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ADVANCES IN SCIENCE AND TECHNOLOGY

VOLUME III

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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

CONTENT

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CARBON FOOTPRINT AND ITS IMPLICATION FOR BETTER FUTURE

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Introduction:

"The only way forward, if we are going to improve the quality of the environment, is to get everybody involved"

- *Richard Rogers*

Climate change is disrupting the weather patterns, leading to extreme weather events. Global warming and water pollution are the major environmental issues responsible for rapid climate change. Going zero waste is a great step towards combating climate change. About 57% of the Indian population does not have access to electricity. Therefore, the need of fossil fuel-based power plants is growing day by day. However, these plants provide energy through a network of inefficient grid services. Therefore, utilization of alternative renewable sources of energyhas become essential to reduce the carbon load of environment. There are numerous examples around the world that use alternative forms of energy successfully. India has a great potential for renewable energy like solar or wind energy. If only efforts are made to try to tap into these bountiful sources, the fossil fuel-based energy requirement of the nation can be met by these renewable resources. Application of renewable natural resources helps to reduce the environmental footprints *viz*. carbon and water footprint. Carbon footprint represents the amount of carbon emitted during the production of a product; likewise, a water footprint represents the amount of water needed to make a product. Awareness of carbon and water footprint among the citizens of a country can help to reduce the negative impact on climate change. Mitigation of carbon emission and conservation of water for sustainable environment can be achieved with the help of carbon and water footprint concept.

Taking into consideration the importance of the environmental footprint, there is a need to providethe knowledge and information regarding carbon footprint among the Indian population. This chapter compiles the definition, calculation and steps to reduce the carbon footprint that may possibly play a significant role in changing the mind-set, norms, habits or manners of the citizens to protect the environment.

Importance of Carbon Footprint concept

Carbon footprint is the total amount of greenhouse gas (GHG) produced by a person, organization, and event or during the manufacturing of any product. Due to rapid urbanization and industrialization, increasing concentration of GHGs at an alarming rate in fast growing countries like India is deteriorating the climate leading to global warming and large-scale shifts in weather conditions. Global warming is the most relevant problem faced by the world.

Production of GHGs from a specific area is affected directly or indirectly, by the ways in which the residents of that area utilize the natural resources as fuels used for food preparation, transportation and consumption of electricity etc. The economic status of the people and the consumption patterns of energy and fuel like electricity, petrol, diesel, LPG and wood are the major governing factor of carbon footprint load. Knowledge of carbon footprint of a particular activity and from all activities as a whole is important in order to take measures and take initiatives to reduce it to the lowest possible level. Raising the awareness of Carbon Footprint among people would trigger development of responsible behaviour to the environment. Thus, aiming at educating the citizens about the concept of carbon footprint to raise awareness and change their behavioural approach toward living style will be a key to achieve a low-emission pathway by reducing carbon footprint. By lowering carbon footprint, one can help contribute in the overall reduction of greenhouse gas emissions. Whether it is cleaner air, a healthier diet, or reduced energy bills, these benefits of reducing your carbon footprint also mean you are doing your bit to combat climate change.

Definition

Carbon footprint is the volume of carbon being emitted by an activity performed by an individual, community or an organisation. The total volume of carbon released in the atmosphere represents the total amount of greenhouse gases (carbon dioxide and methane) that are generated by our actions. In other words, a carbon footprint is the sum of all the global warming emissions brought about by an individual, society or an organization, and is expressed as carbon dioxide equivalent. It is also termed as accumulative greenhouse gas emissions. Carbon Footprint is one of the latest techniques that is widely applied for measuring the potential of GHG to affect the momentum of climate change. Many of our daily activities such as using electricity, driving a car, or disposing of waste - cause greenhouse gas emissions. Together these emissions make up a household's carbon footprint.

Causes

Carbon footprint represents the GHG load due to direct or indirect carbon emission. The main causes of carbon emission into the environment is either natural or anthropogenic. Natural sources include decomposition, ocean release and respiration. Anthropogenic are the human sources, which come from activities like cