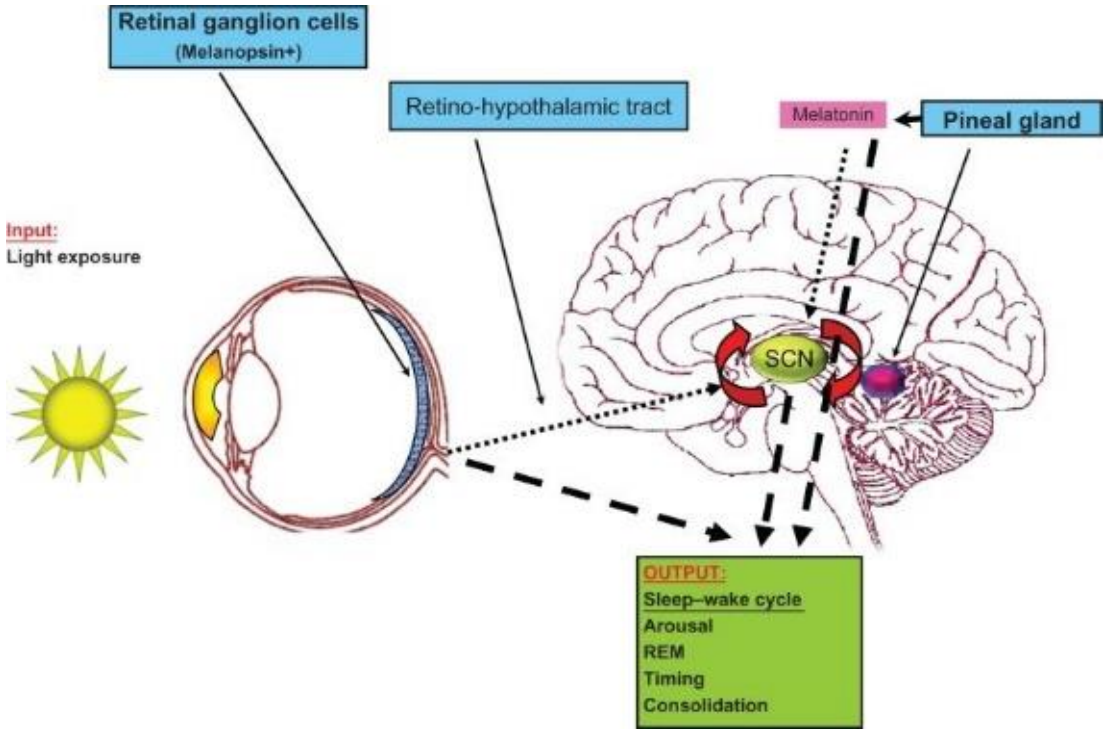

Fig 1: Mechanisms underlying circadian rhythm
Source: Jet lag syndrome: Circadian organization, pathophysiology, and management strategies. August 2010, Nature and Science of Sleep, 2:2-187

Molecular mechanisms

At the molecular level, the core mechanism underlying circadian rhythm involves multiple complex molecular mechanisms involving autoregulatory transcription-translation feedback loops (TTFLs). These loops consist of several genes and proteins, the most important being the two transcriptional activators, CLOCK (circadian locomotor output cycles kaput) and BMAL1 (brain and muscle Arnt-like protein 1 also known as ARNTL), which form a heterodimer and binds to canonical Enhancer Box (E-Box)-sequences containing the consensus sequence CACGTG or noncanonical E-Boxes of clock-regulated genes. This complex promotes the transcription of several clock-controlled genes, including Period (Per) and Cryptochrome (Cry), which encode proteins PER & CRY that act as negative regulators of CLOCK-BMAL1 activity. As the levels of PER and CRY proteins rise, they form complexes and translocate into the nucleus, where they inhibit CLOCK-BMAL1 activity, leading to a decrease in the expression of clock-controlled genes. This negative feedback loop continues throughout the day, resulting in

oscillations of gene expression that drive the circadian rhythm [Huang R. C. (2018)]. Additional molecular mechanisms, including post-transcriptional and post-translational modifications, also play critical roles in regulating the circadian rhythm. For example, phosphorylation of PER and CRY proteins can modulate their stability and activity, providing additional levels of control over the molecular feedback loops. In addition to these core components, a number of accessory proteins and signaling pathways have been shown to modulate circadian rhythms [Buhr *et al*. (2013)]. For example, the nuclear receptor REV-ERBα & REV-ERBβ, as well as the retinoic acid orphan receptor (ROR) (RORα, RORβ, and RORγ), establish another feedback loop. Studies show that while REV-ERBs act as transcriptional repressors of BMAL1 expression, RORs positively regulate the expression of BMAL1. These feedback loops have also led to play a crucial role in regulating the timing of behavioral and physiological processes such as sleep and metabolism. [Fagiani *et al*. 2022]

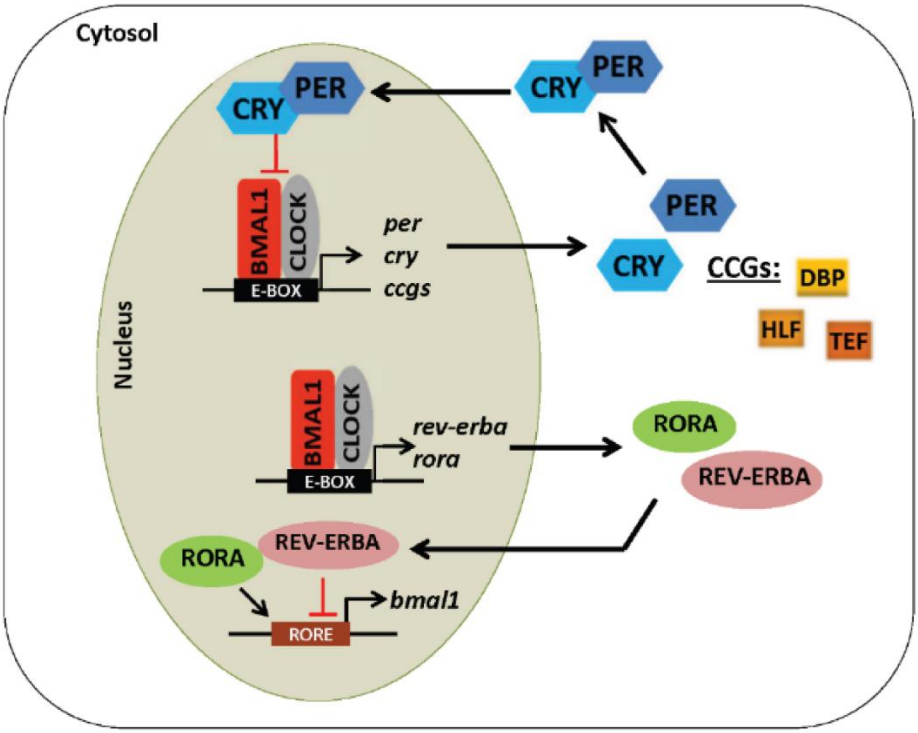

Figure 2: Molecular mechanism of circadian regulation

Source: Udoh, U., Valcin, J., Gamble, K., & Bailey, S. (2015). The Molecular Circadian Clock and Alcohol-Induced Liver Injury. *Biomolecules*, *5*(4), 2504–2537

Hormonal mechanism

Melatonin, a hormone, also plays a significant role in the regulation of circadian rhythms. . Melatonin is synthesized by the pineal gland in response to darkness and acts as a potent entrainer of the circadian clock. Melatonin is also known as ”hormone of darkness” as its level is high during night and low during daytime. Melatonin binds to specific receptors (MT1 & MT2) in the brain and peripheral tissues, signaling the onset of night time and promoting the onset of sleep [Pevet *et al*. (2017)]. Extensive research has established the significant role of melatonin in glucose metabolism and its impact on Diabetes mellitus. It has been demonstrated that melatonin

plays a crucial role in regulating insulin secretion and synthesis. Studies show that melatonin has an antagonistic relationship with insulin secretion, i.e., when the level of melatonin is lower in the day time, the level of insulin is high and vice versa at night. Genetic disorders affecting melatonin receptors have been observed to disrupt the glucose metabolism pathway, thereby increasing the susceptibility to developing type 2 diabetes [Espino et al. (2011)]. The circadian clock is also tightly linked to cellular metabolism. Recent studies have shown that the clock regulates key metabolic pathways, such as glucose and lipid metabolism, and disruption of circadian rhythms has been linked to a variety of health problems, including obesity, cardiovascular disease, and diabetes mellitus [Nagorny & Lyssenko (2012)].

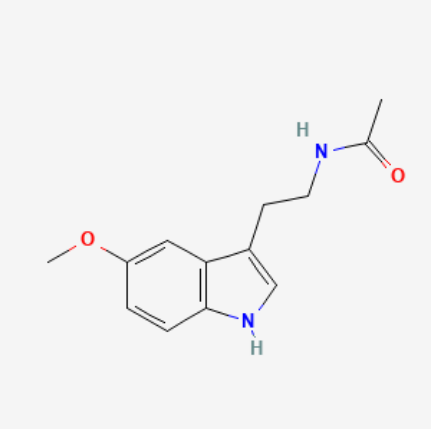

Figure 3: Melatonin
IUPAC Name: N-[2-(5-methoxy-1H-indol-3-yl)ethyl]acetamide
Source: PubChem

Circadian rhythm disruption

Circadian disruption is the alterations in the biological timing occurring at various organisational levels ranging from behavioural changes aligned with environmental changes to cellular as well as physiological changes extending to the changes in molecular level rhythmic patterns [Morris *et al.* (2012)]. Modern lifestyle factors, including shift work, jet lag, and excessive exposure to artificial light, are commonly responsible for disturbances in the circadian rhythm [Smolensky et al. (2015)]. Additionally, irregular eating patterns [Mattson et al. (2014)] can also contribute to disruptions in the circadian rhythm. Some of the major consequences of circadian rhythm disruption are:

- Impairment of sleep quality and quantity: Recent studies indicate that disturbances in the circadian rhythm led to impaired sleep quality and quantity. These disruptions manifest as challenges in initiating sleep, reduced duration of sleep, delayed or advanced sleep onset, fragmented sleep, and an increased risk of sleep-related disorders such as insomnia and obstructive sleep apnea.
- Dysregulation of metabolic processes: - Shift work, jet lag, and other factors that disrupt the circadian rhythm contribute to disturbances in the sleep-wake cycle and eating patterns. These disruptions can consequently increase the risk of developing metabolic

disorders such as insulin resistance, obesity, and Type 2 Diabetes mellitus. , [Stenvers *et al.* (2019)]. Cancer [Lee, Y. (2021], etc.

- Impairment of cognitive function and performance: Disruptions in the sleep-wake cycle can impair attention, memory, problem-solving abilities, and overall cognitive performance. Chronic circadian rhythm disruption, as experienced by shift workers, has also been associated with the increased risk of neurodegenerative diseases. [Fisk *et al.* (2018)].
- Mental health disorders: The changes in sleep-wake cycles associated with circadian rhythm disruption affect the production of hormones, melatonin, serotonin, cortisol etc., which are important in maintaining mental health. Disruption of these hormones leads to mood disorders like major depressive disorder, anxiety, schizophrenia, bipolar disorder etc. [Walker *et al.* (2020)]

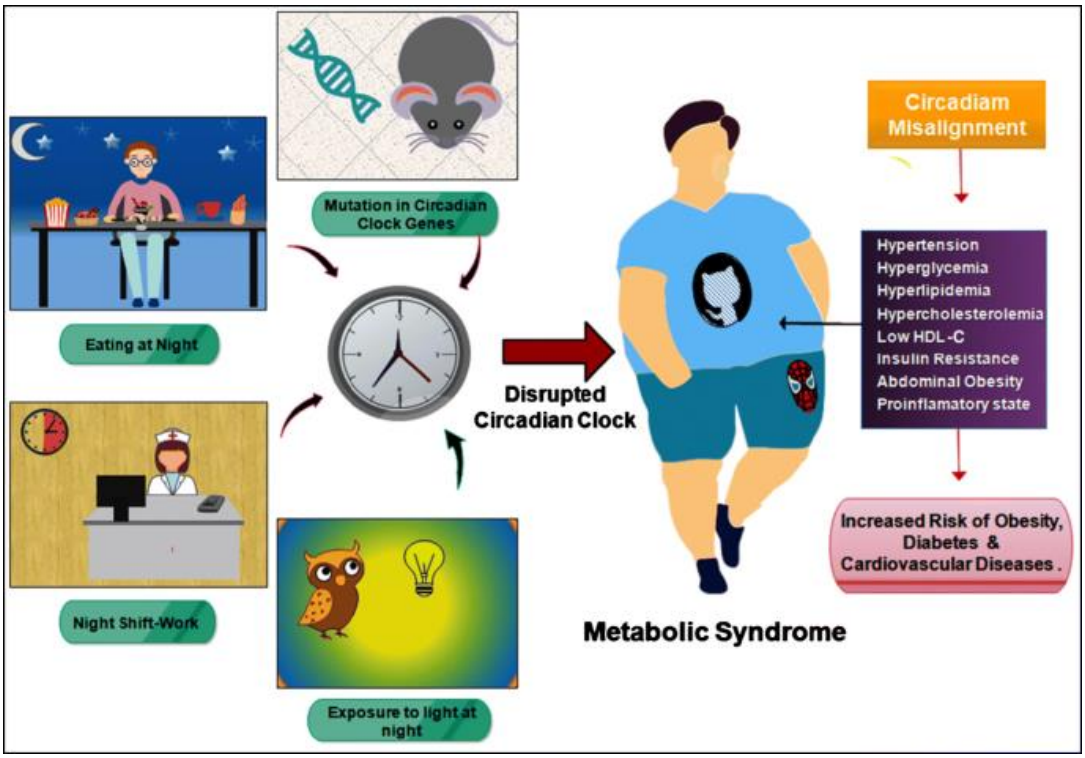

Figure 4: Factors affecting circadian disruption

Source: Fatima, N., & Rana, S. (2020). Metabolic implications of circadian disruption. *Pflügers Archiv-European Journal of Physiology*, *472*, 513-526.

Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in the blood, which can lead to various complications if left untreated. The most common form of diabetes mellitus is type 2 diabetes, which accounts for about 90% of all cases worldwide [Banday *et al.* (2020)]. Hyperglycemia, or elevated blood glucose levels, is a hallmark feature of diabetes mellitus and is the result of impaired insulin secretion due to pancreatic β cell dysfunction, resistance to insulin action, or both. Recent research has highlighted the role of circadian rhythm disruption in the pathogenesis of diabetes mellitus and hyperglycemia.

The molecular clocks that govern circadian rhythms are intimately linked with metabolic pathways, and disruption of these clocks can lead to dysregulation of glucose metabolism. Studies have shown that disrupting the circadian rhythm in mice can lead to impaired glucose tolerance and insulin resistance, even in the absence of changes in body weight or diet [Shi *et al.* (2013)].

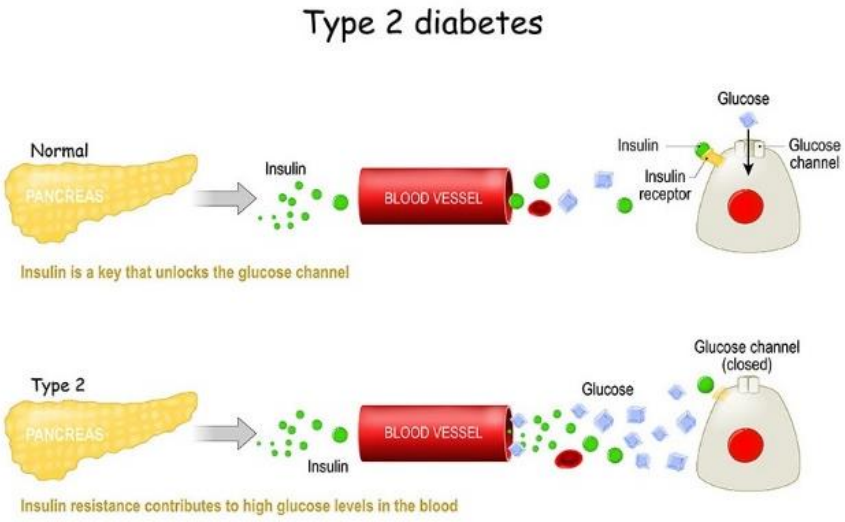

Figure 5: Comparison of Normal and Type 2 Diabetes Mellitus conditions
Source: Cleveland Clinic, Healthline

Link between Circadian Rhythm and Diabetes Mellitus

The link between circadian rhythm disruption and Diabetes Mellitus may be mediated by a variety of mechanisms. Studies show the involvement of the circadian clock in the regulation and expression of genes involved in glucose metabolism, including those encoding for insulin, glucagon, and glucose transporters. This connection has been further elucidated through the use of knockout models targeting circadian genes at both the whole-body and tissue-specific levels, highlighting the correlation between circadian genes such as Clock and Bmal1 and disruptions in glucose metabolism leading to the development of Diabetes Mellitus [Kalsbeek et al. (2014)].

- **Glucose metabolism**

In healthy individuals, Insulin secretion and glucose uptake follow a circadian rhythm, with higher levels of insulin released and glucose uptake in the morning to late afternoon and lower levels at night [Heden & Kanaley (2019)]. Glucose transporter 4 (GLUT4) is the primary glucose transporter in muscle and adipose tissue and glucose transport to endothelial cells is carried out by glucose transporters (GLUT-1, GLUT-3) or by sodium-glucose cotransport (SGLTs). The expression of glucose transporters are directly linked with the circadian control by SCN as well as tissue specific peripheral clocks [Gachon *et al.* (2017)]. This rhythm is important for maintaining glucose homeostasis, as insulin regulates glucose uptake and utilization in the body's cells [Zlacká & Zeman (2021)]. Similarly, glucagon levels, which stimulate glucose production by the liver, are highest during periods of fasting, such as overnight, and lowest after meals. These fluctuations are influenced by diurnal variations in multiple metabolic pathways,

including peripheral insulin sensitivity, β-cell responsiveness, insulin clearance, and glucose effectiveness. Disruptions in these rhythmic patterns can lead to abnormal glucose production and utilization [Poggiogalle *et al.* (2018)]. Animal studies investigating disruptions to the circadian rhythm at environmental, physiological, and molecular levels have demonstrated that the suprachiasmatic nucleus (SCN) directly influences glucose metabolism. Additionally, research utilizing tissue-specific knockout models of the Bmal gene has revealed that peripheral clocks exert varying effects on different tissues. These findings present opportunities for future in-depth investigations in humans [Qian & Scheer (2016)]. Research using rodent models with mutations in clock genes and experimentally induced disruptions to the circadian rhythm has identified several significant mechanisms that contribute to the development of insulin resistance and impaired β-cell function, ultimately leading to the onset of type 2 diabetes mellitus. These mechanisms include impairments in insulin vesicle trafficking, membrane fusion, and processing, alterations in the rate of beta-cell growth, proliferation, and survival, as well as heightened susceptibility to oxidative stress [Rakshit et al. (2014)].

- **Sleep-Wake Cycle**

The sleep-wake cycle plays a vital role in regulating circadian rhythms and glucose metabolism. Disruption of the sleep-wake cycle, such as those caused by shift work or sleep disorders, can lead to circadian rhythm disruption and abnormal glucose metabolism. Similarly, studies have shown that alterations in sleep duration, chronic sleep restriction, excessive sleep, and individuals with sleep disorders, such as obstructive sleep apnea, are at increased risk of developing diabetes mellitus and hyperglycemia [Koren *et al.* (2015)]. A population-based study reported that individuals who worked night shifts and experienced chronic exposure to light during the night had significantly higher risk of developing diabetes mellitus compared to those who worked regular daytime hours [Roestamadji *et al.* (2019)].

Conclusion:

In conclusion, circadian rhythms are endogenous time-keeping processes that can be modified or disrupted by external factors such as exposure to light, feeding habits, variations in sleep-wake cycles, etc. These rhythms are tightly regulated at the molecular level by clock genes (*per, cry, clock, bmal*, etc) using a transcription-translation feedback loop mechanism. Physiologically, timely secretion of hormones like melatonin and cortisol in response to external cues further regulate these cues. Disruptions in these processes, commonly observed in shift workers, individuals with, jet lag, irregular feeding habits, and light exposure at night causes various dysfunctions in metabolic process that leads to mental health disorders such as anxiety, obstructive sleep apnea, depression and various metabolic disorders such as cancer, diabetes mellitus, etc.

Diabetes mellitus is a chronic metabolic syndrome with severe consequences on multiple organ systems in the human body. . Recent research has highlighted the role of circadian rhythm disruption in the pathogenesis of diabetes mellitus and hyperglycemia. Studies show that disruption of the circadian rhythm can lead to dysregulation of glucose metabolism, insulin resistance, impaired glucose tolerance, and impaired β cell function, that results in Diabetes

mellitus. Future research is needed to deepen our understanding of the mechanisms underlying the link between circadian rhythm disruption and hyperglycemia and to develop effective strategies for prevention and treatment of these conditions.

References:

1. Farhud, D., & Aryan, Z. (2018). Circadian Rhythm, Lifestyle and Health: A Narrative Review. *Iranian journal of public health*, *47*(8), 1068–1076.
2. Chen, S. K., Badea, T. C., & Hattar, S. (2011). Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. *Nature*, *476*(7358), 92-95.
3. Huang R. C. (2018). The discoveries of molecular mechanisms for the circadian rhythm: The 2017 Nobel Prize in Physiology or Medicine. Biomedical journal, 41(1), 5–8. https://doi.org/10.1016/j.bj.2018.02.003
4. Buhr, E. D., & Takahashi, J. S. (2013). Molecular components of the Mammalian circadian clock. Handbook of experimental pharmacology, (217), 3–27. https://doi.org/10.1007/978-3-642-25950-0_1
5. Fagiani, F., Di Marino, D., Romagnoli, A. *et al.* Molecular regulations of circadian rhythm and implications for physiology and diseases. *Sig Transduct Target Ther* **7**, 41 (2022). https://doi.org/10.1038/s41392-022-00899-y
6. Pevet, P., Klosen, P., & Felder-Schmittbuhl, M. P. (2017). The hormone melatonin: Animal studies. *Best Practice & Research Clinical Endocrinology & Metabolism*, *31*(6), 547-559.
7. Espino, J., Pariente, J. A., & Rodríguez, A. B. (2011). Role of melatonin on diabetes-related metabolic disorders. *World journal of diabetes*, *2*(6), 82–91. https://doi.org/10.4239/wjd.v2.i6.82
8. Nagorny, C., & Lyssenko, V. (2012). Tired of diabetes genetics? Circadian rhythms and diabetes: the MTNR1B story?. Current diabetes reports, 12, 667-672.
9. Morris, C. J., Aeschbach, D., & Scheer, F. A. (2012). Circadian system, sleep and endocrinology. *Molecular and cellular endocrinology*, *349*(1), 91-104.
10. Smolensky, M. H., Sackett-Lundeen, L. L., & Portaluppi, F. (2015). Nocturnal light pollution and underexposure to daytime sunlight: Complementary mechanisms of circadian disruption and related diseases. *Chronobiology international*, *32*(8), 1029-1048.
11. Mattson, M. P., Allison, D. B., Fontana, L., Harvie, M., Longo, V. D., Malaisse, W. J., & Panda, S. (2014). Meal frequency and timing in health and disease. *Proceedings of the National Academy of Sciences*, *111*(47), 16647-16653.
12. Kim, M. J., Lee, J. H., & Duffy, J. F. (2013). Circadian Rhythm Sleep Disorders. *Journal of clinical outcomes management : JCOM*, *20*(11), 513–528.
13. Stenvers, D. J., Scheer, F. A. J. L., Schrauwen, P., la Fleur, S. E., & Kalsbeek, A. (2019). Circadian clocks and insulin resistance. *Nature reviews. Endocrinology*, *15*(2), 75–89. https://doi.org/10.1038/s41574-018-0122-1
14. Lee, Y. (2021). Roles of circadian clocks in cancer pathogenesis and treatment. *Experimental & molecular medicine*, *53*(10), 1529-1538.

15. Fisk, A. S., Tam, S. K., Brown, L. A., Vyazovskiy, V. V., Bannerman, D. M., & Peirson, S. N. (2018). Light and cognition: roles for circadian rhythms, sleep, and arousal. *Frontiers in neurology*, *9*, 56.
16. Walker, W. H., II, W., & JC, D. AC, and Nelson, RJ 2020. Circadian rhythm disruption and mental health. Transl. *Psychiatry*, *10*(1), 28.
17. Banday, M. Z., Sameer, A. S., & Nissar, S. (2020). Pathophysiology of diabetes: An overview. Avicenna journal of medicine, 10(4), 174–188. https://doi.org/10.4103/ajm.ajm_53_20
18. Shi, S. Q., Ansari, T. S., McGuinness, O. P., Wasserman, D. H., & Johnson, C. H. (2013). Circadian disruption leads to insulin resistance and obesity. Current biology : CB, 23(5), 372–381. https://doi.org/10.1016/j.cub.2013.01.048
19. Kalsbeek, A., la Fleur, S., & Fliers, E. (2014). Circadian control of glucose metabolism. *Molecular metabolism*, *3*(4), 372–383. https://doi.org/10.1016/j.molmet.2014.03.002
20. Heden, T. D., & Kanaley, J. A. (2019). Syncing Exercise With Meals and Circadian Clocks. Exercise and sport sciences reviews, 47(1), 22–28. https://doi.org/10.1249/JES.0000000000000172
21. Gachon, F., Loizides-Mangold, U., Petrenko, V., & Dibner, C. (2017). Glucose homeostasis: regulation by peripheral circadian clocks in rodents and humans. *Endocrinology*, *158*(5), 1074-1084.
22. Zlacká, J., & Zeman, M. (2021). Glycolysis under Circadian Control. *International journal of molecular sciences*, *22*(24), 13666. https://doi.org/10.3390/ijms222413666
23. Poggiogalle, E., Jamshed, H., & Peterson, C. M. (2018). Circadian regulation of glucose, lipid, and energy metabolism in humans. *Metabolism: clinical and experimental*, *84*, 11–27. https://doi.org/10.1016/j.metabol.2017.11.017
24. Qian, J., & Scheer, F. A. J. L. (2016). Circadian System and Glucose Metabolism: Implications for Physiology and Disease. *Trends in endocrinology and metabolism: TEM*, *27*(5), 282–293. https://doi.org/10.1016/j.tem.2016.03.005
25. Rakshit, K., Thomas, A. P., & Matveyenko, A. V. (2014). Does disruption of circadian rhythms contribute to beta-cell failure in type 2 diabetes?. Current diabetes reports, 14(4), 474. https://doi.org/10.1007/s11892-014-0474-4
26. Koren, D., O'Sullivan, K. L., & Mokhlesi, B. (2015). Metabolic and glycemic sequelae of sleep disturbances in children and adults. *Current diabetes reports*, *15*, 1-10.
27. Roestamadji, R. I., Nastiti, N. I., Surboyo, M. D. C., & Irmawati, A. (2019). The Risk of Night Shift Workers to the Glucose Blood Levels, Saliva, and Dental Caries. *European journal of dentistry*, *13*(3), 323–329. https://doi.org/10.1055/s-0039-1697211

PHYLOGENETIC RELATIONSHIP BETWEEN THE ORGANISM WITH THE HELP OF MITOCHONDRIAL GENES AND DIFFERENT MARKERS

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Introduction:

Living life is a very beautiful event in this universe, whether it is single-cell or multicellular, all share the memories and characteristics of their evolutionary development. Their journey to evolve from single cells to complex organisms tells many things. Phylogenetic relations are used to distinguish or compare within or between organisms.

Phylogenetic relationships refer to the evolutionary relationships among different species, groups, or individuals based on their shared characteristics, genetic information, and ancestry or the evolution of a genetically linked collection of species as opposed to the growth of each individual organism may be summed up as phylogeny (Dissanayake *et al.*, 2020). Determining the phylogenetic relationships among different species is important in fields such as biology, ecology, and evolution, as it helps to understand the origin and diversification of life on Earth, and can inform conservation efforts. One of the pioneers of modern phylogenetics was the American evolutionary biologist Willi Hennig, who developed the concept of cladistics in the 1950s. Cladistics is a method of phylogenetic analysis that uses shared derived characteristics, or synapomorphies, to group organisms into monophyletic clades. Hennig's work laid the foundation for many of the phylogenetic methods and techniques that are used today. To find the answers to a variety of questions related to the evolutionary point of view, detailed knowledge of phylogeny is necessary which allows us to identify organisms by revealing the historical pattern of speciation and divergence, which reveals the pattern of evolutionary relationships and to understand the formation of various different continent and the biogeographic changes (Noonan and Sites, 2010), as well as evolutionary changes in morphological features or behaviors of various species of fish (Ingley *et al.*, 2012).

Phylogenetic research can also shed light on the biogeography of different species. By studying the evolutionary history of organisms, researchers can understand how and when different groups of organisms moved across geographic regions, and how this movement may have influenced the distribution of species we see today. It can help identify the genetic factors that make fish species more susceptible or resistant to certain diseases. This information can help farmers select fish breeds that are less likely to be affected by specific diseases, reducing the risk of epidemics and improving the overall health of the fish population. The studies can help identify the genetic diversity within fish populations, which is essential for the success of breeding programs. By understanding the evolutionary relationships among different fish

populations, farmers can select breeding pairs that are genetically diverse, improving the overall genetic health of the fish population.

In the past, phylogenetic relationships and identifications were often inferred based on morphological features (Rosen and Bailey, 1963) such as the shape of an animal's bones, or the structure of its organs, because they are easier and not expensive (Batubara *et al.*, 2018) yet, this approach has some limitations. For example, the body parts of the organisms which have to be identified must be complete and it is difficult or complicated to interrelate between the nominal species and their family by poorly defined and environmentally variable physical characteristics. Therefore, substantial phylogenetic links cannot be provided by the conventional method based on morphological features. It is essential to do research that makes use of novel methodologies to establish evolutionary links between organisms and their classification. But in recent years, as molecular biology has advanced and genetic data has become more widely available, advances in computational and statistical methods have made it possible for researchers to analyse larger and more complex data sets and to take into account more sources of genetic data, including whole genome sequences. Today, single and multiple genomic region DNA barcodes have been established as useful for species identification, evaluating taxonomic diagnoses, and examining evolutionary linkages.

Important genes and markers used in phylogeny

Coding DNA Sequences (CDS): Genes in the genome that code for proteins can be located in CDS regions. These genes produce mRNA, which is then translated into proteins. Researchers can compare the protein amino acid sequences of several species to deduce their evolutionary relationships using phylogenetic analysis based on CDS.

Non-Coding DNA Sequences: The parts of the genome that do not encode proteins are known as non-coding DNA regions. For phylogenetic analysis, these regions can offer useful information. Some instances are:

- **Introns:** Genes contain non-coding sections called introns. The evolutionary history of gene duplications, rearrangements, and splicing mechanisms can be understood by comparing intron sequences.
- **Intergenic regions:** Intergenic regions, which are found between genes, frequently have regulatory components. Based on their regulatory sequences, these areas can be compared to help identify how closely related certain species are.

Mitochondrial DNA (mtDNA): Eukaryotic cells have mitochondria, which contain mitochondrial DNA, which has a unique collection of genes. mtDNA is valuable for analyzing phylogenetic relationships, especially in animals, due to its maternal inheritance pattern and quicker evolutionary pace.

Chloroplast DNA (cpDNA): The chloroplasts of organisms that engage in photosynthetic processes, such as plants and algae, contain chloroplast DNA. Due to its maternal inheritance pattern and relatively slow pace of evolution, cpDNA is frequently utilized in plant phylogenetic investigations. Like mtDNA, it contains its own set of genes.

Single-Copy Nuclear Genes: Each haploid genome has a single copy of these genes, which are present in the nuclear genome. Since single-copy nuclear genes change more slowly than quickly evolving genes, they can provide details about complex evolutionary relationships.

Retrotransposons: Retrotransposons are genetic components that can travel around the genome and reproduce on their own. Inferring evolutionary relationships and locating shared ancestry among species can be done with the aid of phylogenetic analysis based on retrotransposon insertions.

Amplified Fragment Length Polymorphisms (AFLPs): PCR amplification of genomic DNA, followed by restriction digestion and selective amplification, produces AFLPs, which are DNA markers. They are frequently employed in plant and microbial phylogenetic research and provide information on genetic variation.

Restriction Fragment Length Polymorphisms (RFLPs): RFLPs are DNA markers that are produced as a result of changes in the DNA sequence that restriction enzymes can recognize and cut. They can be used to compare whether particular DNA fragments are present in various species or not.

Mitochondrial genes

Mitochondrial genes are frequently employed in phylogenetic research because they are inherited from the mother, making it possible to track maternal lineages. While it is widely known that climatic factors have a substantial influence on how the genes encoding mitochondrial proteins have developed, this influence was seen in Teleostei (Sun *et al.*, 2011). It was simple to clarify the phylogenetic links and test the prior theory as well as the classification in the instance of *Cyprinella* by employing these genes. A frequently used mitochondrial gene, particularly in animal studies, is the cytochrome c oxidase subunit I (COI) gene.

Mitochondria are two layered organelles found in most cells that are responsible for producing ATP, the energy currency or the powerhouse of the cell. They have their own genome, separate from the nuclear genome, and are inherited maternally in most organisms. Mitochondrial DNA (mtDNA) is circular, double-stranded DNA (about 16-20kb) that contains 13 protein-coding genes (PCGs), 22tRNA genes (tRNA), and two RNA genes (rRNA) (Lu *et al.,* 2019). Mitochondrial genes have been widely used in phylogenetic studies to investigate the evolutionary relationships among species. mtDNA is a very promising genetic marker for studying population structure and phylogenetic research, either by itself or in conjunction with other nuclear markers like microsatellites. In some individuals more than one type of mitochondria is present, this condition is called heteroplasmy (Moritz, 1991).

Since mtDNA is inherited from the mother, its recombination rate is very low and its replacement rate is higher than that of nuDNA (Brown *et al.,* 1979). As a result, mtDNA sequences are frequently used in phylogenetic studies because they evolve much more quickly than nuclear DNA and are highly conservative and informative, especially for closely related species. Previous research using restriction enzymes on the chosen sequences had provided a crucial hint that mtDNA changes 5–10 times more quickly than the single copy of nuclear genes (Perler *et al.,* 1980). According to Wilson *et al*. (1985), an increased frequency of point and length mutations is the reason for this evolution.

These two mitochondrial genes are mostly used in phylogenetic analysis:

a) Cytochrome b (Cyt b)

Cytochrome b (Cyt b) is a mitochondrial gene, and it is a commonly used molecular marker in fish phylogeny (Elvyra *et al.,* 2020) because the codons are based on position and have more conserved and diverse regions, the Cyt b gene is utilized as a genetic marker (Farias *et al.,* 2001). The Cytochrome B (Cyt b) gene, which is encoded by mtDNA, is necessary for the transfer of electrons in the respiratory system. The Cyt b gene has been used in prior research to do genetic analyses of several animal species (Megarani *et al.,* 2020). It is inherited from the mother, making it possible for scientists to use it to explore the maternal lineages of many animals (Parson *et al.,* 2000). The Cyt b gene has a moderate evolutionary level and a distinctive evolutionary pattern, making it an attractive candidate for phylogenetic evolution studies at the intraspecific and interspecific levels. Megarani and colleagues 2020.

Studies using Cyt b have helped to resolve the evolutionary relationships among many animal groups. The phylogenetic data of cytochrome b has been studied at some systematic taxonomy levels of taxa.

b) Cytochrome C Oxidase I (COI)

The mitochondrial cytochrome c oxidase subunit I (COI) gene has proven to be a valuable molecular marker for studying the evolutionary relationships among different taxa due to its high level of variability and conserved function. The COI gene is located in line 5489-7039 of mitochondrial DNA (mtDNA) and is responsible for encoding a protein with a base length of up to 1551 starting at the start codon "GTG" and ending with the stop codon "TAA" (Liu *et al.,* 2014). Since recent years, the target gene cytochrome c oxidase subunit I (COI) has been widely used in one common molecular identification method called DNA barcoding. According to Panday *et al.,* (2014), characteristics like the quick accumulation of mutations and the low rate of recombination make it especially useful for identifying various species. By merely enhancing genomic resolution, the examination of COI sequences exposes important traits of an organism that could otherwise be easily overlooked in whole genome sequencing or even morphological identification (Hossain *et al.,* 2021). According to additional research, the cytochrome c oxidase subunit I (COI) genes found in mitochondria have been adopted worldwide as standard tools for molecular taxonomy and animal identification and it may be possible to discriminate between different species using 658 bp segments based on the COI gene (Hebert *et al.,* 2003). A greater variety of animal species may now be specifically identified because of the creation of extensive DNA barcode sequences and the existence of massive databases (Alfonsi *et al.,* 2013). Hence, the aforementioned instances further show how useful the mitochondrial COI gene's DNA barcoding approach is for recognizing, categorizing, and evaluating the development of species. A number of taxa have been successfully classified using COI. By analyzing the sequences of this gene, researchers can infer phylogenetic relationships and reconstruct the evolutionary history of organisms. Variation in the mitochondrial cytochrome c oxidase subunit I (COI) has been investigated in a variety of species to clarify the population's genetic structure, (Jiang *et al.,* (2011) used the mitochondrial COI gene to check the monophyly and phylogenetic relationship within the genus of *Glyptothorax*. Based on DNA barcoding of the COI gene, Parvez *et al.,* (2020) discovered significant genetic differences between native and foreign *A. testudineus*, and

to determine the degree of genetic variation among the climbing perch now available in Bangladesh.

Conclusion:

The study of phylogenetics makes it possible to comprehend the development of distinct groups of species, specifically how they altered and diversified over time. We can better understand the broad patterns and processes of evolution as well as the origins of specific traits, behaviors, and ecological interactions by using this knowledge. It can support efforts for management and conservation. The information can be used to set conservation goals and develop strategies for preserving genetic diversity within and between species. Additionally, phylogenetics can be used to determine which species are most vulnerable to extinction and which species may be crucial for preserving ecosystem function. But first, the specific species must be identified, and the phylogeny aids in choosing the correct one.

The molecular approach is ideal for studying phylogeny because it ensures greater accuracy while minimizing the possibility of errors. Because mitochondrial DNA is inherited from the mother, meaning that the mtDNA sequence is the same for all members of a maternal lineage, it is a better choice for this study than nuclear DNA. This makes mtDNA particularly useful for figuring out how populations and species have changed over time. Over time, mtDNA produces more genetic variety because it evolves more swiftly than nuclear DNA. Because of this, mtDNA can be utilized to look into more recent evolutionary occurrences and to elucidate the connections between species that are closely related. It has been shown that the mitochondrial cytochrome c oxidase subunit I (COI) gene is a useful genetic marker for researching the evolutionary links between various animal species.

References:

1. Alfonsi, E., Méheust, E., Fuchs, S., Carpentier, F.G., Quillivic, Y., Viricel, A., Hassani, S. and Jung, J.L., 2013. The use of DNA barcoding to monitor the marine mammal biodiversity along the French Atlantic coast. *ZooKeys*, *365*(Special Issue), pp.5-24.
2. Batubara AS, Muchlisin ZA, Efizon D, Elvyra R, Fadli N, Irham M. 2018. Morphometric variations of the genus *Barbonymus* (Pisces, Cyprinidae) harvested from Aceh Waters, Indonesia. Fish Aquat Life 26: 231-237.
3. Brown, W., George, M. J. & Wilson, A. C. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76, 1967–1971. https://doi.org/10.1073/pnas.76.4.1967 (1979).
4. Elvyra, R., SOLIHIN, D.D., AFFANDI, R., JUNIOR, M.Z. and SUHENDRA, M., 2020. Molecular characteristics and phylogenetic relationships of silurid catfishes (Kryptopterus, Ompok and Phalacronotus) from the Kampar River, Indonesia, based on the cytochrome b gene. *Biodiversitas Journal of Biological Diversity*, *21*(8).
5. Farias IP, Orti G, Sampaio I, Schneider H, Meyer A. 2001. The cytochrome b gene as a phylogenetic marker: the limits of resolution for analyzing relationships among Cichlid fishes. Mol Phylogenet Evol 53: 89-103.
6. Hebert, P.D.N., A. Cywinska, S.L. Ball. 2003. Biological identifications through DNA barcodes. Proc. Royal Soc. London. Series B: Biol. Sci. 270(151). 313-321

7. Hossain, Z., Sanzida, N.J., Akhand, M.A.A. and Ahmed, M.S., 2021. DNA barcoding of threatened fishes of Bangladesh: DNA barcoding of threatened fishes of Bangladesh. *Bioresearch Communications*, *7*(2), pp.990-998.
8. Ingley, S.J., Bybee, S.M., Tennessen, K.J., Whiting, M.F., Branham, M.A., 2012. Life on the fly: phylogenetics and evolution of the helicopter damselflies (Odonata, Pseudostigmatidae). Zool. Scr. 41, 637–650.
9. Jiang, W., Ng, H.H., Yang, J. and Chen, X., 2011. Monophyly and phylogenetic relationships of the catfish genus Glyptothorax (Teleostei: Sisoridae) inferred from nuclear and mitochondrial gene sequences. *Molecular Phylogenetics and Evolution*, *61*(2), pp.278-289.
10. Liu, S., Yang, Z., Wang, D. dan Liu, M. 2014. The mitochondrial genome of Mystacoleucus marginatus (Cypriniformes, Cyprinidae). Mitochondrial DNA.
11. Megarani, D.V., Nugroho, H.A., Andarini, Z.P., Surbakti, Y.D.R.B. and Widayanti, R., 2020. Genetic characterization and phylogenetic study of Indonesian indigenous catfish based on mitochondrial cytochrome B gene. *Veterinary world*, *13*(1), p.96.
12. Moritz, C., 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3, 401–411.
13. Panday, R., Jha, D.K., Thapa, N., Pokharel, B.R., and Aryal, N.K. 2014. Forensic wildlife parts and their products identification and individualization using DNA barcoding. The Open Forensic Science Journal 7: 6-13.
14. Parson, W., Pegoraro, K., Niederstatter, H., Foger, M. and Steinlechner, M. (2000) Species identification by means of the cytochrome B gene. Int. J. Legal Med., 114(1-2): 23-28.
15. Parvez, I., Mahajebin, T., Clarke, M.L., Chhanda, M.S. and Sultana, S., 2020. Genetic variation of native and introduced climbing perch *Anabas testudineus* (Bloch, 1792) derived from mitochondrial DNA analyses. *Ecological Genetics and Genomics*, *17*, p.100067.
16. Perler, F., Efstratiadis, A., Lomedico, P., Gilbert, W., Kolodner, R. and Dodgson, J., 1980. The evolution of genes: the chicken preproinsulin gene. *Cell*, *20*(2), pp.555-566.
17. Rosen, D.E., Bailey, R.M., 1963. The poeciliid fishes (Cyprinodondiformes): their structure, zoogeography, and systematics. Bull. Am. Mus. Nat. Hist. 126.
18. Schönhuth, S. and Mayden, R.L., 2010. Phylogenetic relationships in the genus Cyprinella (Actinopterygii: Cyprinidae) based on mitochondrial and nuclear gene sequences. *Molecular Phylogenetics and Evolution*, *55*(1), pp.77-98.
19. Sun, Y.B., Shen, Y.Y., Irwin, D.M., Zhang, Y.P., 2011. Evaluating the roles of energetic functional constraints on teleost mitochondrial-encoded protein evolution. Mol. Biol. Evol. 28, 39–44.
20. Wilson, A.C., Cann, R.L., Carr, S.M., George, M., Gyllensten, U.B., Helm-Bychowski, K.M., Higuchi, R.G., Palumbi, S.R., Prager, E.M., Sage, R.D. and Stoneking, M., 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society*, *26*(4), pp.375-400.

EXPLORING THE WORLD OF RADIATION: SOURCES, EFFECTS, AND APPLICATIONS

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Introduction:

All living things are constantly exposed to cosmic rays from space as well as natural sources of radioactivity near the earth's surface (Durante & Cucinotta,2008). Radiation is a form of energy that is emitted by a source through rays or high-speed particles. It involves the transfer of energy from a source to its surroundings or to a distant receiver. Natural radiation is produced by a variety of radioactive substances that are abundant in soil, water, air, and in the body. People regularly consume and breathe in radiation through their food, water, and environment. Radiation Biology or Radiology is the study of the action of ionizing radiation on living things (Hall & Giaccia, 2012).

W.C. Roentgen, a German physicist, discovered a new type of ray emitted from a gas discharge tube in 1895 that could blacken photographic films in light-tight containers, which he named X- rays. After a new branch of science called Diagnostic Radiology was formed, X-ray technology spread like wildfire over Europe and the United States. After the discovery of X-rays, in 1906 the first case of skin cancer was reported.

Radiation can damage cells and tissues by direct or indirect actions. Direct action can damage by direct deposition of energy at target bio-molecules like DNA, RNA, and Lipid in the cells. On the other hand, indirect action can be described as the condition, where radiation can damage non-targeted molecules of the cells i.e., water and generated hydroxyl radicals, that further oxidize the vial molecules present in the vicinity (Ward, 1988).

Source of radiation

Radiation can come from both natural and man-made sources. Cosmic, radon, and thorium are examples of natural radiation, while x-rays, CT scans, and nuclear medicine are examples of man-made radiation.

Natural radiation: Heaviest elements such as Uranium, Radium, Polonium, Thorium, and Potassium found in the earth's crust emit energy through the process of nuclear decay. Cosmic radiation is also a source of natural background radiation and this radiation constantly exposed by processing occurs in the sun, other stars and throughout the universe. Radon is a tasteless, colorless, odorless gas present in nearly all rocks and soils. It is produced by the nuclear decay of radium. It is the most damaging source of radiation. Internal radiation is also a type of natural radiation which occurs in the human body due to the presence of radioactive element potassium (k-40) and carbon-14(C-14) from birth till death. Radioactive elements such as carbon and potassium are present in very small quantities in the human body. They enter our body through

food we eat, water we drink and air we breathe. This radioactive element gets incorporated into tissues and organs throughout the body. All natural sources of radiation are exposed to approx. 2.4mSv/year(IAEA,2011).

Man-made source: Today, man-made sources of radiation approximately consist of about 21% of our total exposure. In industrialized countries, it can be as high as 50% due to better access to medical imaging. The largest source of man-made radiation used in medical applications. In medical application, the largest exposure is from diagnostic x-rays, which are used to determine the physical injury.

Effects of radiation

Radiation can affect the body in several ways, and the adverse health effects of exposure to radiation can result from natural, planned (medical, occupational) or accidental situations and it may be external, internal (inhalation, ingestion or absorption via a contaminated wound), or a combination of both. These adverse health effects can range from mild effects, such as skin reddening, to serious effects such as cancer and death, depending on the amount of radiation absorbed by the body (the dose), the type of radiation, the route of exposure, and the length of time a person was exposed (Balentova *et al.*,2015). Today, the most common artificial sources of human exposure to radiation are X-ray machines and radiopharmaceuticals used for diagnostic or radiotherapy and other medical devices. Exposure to above-normal levels of radiation can lead to fatigue, nausea and vomiting, and changes in the blood. Exposure to very large doses of radiation can lead to radiation sickness, with symptoms such as loss of appetite, hair loss, diarrhea, or even death within a few days or months (Dubois, Andre, et al.,1988). This is called Acute Radiation Syndrome. Exposure to lower doses of radiation may lead to an increased risk of developing cancer or other adverse health effects later in life.

Bio monitoring of exposure to radiation

Generally, two main effects are observed based on duration of exposure of radiation:

1. Short term effect
2. Long term effect

Short term effect: In an acute dose, radiation is delivered to the body over a short period of time. If the amount of radiation exposed is large enough, acute doses may result in effects that can be marked themselves within a period of hours or days. As the latent period between the radiation exposure and the onset of effects, is relatively short and grows progressively shorter as the level of dose increases. Acute dose of radiation can cause clearly identifiable symptoms; these are referred to as acute radiation syndrome.

Long term effect: long term effects of radiation are those in which we can manifest those years after the original exposure. The latent period of this is much longer than the acute radiation syndrome. From the point of view of public health significance, the possibility of long-term effects on the larger number of receiving low, chronic exposure can cause greater health defects than the short- term radiation effects from acute exposures which involve only a few individuals. In long term radiation, no unique disease can be observed. These can be identified after a long

study on long term effects of radiation. A large amount of data is available for animals, but quite less for humans.

Types of radiation

Radiation is classified into two main categories:

3. non-ionizing radiation (cannot ionize matter)
4. Ionizing radiation (can ionize matter).

Non-Ionizing radiation: It is a type of energy that is released by a source and travels across space in the form of electromagnetic waves with certain wavelengths. As a result, it appears in nature as a part of 5 the spectrum and at a lower frequency than ionizing radiation (x-rays). It starts at the low end of the spectrum with extremely low frequency (ELF) electromagnetic fields (EMF), proceeds to radiofrequency and microwave, and then rises significantly to visible light, ultraviolet (UV), and infrared (IR). Unlike ionizing radiation, the energy it delivers is feeble and hence incapable of causing ionization in the human who is exposed. As a result, it is known as non-ionizing radiation (NIR), and the hazard it produces is plainly less hazardous than that of ionizing radiation (Hladik,et al.,2016).

Ionizing radiation

Ionization is the process of ion production by ejection of electrons from atoms and molecules after exposure to high temperature, electrical discharges or electromagnetic and nuclear radiation. Ionizing radiation is subdivided into electromagnetic radiation (X-rays and gamma rays) and particulate radiation including neutrons and charged particles (alpha and beta particles). Types of lonizing Radiation- 1. Alpha Radiation 2. Beta Radiation 3. Gamma Radiation (Photon)

Alpha Radiation

These are the positively charged part of the atoms that are emitted by the radioactive elements. Alpha-particle emitting radionuclides are of interest in targeted therapy because of the short range and high linear energy transfer (LET) of these emissions.

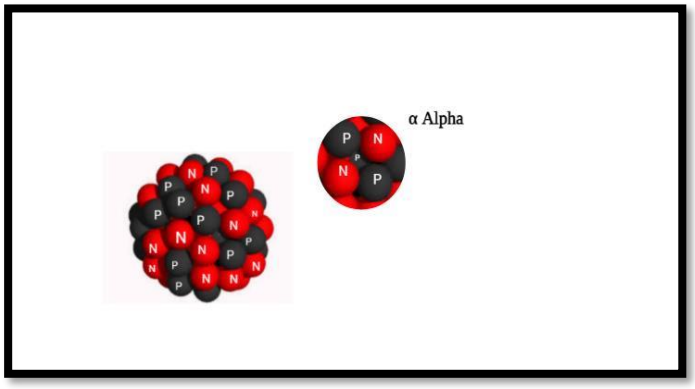

Fig. 1: The release of an alpha particle from an atom's nucleus

Beta radiation

Beta radiation takes the form of either an electron or a positron (a particle with the size and mass of an electron, but with a positive charge) being emitted from an atom. Due to the

smaller mass, it can travel further in air, up to a few meters, and can be stopped by a thick piece of plastic, or even a stack of paper. It can penetrate skin a few centimeters, posing somewhat of an external health risk. However, the main threat is still primarily from internal emission from ingested material.

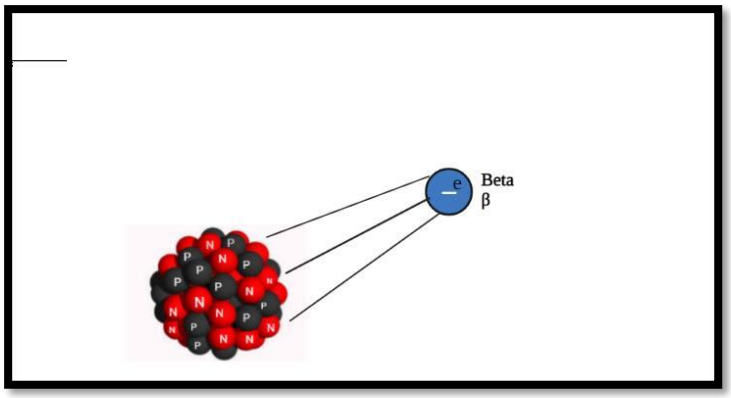

Fig. 2: The release of a beta particle from an atom's nucleus

Gamma radiation

Gamma radiation, unlike alpha or beta, does not consist of any particles, instead consisting of a photon of energy being emitted from an unstable nucleus. Having no mass or charge, gamma radiation can travel much farther through air than alpha or beta, losing (on average) half its energy for every 500 feet. Gamma waves can be stopped by a thick or dense enough layer material, with high atomic number materials such as lead or depleted uranium being the most effective form of shielding.

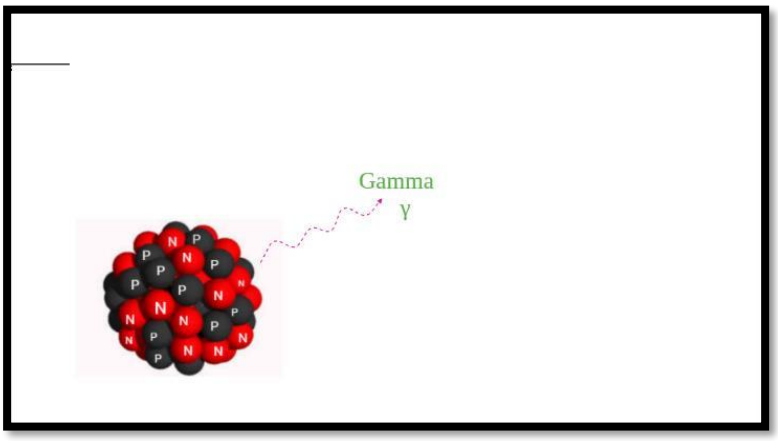

Fig. 3: The release of a powerful wave from an atom's nucleus

Application field of radiation

- Sterilize medical equipment
- Sterilize food (irritated food)
- Used as tracers in medicine
- Radio Therapy- In oncology, to kill cancerous cells.
- Generate nuclear reactions and use it in the development of bombs.

Future perspectives:

Considering today's scenario, a significant population of human beings, especially cancer patients encounter ionising radiation either unintentionally and/or as means of diagnosis and treatment. Further on, ionising radiation is something, which is also being exploited for military use by countries including India. Radioprotection like lead- shielding is used to 'prevent' radiation exposure, but there is an immense need for 'treatment' of radiation-induced damage, as radiation exposure at most times is unforeseen and inevitable. The damaging effects caused by these ionizing radiations when left untreated can lead to severe problems and health issues like Inflammatory bowel disease (IBD), Crohn's disease, Ulcerative colitis, etc. Some counter or alternative measures must be developed to reduce such damages effectively and efficiently without any compromise done with the health of an individual.

There is a vast scope of development of new and emerging technologies which can prove to be of great significance and can be beneficial, economical, time effective, easy to perform and most importantly, technologies that can produce quality research within Radiation Biology studies as radiation biology seems to have a major impact on clinical radiation therapy by providing a rationale for implementation of new treatment strategies and for clinical concepts or practices thereby increasing their acceptance. Radioprotection is one of the techniques to prevent damage of the cells and tissues caused by the ionizing radiation. Radioprotectors are the compounds that are designed with an aim to reduce the damage of these radiations in the normal cells and tissues. Radio mitigation is another such technique where in cells are treated with certain radio frequency to reduce the severity or risk of damage in the cells. Several new innovations and emerging techniques can substantiate wonders in radiation biology

Refrences:

1. Durante, M., & Cucinotta, F. A. (2008). Physical basis of radiation protection in space travel. Reviews of Environmental Health, 23(4), 251-261.
2. Hall, E. J., & Giaccia, A. J. (2012). Radiobiology for the radiologist. Lippincott Williams & Wilkins.
3. Ward, J. F. (1988). DNA damage as the cause of ionizing radiation-induced gene activation. Radiation research, 116(1), 118-121.
4. IAEA (International Atomic Energy Agency). (2011). Radiation protection and safety of radiation sources: International basic safety standards. IAEA Safety Series No. GSR Part 3.
5. Balentova, S. and Adamkov, M. (2015) "Molecular, cellular and Functional effects of radiation- induced brain injury: A review," International Journal of Molecular Sciences MDPIAG,pp.27796– 27815.
6. Dubois, Andre, et al. "Prevention and treatment of the gastric symptoms of radiation sickness." Radiation research 115.3 (1988): 595-604.
7. Hladik, D.andTapio,S.(2016)"Effects of ionizing radiation on the mammalian brain," Mutation Research - Reviews in Mutation Research, 770,pp.219–230.

DEVELOPMENT OF PLANT-BASED NATURAL PRODUCTS AS POTENTIAL THERAPEUTIC AGENTS USING MODERN TECHNIQUES

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Abstract:

Natural products are the important source of new lead compounds in drug discovery research. Several drugs currently being used as therapeutic agents have been developed from natural sources. The plant sources are specifically important. Different approaches utilised in the selection, authentication, extraction/isolation, biological screening, and analogue development through the application of modern drug-development principles of plant-based natural products are discussed here. Furthermore, modern approaches including molecular modeling, virtual screening, natural product library, and database mining are being used for improving natural product drug discovery research. Growing research interest in natural product drug discovery clearly indicated that natural products will play important role in the future development of new therapeutic drugs.

Keywords: Natural products, hit, lead, SAR studies, drug discovery.

Introduction:

Several plant species like opium (Papaver somniferum), myrrh (Commiphora species), and licorice (Glycyrrhiza glabra) have been introduced in the clay tablets from Mesopotamia in 2600 BC. These plants are still for the treatment of various diseases used either alone or as one of the ingredients of herbal formulations. Furthermore, bioactive organic compounds from natural sources were used in the past and also used still now to treat various diseases. These bioactive compounds as well as serve as lead molecules for the development of new synthetic and semisynthetic drugs with improved efficacy. Several such bioactive molecules entered in clinical application include morphine, codeine, noscapine, papaverine, quinine, artemisinin, paclitaxel, etc. (Newman *et al.*, 2000).

These types of bioactive natural products and related drugs are cilinically used for the treatment of most of the popular human diseases, including infectious diseases, cancers, peptic ulcers, as immunomodulators, anticoagulants, antioxidants, respiratory, digestive and cardiovascular system-related diseases, antidiabetics, etc. (Newman *et al.*, 2003). To discover a new chemical entity (NCEs), one of the four major natural sources included plants, marine, animals, and microorganisms (fungi and bacteria). Generally, the compounds which is isolated from natural sources having some unique structural characteristics. Owing to these unique characteristics of the compounds, the development of analogues either to improve potency and pharmacokinetic properties or reduce toxicity is important research area in medicinal chemistry. This chapter will highlight the modern approaches used for the discovery of natural products derived drug molecule for candidate selection, bioactivity-guided extraction, and fractionation,

biological screening, and finally to identify potential lead compounds for specific biological activity.

Selection of plants for screening:

The first and most important steps in drug discovery involves the selection of plant candidates for extraction/isolation of active principles and screening for biological activities. According to Fabricant and Farnsworth (2001), out of approximately 250,000 available species of higher plants, only 15% were taken to phytochemical screening and about 6% of the plant species were estimated for their biological properties (Fabricant *et al.,* 2001). The following techniques are generally being pursued by researchers worldwide for this purpose.

1. Selection based on ethnopharmacological knowledge

This approach is based on the ethnomedicinal usage history of the plants, for example, andrographolide was isolated from the plant *Andrographis paniculta*, which was used for the treatment of dysentery in ethnomedicine. Moreover, a number of active constituents, including berberine, morphine, and picroside from *Berberis aristate*, *Papaver somniferum*, and *Picrorrhiza kurroa* were isolated through this approach. In this approach, the candidate plants are being selected on the basis of observation, description, and even some experimental evaluation. It may involve the study of botany, chemistry, pharmacology, biochemistry, archaeology, anthropology, and the historical background of the plant (Katiyar *et al.,* 2012).

2. Random approach

In this approach, the plants are mainly selected randomly from the local/national regions, and then the selected plants are screened for target bioassays. In addition to that, any of the target chemical classes of compounds such as flavonoids, alkaloids, polysaccharides, etc., may also be screened. This is a trial and error method for general screenings and provides a high chance of finding a lead comounds. It is simple to select the plant candidates through this approach. However, the major drawbacks of this approach are that it does not provide any prior information regarding the biological activity of the selected species.

3. Approach based on traditional system of medicine

The Country like China and India have a high knowledge of well-documented records of traditional/herbal medicines. It is based on a codified system of medicines from botanical sources. Here, the concept of pharmaceutical formulations was more developed in the traditional codified system as compared to ethnomedicinal practices, where the products were used mainly as crude extracts such as decoction and juices. The term standardization was common in the traditional system of medicine. Lastly, the ethnomedicinal practices are generally controlled by a small fraction of the community and are localized in nature; on the other hand, the traditional system is much institutionalized. Some of the important examples of natural products discovered by adopting the approach based on the codified system of medicine include bacosides from *Bacopa monnieri*, artemisinin from *Artemesia alba, boswellic acid* from Boswellia serrata, and reserpine from *Rauwolfia serpentine* used as a memory enhancer, antimalarial, anti-inflammatory, and antihypertensive agents, respectively (Katiyar *et al.,* 2012).

Authentication of plant

The authentication of plant materials may be achieved through the application of one or more of the methods involving taxonomic, macroscopic, microscopic, chromatographic, spectroscopic, chemometric, immunoassays, and DNA fingerprinting analysis. Depending on the type of adulterants and closeness of the chemical constituents, a simple method such as examining the organoleptic properties may be enough to authenticate certain drugs, while a highly sophisticated method may be requiredby some other drugs. Therefore, it is up to researchers to select a suitable procedure for the material of interest (Smille *et al.,* 2010).

Extraction and isolation of natural compounds using biological-activity guided fractionation

In this approach, the fractionation of extract is based on biological activity rather than a class of compound of interest which involves step-by-step separation of the plant extract. On the basis of physicochemical properties and screening for biological activity, further fractionation and screening are followed. In the first round, all the fractions are screened for biological activity, and only the fractions possessing significant activity are further processed until the achievement of the pure isolate, responsible for target biological activity. The chemical characterization and structural elucidation are performed after the identification of the active isolates (Shams Eldin *et al.,* 2018). The biological activity-guided isolation method has been used for the discovery of a variety of plant-derived natural products, including anticancer agents, camptothecin, and paclitaxel from Camptotheca acuminata and Taxus brevifolia, respectively (Kinghorn *et al.,* 1994).

1. Parallel approach

This approach is used when the selected plants are known for their biological activities from traditional or ethnopharmacological knowledge. The active compounds responsible for the target activity are isolated from the crude plant material as described in Figure 1. The extraction, isolation/purification is generally performed in the following three stages.

1.1. Extraction

Initially, at least three fractions of extracts, for example, 100% aqueous, 100% ethanolic, and 50% aqueous −50% ethanol extracts are collected and evaluated for target biological activity in the primary screening.

1.2. Fractionation

The most active extract(s) are extracted into sub-fractions in the sequence of decreasing polarity of a solvent such as butanol, chloroform, and hexane. The sub-fractions are further evaluated for biological activity.

1.3. Isolation and Purification

The most active sub-fraction(s) obtained at stage 2 are subjected to chromatographic separation to isolate the compounds of interest. Each compound is purified through appropriate purification techniques such as column chromatography, preparative HPLC, etc., and screened for target biological activity. Chemical structures of the compounds exhibiting optimum

biological activity are elucidated by using modern techniques such as NMR spectroscopy, Mass spectrometry, LC-MS, etc.

2. Sequential approach

This approach is mainly used for the plants selected by random selection strategy and the biological activity of the selected plant is not known. The extraction/fractionation, isolation, and biological screening processes used in this approach have been summarized in Figure 2. The experiment can be divided into two stages as follows.

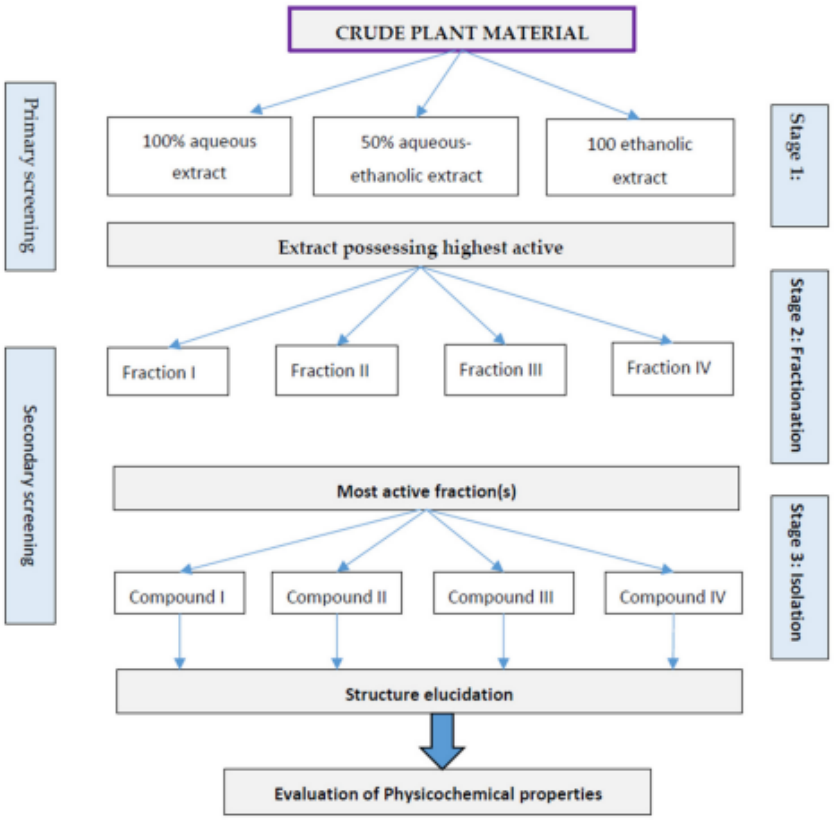

Fig. 1: An outline of parallel approach for biological activity guided fractionation of plant extracts

2.1. Extraction and Fractionation

In this stage, the extraction of the plant material and fractionation of the extracts take place simultaneously. The extractions are performed in the solvent systems of increasing polarity and fractions are collected in a sequential manner using petroleum ether, chloroform, ethyl acetate, ethanol, and water, for instance. All the fractions are screened for target biological activity.

2.2. Isolation and Purification

The fractions from the stage 1 experiment possessing the highest biological activity are selected, and the compounds responsible for specific biological activity are isolated by following the techniques mentioned in the previous approach. The isolated compounds are analyzed for structure elucidation by using modern techniques such as LC-MS, NMR spectroscopy, FT-IR spectroscopy, and Mass spectrometry. As shown in Figure 3, the primary screening is done in the

first stage to evaluate the efficacy, whereas the secondary screening is mainly oriented to the evaluation of the mechanism of action and may involve *in-vitro* screening at molecular levels. In both approaches, the extraction of the plant material is performed by using a range of polar and non-polar solvents. However, their method of extraction and fractionation remained largely invariable. In general, the nature of chemical constituents present in the extract or fraction can be predicted depending on the polarity of the solvent used. Low polar lipophilic compounds such as oils, fatty acids, steroids, hydrocarbons, and low polarity terpenoids are extracted in non-polar solvents such as n-hexane and ether; whereas, the medium polar compounds such as phenolics and alkaloids are usually present in ethyl acetate and chloroform extracts. The highly polar compounds such as sugars, flavonoids, glycosidic alkaloids, and small carboxylic acids are generally extracted in aqueous, methanol or ethanol extracts.

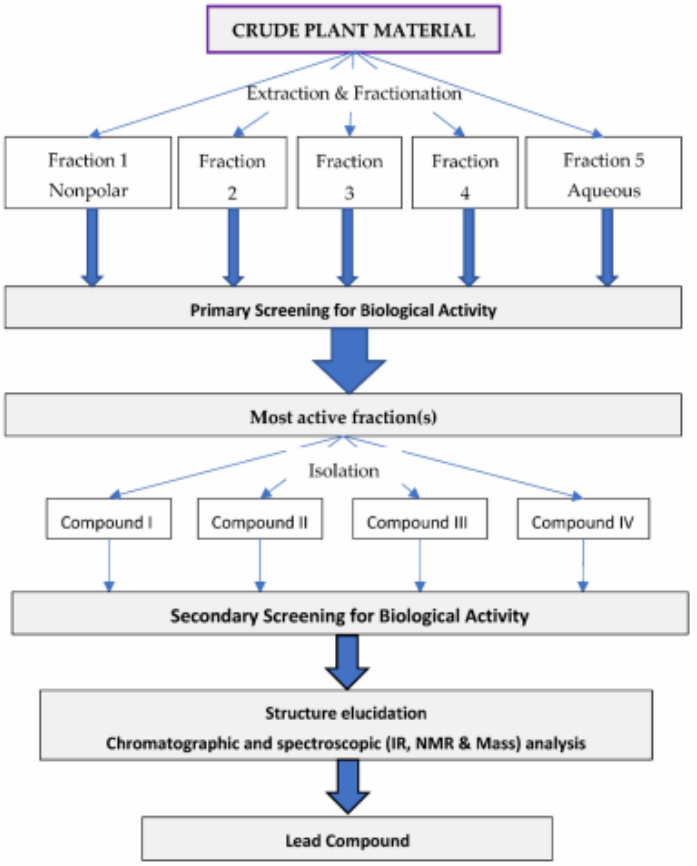

Fig. 2: An outline of sequential approach for biological activity guided fractionation of plant extract

Structure elucidation of isolated compounds

At present, spectroscopic analyses have become the power tool in the structural determination of phytochemicals. After a preliminary biological screening of extracts, the bioactive ones can be rapidly fractionated by high-performance liquid chromatography, subsequently, the chemical characterization of the fractions is determined by liquid chromatography-mass spectrometric (LC-MS) and nuclear magnetic resonance (NMR) spectroscopic analysis. LC-MS/MS is one of the most important techniques used for

phytochemical profiling of the active fractions, which integrates good chromatographic separation efficiency with excellent characterizing capacity of mass spectrometry. Characterization of the structure of extracts, as well as pure bioactive molecules, are performed by the data obtained from a wide range of spectroscopic techniques such as FT-IR spectroscopy, NMR spectroscopy, and Mass spectrometry. In addition to these main techniques, data from X-ray diffraction, Optical rotatory dispersion, and chemical examination may also be helpful. A combination of HPLC with the above techniques has led to the development new technique useful in differentiating between known and unknown compounds and characterizing the full constituents of the natural products directly from the crude extract with a minimum amount of sample and time.

Biological screening of extracts/fraction/isolates

Generally, the natural products are screened for their biological activities on the basis of their reported ethnopharmacological and traditional uses. For instance, the medicinal plant traditionally used for the management of diabetes may be assayed for hypoglycemic effect and the traditional use is scientifically rationalized, once a very good 'hit' molecule has been achieved. However, most of the time the activity is not replicated in *in-vitro* screening. Most of the natural products are low-yielding, so the biological screening of such compounds can be performed by a series of bioassay methods that provide fast and sensitive results. These assays are performed by using several animal or human cell lines and microorganisms. A number of accurate and efficient instruments have been developed in this regard (Hamid *et al.,* 2004). Generally, the practical yield of bioactive pure compounds from natural sources is extremely low, and it is really difficult to supply them in adequate amounts needed for animal screening. On the other hand, potential hits may be regarded as unsafe on the basis of toxic effects observed in cell-based screening, which may have been revealing good safety profiles in the animal body due to detoxification in the liver (Liska *et al.,* 1998). After determining the biological profile of the new natural products; identification of their target of action in the biological system, physiological pathways they interact with and hence, mechanism of action should be established. The SAR studies provide preliminary information regarding the mode of drug-target interaction and identification of analogues showing higher potency than the parent compound. A compound exhibiting LC_{50} and IC_{50} values at micro or nano mole levels is considered to be potent. However, the biological profile of a true drug candidate should not rely only on a single *in-vivo* screening, rather multiple screenings are necessary to confirm its efficacy (Gray *et al.*, 2012).

Molecular modeling and natural product database

The discovered bioactive natural products can be used as lead compounds for the optimization of structural features to develop new and more effective analogues by applying molecular modeling and combinatorial chemistry. Furthermore, the natural products present along with other compounds as a family of structurally related molecules; therefore, a number of homologues may be obtained from one source which can provide SAR-related information. In the modern drug discovery of natural products, the isolated new compounds possessing acceptable bioactivity are subjected to SAR studies and molecular modeling processes to design

and develop analogues with more potency, fewer toxic effects, and better pharmacokinetic profiles. The study can also reveal that interaction with certain enzymes may influence the test compounds' biological activity. The analogues with the best druggability may be synthesized in the laboratory and evaluated by a number of *in-vitro* and *in-vivo* biological assays (Kitchen *et al.*, 2004). The overall, process of design and development of analogues from a naturally isolated lead compound can be summarized as below.

1. In-Silico ligand construction and preparation

Molecular modeling procedures require optimized 3D structures of ligands in PDB format. Natural product databases and other databases like PUBCHEM, ZINC are reliable sources for retrieving the structures of known natural compounds in different acceptable formats like SDF., mol., mol2, PDB, etc. (Sorokina *et al.,* 2020). Structures obtained must be optimized for geometry so as to possess minimum energy. Energy minimization can be performed prior to docking in docking software like AutoDock Vina and Discovery Studio, or independent structure building and optimization software like Chimera, Chem 3D Ultra, Avogadro, etc., can be applied (Liao *et al.*, 2011).

2. Target preparation

Preparing or downloading the 3D structures of target molecules such as proteins (example: human serum albumin), receptors (examples: PPAR-α and PPAR-γ), and enzymes (examples: cyclo-oxygenase, topoisomerase II, and protein kinase) from Protein Data Bank (PDB) and optimized for geometry and energy. The binding site to be defined and standard scores calculated for natural ligands present.

3. Docking

The 3D structures of natural products are docked against the target structure by using docking software and ranked according to the binding energy. Widely used docking tools include AutoDock, AutoDock Vina, FlexX, Discovery Studio, and MDock. A prerequisite for docking is the optimized 3D structures of the ligands and targets in PDB format. Docking utilizes several search algorithms, for example, the Lamarckian genetic algorithm for identifying the best binding conformation of the ligand. Post docking studies to analyze inter-molecular interactions are essential to substantiate the results obtained from docking.

4. Identification of hit molecule

After docking simulation, the results are analyzed and the top interactions are identified based on the energy scores. Usually, the top 10 scores are further subjected to molecular dynamics (MD) simulation studies. In this regard, two systems i.e., (I) Apo (uncomplexed) protein/receptor, (II) protein/receptor complexed with interacting compound (predicted by docking study) can be submitted to MD simulation. According to the ranking of ligands and their interactions with the target, the hit molecules having a high affinity towards the target are identified.

5. Optimization of hits

The optimization process of hits is performed by observing better affinity towards the target by preparing various analogues of hits and the hits showing best affinity are developed and

various drug-like properties viz; stability, pharmacokinetic and pharmacodynamic properties can be studied by using QSAR software (Devillers *et al.*, 2013). Alternative to the extraction and biological assay of natural products, in modern drug discovery, a collection of a large number of compounds derived from natural sources possessing diverse chemical structures are tested through virtual screening or *in-silico* study. Through the application of virtual screening, potential hits can be identified for a target biological activity and a good SAR can be established through a lead optimization process. In this way, virtual screening can filter down the number of compounds for the real test through bioassay (Stockwell *et al.*, 2004). Such compounds can be synthesized in the laboratory or purchased it from any commercial sources. DNP, Phytopure, ChemSpider, Natural Product Alert, Tim Tech Natural Products, etc., are some of the commonly available natural product libraries and databases (Calixto *et al.*, 2019). The overall approach to drug discovery and development from natural sources has been summarized in Figure 3.

Drug discovery from plants

Natural products remain the best sources of drugs and drug leads. About 35% of the annual global market of medicine is either from natural products or related drugs; mainly including plants (25%) followed by microorganisms (13%) and animal (3%) sources [25]. Between 1983 and 1994, the USFDA approved 520 new drugs, of that about 39% were natural products or drugs derived from them, while this proportion was about 60–80% in the case of antibiotics and anticancer agents (Cragg *et al.*, 1997).

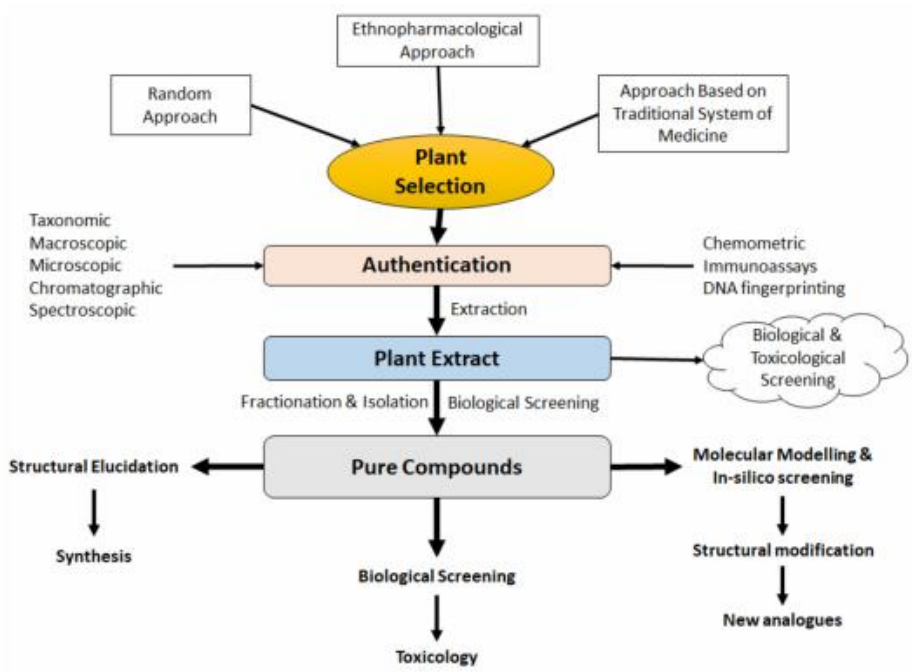

Fig. 3: The overall approaches in modern drug discovery and development process from botanical sources

Newman and Cragg (2016) reported that, of the 1562 drugs approved by USFDA between 1981 and 2014, 64 were pure natural products, 141 were herbal mixtures, 320 were derived from natural products, and 61 were synthetic drugs prepared by exploring the

pharmacophores of natural products; these constitute 4%, 9.1%, 21%, and 4%, respectively, of the total approved drugs. The examples of worldwide best-selling natural products derived medicines include antibiotics and antifungal agents, erythromycin, clarithromycin, amoxycillin, amphotericin B; anticancer agents, paclitaxel, docetaxel, and campothecin; cholesterol-lowering drugs, atorvastatin, simvastatin, lovastatin; immunosuppressant, tacrolimus, and cyclosporin A and antihypertensive agents, captopril, and enalapril (Li *et al.,* 2009). Here we highlights various natural products collected from different sources and their biological activities.

1. Anti-inflammatory natural products

Inflammation is associated with diseases like cancer and diabetes. Pro-inflammatory cytokines (TNF-α and IL-1β) and NO are considered as the key mediators in inflammatory conditions like rheumatoid arthritis, sepsis etc. Thus, inhibition of pro-inflammatory cytokines and NO production are important targets for treatment of inflammatory disorders. Various plants used for the treatment of inflammatory conditions, have been found to inhibit TNF-α and IL-1β production in various *in vitro* models (Paul *et al.,* 2006). Withanolides from Withania somnifera are found to be active in arthritis and are potent inhibitors of angiogenesis, inflammation and oxidative stress. Alkaloid, berberine found in the plant Berberis aristata, has been found to inhibit NFκB, COX-2, TNF-α, IL-1β, IL-6 responsible for the potent anti-inflammatory activity of this plant. Other examples include nimbidin from neem (Azadirachta indica) and embelin a constituent of vidang (Embelia ribes) which has been reported to show potent antiinflammatory activity in experimental animals.

2. Cardiovascular natural products

A number of plants are reported to contain cardiac glycosides. They inhibit the membrane bound sodium-potassium ATPase pump resulting in depletion of intracellular potassium and an increase in serum potassium, causing decrease in the electrical conductivity as well as the heart rate, and increasing cardiac output. Yellow oleander plant (*Thevetia neriifolia*) is reported to contain thevitin A, B and peruvoside which are potent cardiac glycosides found in all parts of the plant and with high concentration in the fruits. *Rauwolfia serpentina* was first tested in India for antihypertensive activity (Vakil *et al.,* 1949). However, the active principle Reserpine was isolated and studied abroad. It exhibits its action by inhibiting monoamine oxidase (MAO). *Terminalia arjuna bark* has been used for the treatment of symptoms similar to angina in the traditional Indian system of medicine.

3. Anti-diabetic natural products

Charantin, a steroidal saponin isolated from this plant is reported to have an insulin-like activity, responsible for its hypoglycemic effect. Besides, charantin stimulates the release of insulin and blocks glucose formation in the bloodstream, suggesting its beneficial effects in non-insulin-dependent diabetes (Krawinkel *et al.,* 2006). Gymnema sylvestre (gurmar), is another plant that has been used traditionally for the treatment of diabetes. Gymnemic acid IV, obtained from leaves of Gymnema sylvestre has been reported to show strong hypoglycemic activity in animals models of diabetes comparable to glibenclamide.

4. Anti-obesity natural products

Tea polyphenolics like (-)-epigallocatechin 3,5-diogallate (IC_{50}; 0.098 µM), oolonghomobisflavan A (IC_{50}; 0.048 µM), oolongtheanin 3'-O-gallate (IC_{50}; 0.068 µM) and theaflavin 3,3'-O-gallate (IC_{50}; 0.092 µM) show a potent pancreatic lipase inhibitory activity (Nakai *et al.,* 2005). 3-Methyletherganglin and 5-hydroxy-7-(4'-hydroxy-3'-Methoxyphenyl)-1-phenyl-3-heptanone isolated from Alpinia officinarum have shown significant pancreatic lipase inhibitory activity *in vitro*. These compounds also showed a strong hypolipidemic effect in Triton WR-1339 induced hyperlipidemic mice.

5. Anti-malarial natural products

A number of medicinal plants have been used traditionally in the treatment of malaria. Several biflavonoids have been reported from Selaginella bryopteris which have been investigated for their anti-protozoal activity *in vitro* against K1 strain of *Plasmodium falciparum*. Nimbolide has been identified as the active antimalarial principle of this plant (EC_{50}; 0.95 ng/ml, P. falciparum K1) (Rochanakij *et al.,* 1985). Besides, gedunin (IC_{50} 720 ng/mL, P. falciparum D6) and its dihydro derivative have been found to be active (IC_{50}; 2630 ng/ml).

6. Anti-leishmanial natural products

Diospyrin isolated from Diospyros spp. has been a very potent antileishmanial natural product with IC_{50} of 1µg/ml against Leishmania donovani (Hazra *et al.,* 1987), this compound was also found to inhibit the type I DNA topoisomerase of L. donovani parasite. Plumbagin from Plumbago spp. is perhaps the most potent antileishmanial natural product with an IC_{50} of 0.42µg/ml against L. Donovani. Piperine, which is found in many piper species, has been shown to be active against promastigotes of L. donovani with activity comparable to pentamidine (Kapil *et al.,* 1993). Amarogentin, isolated from Swertia chirata has been found to inhibit L. donovani topoisomerase I.

7. Anti-viral natural products

HIV is the leading cause of death in African continent and the disease is increasing at an alarming rate in India. Several natural products particularly alkaloids, phenolics and terpenes have shown a promising anti HIV activity. Theasinensin D, a phenolic compound found in tea (*Thea sinensis*) has been shown to exhibit a good anti-HIV activity with IC_{50} of 8µg/ml. The common phytosterols ursolic acid and oleanolic acid found in many plants has also been reported to show anti-HIV activity with an IC_{50} of 8µM and 21.8µg/ml (Min *et al.,* 1999). Gallic acid, chebulagic acid and other galloyl glucoses isolated from *Terminalia chebula* have been reported to show a promising HIV integrase inhibitory activity.

8. Anti-neoplastic natural products

A diterpenoid precalyone isolated from Roylea calycina has been reported to show activity against P388 lymphoid leukaemia. The other compound studied for anticancer activity is Tagitinin F, a germacranolide isolated from Tithonia tagitiflorahas has been also found to be active against lymphocytic leukaemia (Pal *et al.,* 1976). Flavopiridol, a semi-synthetic flavonoid derived from rohitukine found in the plant Dysoxylum binectariferum is the first cyclin-dependent kinase (CDK) inhibitor to be tested in clinical trials (Sedlacek *et al.,* 2001).

Podophyllotoxin, a lignan isolated from this plant has been found to be responsible for the anticancer activity. A flavanol glycoside tephdidoside from Tephrosia candida has been found to be active against human epidermoid carcinoma of nasopharynx (Sarin *et al.*, 1976).

Conclusions:

A number of successful therapeutic agents have been obtained directly from plant sources or developed from naturally derived lead molecules. Due to renewed interest of the medicinal chemists in natural product drug discovery, a number of new approaches accompanied by technological advancement for selection, identification, isolation, characterization, and biological screening of natural products have been developed. These new approaches could minimize the technical drawbacks associated with natural product development and address the challenges encountered in the discovery and development of new natural products owing to the complex behavior of natural products. In the future, the new techniques on natural product drug development will minimize the challenges and enhance the success rate in the drug discovery process.

References:

1. Newman, D.J.; Cragg, G.M.; Snader, K.M., 2000.The influence of natural products upon drug discovery. *Nat. Prod. Rep*.17(3), 215–234.
2. Newman, D.J.; Cragg, G.M.; Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* 66 (7), 1022–1037.
3. Fabricant, D.S.; Farnsworth, N.R., 2001. The value of plants used in traditional medicine for drug discovery. *Environ. Health Perspect*. 109 (1), 69–75.
4. Katiyar, C.; Gupta, A.; Kanjilal, S.; Katiyar, S., 2012. Drug discovery from plant sources: An integrated approach. *AYU*. 33(1), 10–19.
5. Smille, T.J.; Khan, I.A., 2010. A Comprehensive approach to identifying and authenticating botanical products. *Clin. Pharm. Therap*. 87(2), 175–186.
6. Shams Eldin, S.M.; Radwan, M.M.; Wanas, A.S.; Habib, A.M.; Kassem, F.F.; Hammoda, H.M.; Khan, S.I.; Klein, M.L.; Elokely, K.M.; ElSohly, M.A., 2018. Bioactivity-guided isolation of potential antidiabetic and antihyperlipidemic compounds from Trigonella stellata. *J. Nat. Prod.* 81(5), 1154–1161.
7. Kinghorn, A.D. The discovery of drugs from higher plants. In The Discovery of Natural Products with Therapeutic Potential; Gullo, V.P., Ed.; Butterworth-Heinemann: Oxford, UK, 1994; pp. 81–108.
8. Hamid, R.; Rotshteyn, Y.; Rabadi, L.; Parikh, R.; Bullock, P., 2004. Comparison of alamar blue and MTT assays for high through-put screening. *Toxicol. In Vitro*, 18(5), 703–710.
9. Liska, D.J., 1998. The detoxification enzyme systems. *Altern. Med. Rev*. 3(3), 187–198.
10. Gray, A.I.; Igoli, J.O.; Edrada-Ebel, R. Natural products isolation in modern drug discovery programs. In Natural Products Isolation, Methods in Molecular Biology; Sarker, S.D., Nahar, L., Eds.; Humana Press: Totowa, NJ, USA, 2012; Volume 864, pp. 515–534.

11. Kitchen, D.B.; Decornez, H.; Furr, J.R.; Bajorath, J., 2004. Docking and scoring in virtual screening for drug discovery: Methods and applications. *Nat. Rev. Drug Discov.* 3(11), 935–949.
12. Sorokina, M.; Steinbeck, C. Review on natural products databases: Where to find data in 2020. *J. Cheminform.* 2020, *12*, 20.
13. Liao, C.; Sitzmann, M.; Pugliese, A.; Nicklaus, M.C., 2011. Software and resources for computational medicinal chemistry. *Future Med. Chem.* 3(8), 1057–1085.
14. Devillers, J., 2013. Methods for building QSARs. *Methods Mol. Biol.* 930, 3–27.
15. Stockwell, B.R., 2004. Exploring biology with small organic molecules. *Nature*, 432(7019), 846–854.
16. Calixto, J.B., 2019. The role of natural products in modern drug discovery. An. Da Acad. Bras. De Ciências, *91*, e20190105.
17. Newman, D.J.; Cragg, G.M., 2016. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.* 79(3), 629–661.
18. Cragg, M.G.; Newman, D.J.; Snader, K.M., 1997. Natural products in drug discovery and development. *J. Nat. Prod.* 60(1), 52–60.
19. Li, J.W.; Vederas, J.C., 2009. Drug discovery and natural products: End of an era or an endless frontier? *Science*, 325(5937), 161–165.
20. Paul, A. T.; Gohil, V. M.; Bhutani, K. K., 2006. Modulating TNFalpha signaling with natural products. *Drug Discov Today*, 11(15-16), 725-732.
21. Vakil, R. J. A., 1949. clinical trial of Rauwolfia serpentina in essential hypertension, *Br Heart J*, 11(4), 350-355.
22. Krawinkel, M. B.; Keding, G. B., 2006. Bitter gourd (Momordica Charantia): A dietary approach to hyperglycemia. *Nutr Rev*,64(7), 331-337.
23. Nakai, M.; Fukui, Y.; Asami, S.; Toyoda-Ono, Y. Iwashita, T.; Shibata, H.; Mitsunaga, T.; Hashimoto, F.; Kiso, Y., 2005. Inhibitory effects of oolong tea polyphenols on pancreatic lipase *in vitro*. *J Agric Food Chem*, 53(11), 4593-4598.
24. Rochanakij, S.; Thebtaranonth, Y.; Yenjai, C.; Yuthavong, Y., 1985. Nimbolide, a constituent of Azadirachta indica, inhibits Plasmodium falciparum in culture. *Southeast Asian J Trop Med Public Health*, 16(1), 66-72.
25. Hazra, B.; Saha, A. K.; Ray, R.; Roy, D. K.; Sur, P.; Banerjee, A., 1987. Antiprotozoal activity of diospyrin towards Leishmania donovani promastigotes *in vitro*. *Trans R Soc Trop Med Hyg*, 81(5), 738-741.
26. Kapil, A., 1993. Piperine: a potent inhibitor of Leishmania donovani promastigotes *in vitro*. *Planta Med*, 59(5), 474.
27. Min, B. S.; Jung, H. J.; Lee, J. S.; Kim, Y. H.; Bok, S. H.; Ma, C. M.; Nakamura, N.; Hattori, M.; Bae, K., 1999. Inhibitory effect of triterpenes from Crataegus pinatifida on HIV-I protease. *Planta Med*, 65(4), 374-375.
28. Pal, R.; Kulshreshtha, D. K.; Rastogi, R. P., 1976. Antileukemic and other constituents of Tithonia tagitiflora Desf. *J Pharm Sci*, 65(6), 918-920.

29. Sedlacek, H. H., 2001. Mechanisms of action of flavopiridol. *Crit Rev Oncol Hematol*, 38(2), 139-170.
30. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D., 1989. Isolation and structure of the strong cell growth and tubulin inhibitor combretastatin A-4. *Experientia*, 45(2), 209-211.
31. Sarin, J. P. S.; Singh, S.; Garg, H. S.; Khanna, N. M.; Dhar, M. M., 1976. A flavonol glycoside with anticancer activity from Tephrosia candida. *Phytochemistry*, 15(1), 232-234.
32. Mohana, K.; Purushothaman, K. K.; Susan, T., 1985. Drug potential of echitamine chloride in cancer chemotherapy. *Bull Med Ethnobot Res*, 6(8), 124-129.

A COMMENTARY ON CHARACTERIZATION OF FERRITES

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Abstract:

Throughout the different stages of development of ferrite materials and devices, characterization plays an essential role. This process involves a range of techniques, including structural, spectroscopic, and magnetic measurements, which aim to provide scientific insight into the material. In addition, electrical measurements can be used to assess the quality and performance of ferrites for their intended applications. This article provides an overview of several techniques used to characterize ferrites, such as X-ray diffraction, Mössbauer spectroscopy, Raman spectroscopy, M-H loop, resistivity measurement, and complex permeability measurement. The article briefly highlights the usefulness of these techniques for characterizing ferrites.

Keywords: Ferrites, Characterization

Introduction:

The development of ferrites comprises several critical stages, including modeling, synthesis, and characterization. Characterization is essential in establishing the composition-structure-property relationship for scientific understanding, as well as assessing the material's performance, quality, and reliability for technological applications. This chapter focuses on the structural, spectroscopic, and magnetic characterization techniques used for scientific understanding, as well as the electrical characterization techniques used for application-level understanding. The chapter compiles commonly employed techniques and related scientific approaches for extracting structure-property data and evaluating the technological performance of ferrite materials. However, it is worth noting that the discussions in this chapter are limited to specific aspects of commonly used characterization techniques in ferrite research, rather than being comprehensive.

Characterization techniques play a crucial role in understanding the structural and elemental composition of synthesized ferrite materials. These techniques include diffraction, microscopic, and spectroscopic methods that provide information about atom arrangement, structural order, defects, impurities, morphological aspects, and elemental analysis. The resolution of these techniques varies according to the extent of their excitation wavelength. In addition, electrical measurements provide insights into a material's behaviour and its suitability for specific applications. The composition and chemical variations of materials lead to different electrical characteristic responses, which can be useful in research and development of targeted materials. These measurements rely on precise instrumentation, suitable test fixture, measurement techniques, and test standards, as well as software. The following sub-sections

provide a brief and point-wise discussion of the capabilities of characterization techniques from various viewpoints.

Characterization of Ferrite

1. Structural, morphological and elemental characterization

1.1 X-ray powder diffraction

Polycrystalline bulk material can be obtained by annealing ferrite materials at high temperatures for a certain period. The X-ray powder diffraction (XRD) technique is commonly used to identify the material or, more specifically, the crystalline phases in these materials. When a collimated monochromatic X-ray beam is directed at polycrystalline ferrite material, diffraction occurs based on Bragg's law.

$$2d_{hkl}Sin\theta_{hkl} = n\lambda \quad (1)$$

where, d_{hkl} is the d-spacing between (hkl) plane and θ_{hkl} is the angle between the atomic planes and the incident (and diffracted) X-ray beam.

The pattern resulting from diffraction under the specified circumstances can serve as a distinct identifier for the phase of a particular material. The Joint Committee on Powder Diffraction Standards (JCPDS) and Crystallography Open Database (COD) maintain a vast database, which enables pattern recognition through either manual means or computer-based algorithms. This technique is useful in a range of inquiries, such as detecting crystalline phases and undesired impurities, identifying lattice-parameter, and determining lattice-type and reflection conditions. In addition to the existing databank, the mathematical and analytical methods outlined in Cullity's textbook can be used to index the pattern and calculate the lattice parameter of any cubic and hexagonal ferrite material [1]. Furthermore, the Scherrer formula was employed to estimate the crystallite size based on the line broadening of the most robust diffraction peak.

From Scherrer formula:

$$D = \frac{K\lambda}{B\cos\theta} \quad (2)$$

where, $K = 0.9$ is the shape factor for spherical crystallite, λ is the excitation wavelength, B is the line broadening at half the maximum intensity (FWHM) in radians and θ is the Bragg angle and D is the crystallite size, smaller or equal to the grain size. It is crucial to comprehend the combined impacts of these phenomena and the gathered data on the lattice to accurately decipher the produced ferrite material.

1.2 Raman spectroscopy

Raman spectroscopy, named after its inventor Prof. C. V. Raman, is a technique similar to XRD and Mössbauer spectroscopy in offering a distinctive identification of the material being studied. This method is utilized to investigate the molecular structure of crystalline substances by inelastic scattering of monochromatic light interacting with phonons, leading to a Raman shift, where the energy of the scattered radiation differs from the incident radiation. In the study of polycrystalline ferrites using Raman spectroscopy, the vibrations observed in the atoms and lattices are primarily vibrational and discussed in this subsection. Compared to other vibrational

methods like FTIR, Raman spectroscopy allows for easy sample preparation and is highly versatile in terms of speed and characterization ease.

In a typical micro-Raman experiment, suitable laser light is used to irradiate the ferrite, and the inelastically scattered light is then collected. The intensity of the scattered light is plotted graphically as a function of Raman shift in wavenumber, which results in a spectrum comprising several symmetry-allowed peaks referred to as Raman active modes. These modes correspond to one or more vibrational modes of the solid. However, the total number of peaks is usually less than or equal to the number of Raman active modes, either due to degeneracy of a few modes or low intensity of some modes that makes them difficult to measure. In solid-state investigations, Raman activity is a function of the space group symmetry of crystalline solids, and the corresponding lattice vibrations provide information on the strength of inter-atomic and intermolecular bonds, chemical composition/environment, degree of crystallinity, and the mechanical strains present in the solid. The vibrational characteristics are unique to different molecules and their crystal structure, allowing identification of the material's composition and structure. Raman spectroscopy can also provide valuable information on the static or dynamic disorder of an atom on a specific site [2].

1.3 Scanning Electron Microscopy (SEM)

The scanning electron microscope utilizes a focused beam of high-energy electrons to generate various signals at the surface of ferrites. The interaction of electrons with ferrites provides information about the sample, including its morphology, chemical composition, and crystalline structure. Advanced electron microscopes, such as field emission SEMs (FE-SEM), can probe the material under inspection at high magnification of up to 800,000x, with secondary electron image resolution of approximately 1 nm at 15 kV and 2 nm at an accelerating voltage as low as 1 kV. The SEM produces two primary types of images, secondary electron images and backscattered electron images, which are generated by different mechanisms. Secondary electrons (SE) are emitted when the energy of the emitted electron is less than approximately 50 eV, while backscattered electrons (BSE) refer to electrons that exit the specimen with energy greater than 50 eV. BSE images have built-in contrast caused by elemental differences, providing information on the morphological aspects of powder and the topographical features of pellets and thick-film prints. Additionally, energy-dispersive X-ray spectroscopy (EDS) can be used for elemental mapping or spot chemical analysis.

2. Magnetic characterization: Vibrating sample magnetometer

Ferrites, which are oxide materials exhibiting ferrimagnetism, display spontaneous magnetization even in the absence of an external magnetic field. This phenomenon arises due to the spontaneous ordering of atomic magnetic moments, driven by the exchange interaction among electron spins. Therefore, when characterizing a ferrite material, the most crucial properties to be determined are its intrinsic magnetic properties such as saturation magnetization and coercivity. These properties can be measured by observing the magnetic moment of the sample as a function of an external magnetic field using SQUID or VSM instruments.

A typical VSM measurement involves plotting the bulk magnetization (M) against the applied magnetic field (H), with the total magnetization M resulting from the magnetic moment of each atom of the ferrite material being normalized by volume V. The resulting graph displays emu/gm versus applied magnetic field strength in Oersted. The M-H hysteresis curve provides valuable information on the ferrite's saturation magnetization (σs), remanent magnetization (σr), and coercivity (Hc). While the graph is important to materials scientists for understanding the material, engineers are more interested in the B-H hysteresis curve from a technological point of view. This curve is similar in nature to the M-H hysteresis curve, but takes into account magnetic induction rather than the magnetization behavior of the material seen in the M-H curve. The magnetic induction includes both the intrinsic magnetization as well as the applied magnetic field, which is given by the equation

$$B = \mu_0(H + M) \quad (3)$$

where, μ_0 is the permeability of the free space.

3. Electrical characterization

3.1 Measurement of DC resistivity

The resistivity of ferrite materials is a crucial factor that affects the penetration of microwave energy through them. In comparison to ferromagnetic metals with resistivity levels of approximately 10^{-5} Ω-cm, ferrites exhibit exceptional properties for microwave applications. Hence, determining the resistivity is a crucial aspect in determining the suitability of the material for microwave applications.

The direct measurement of leakage current through a material is carried out using volume resistivity. To do this, a sample is placed between two electrodes and a known potential is applied between them. The current resulting from this is then measured using a Keithley electrometer 6517A along with a resistivity jig that has been custom-made. Depending on the size and shape of the specimen, an electrode configuration can also be made to order, with detailed instructions available in the Keithley Application note series [3]. This procedure is crucial in assessing the suitability of a material for microwave applications, where low resistivity is desired over ferromagnetic metals.

As preferred, the alternative polarity test at ±40 and ±100 volts for 60 seconds was performed for each measurement to eliminate the background currents. The resistivity is calculated from the geometry of the electrodes and the thickness of the sample:

$$\rho = \frac{K_v}{t} \cdot \frac{V}{I} \quad (4)$$

where: ρ = volume resistivity (Ω-cm)

K_v = the effective area of the guarded electrode (cm^2) i.e.

$$K_v = \pi \left(\frac{D_1}{2}\right)^2 \quad (5)$$

V = applied voltage (volts)

I = measured current (amperes)

t = sample thickness (cm)

The resistivity of a material can be utilized to gain insights into various phenomena such as dielectric breakdown, dissipation factor, and mechanical continuity. The sintered spinel and hexagonal ferrite materials exhibit a dc resistivity equal to or greater than 10^9 Ω-cm, which effectively reduces losses due to dielectric and eddy currents at high frequencies.

3.2 Measurement of complex permeability

The ability of a magnetic core to concentrate lines of magnetic flux is known as its permeability. However, at high frequencies, magnetic materials such as ferrite can cause some magnetic loss and delayed induction of magnetic flux. The effective permeability of a core depends on its geometry and the type of materials used. Magnetic materials with high permeability provide high inductance values for a given core shape, size, and material, and a given core winding as compared to low permeability materials. This characteristic is important for achieving high inductance density in modern magnetic components. Therefore, high permeability materials are essential for constructing inductors. Permeability also describes how a material interacts with the magnetic field generated by high-frequency alternating current.

At high frequencies, the permeability is defined as complex relative permeability (μ_r^*).
where:

$$\mu_r^* = \mu_r' - \mu_r'' \quad (6)$$

$$tan\delta = \frac{\mu_r''}{\mu_r'} \quad (7)$$

The real component of the complex relative permeability, μ_r', is a measure of the permeability with magnetization in phase with the alternating magnetic field. This component describes the energy stored in the ferrite from the alternating-current magnetic field. On the other hand, the imaginary component of the complex relative permeability, μ_r'', represents magnetization that is out-of-phase with the alternating magnetic field, which leads to energy loss to the magnetic field. The ratio of the imaginary component (μ_r'') to the real component (μ_r') is known as the magnetic loss tangent ($tan\delta$) and is a measure of the inefficiency of the ferrite material. Permeabilities are typically plotted as a function of frequency, which is referred to as permeability dispersion or permeability spectrum. The use of high permeability materials is essential for modern magnetic components as they allow for higher inductance density for a given core shape, size, and material. One of the simplest permeability measurement techniques is *inductance method*, in which the toroidal core is coiled with a wire to form a single-turn inductor and the complex relative permeability is calculated from the inductance value [4].

Conclusion:

Ferrites are a type of ferrimagnetic ceramic material that have excellent magnetic properties and are utilized in numerous electronic devices. The initial step in characterizing ferrites involves phase confirmation, which can be done quickly and easily using techniques like X-ray diffraction and Raman spectroscopy. These methods utilize unique fingerprinting techniques to identify specific phases and impurities in synthesized ferrite materials. The intrinsic magnetic properties of ferrites, including saturation magnetization, remanent magnetization, and coercivity, can be determined from the M-H hysteresis loop. These properties

are dependent on the chemistry and crystal structure of the ferrites. The correlation of intrinsic magnetic properties with structural and spectroscopic properties leads to a fundamental understanding of magnetization and associated phenomena, such as domain theory. Additionally, the M-H loop is important in designing ferrite materials for various applications, such as hard and soft ferrite.

The crucial properties of ferrites are their complex permeability, saturation magnetization, and resistivity. Ferrites are superior to ferromagnetic metals in high frequency/microwave applications due to their high resistivity. The permeability of ferrites under the influence of high frequency alternating-current provides properties that surpass those of their counterparts, making them the preferred choice for microwave applications. Measuring the permeability not only assesses the material's performance but also helps in understanding its magnetization dynamics. In summary, characterizing ferrites is a crucial step in optimizing their magnetic properties, from both a scientific and technological perspective.

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References:

1. B. D. Cullity, “Elements of X-ray diffraction”, Reading, Messachusetts: Addison-Wesley Pub. Co. Inc., 1956.
2. J. Kreisel, G. Lucazeau, and H. Vencent, “Raman spectra and vibrational analysis of $BaFe_{12}O_{19}$ hexagonal ferrite”, J. Solid. State Chem., vol. 137, pp. 127-137, 1998.
3. Keithley Instruments Inc., “Volume and surface resistivity measurements of insulating materials using the model 6517A electrometer/high resistance meter”, Application Note Series no. 314 (no. 1680), 2001.
4. Agilent Technologies, “Theory on material measurement (Magnetic material)", Agilent E4991A RF Impedance/Material Analyzer (Operation Manual)”, pp. 428-432, 2006.

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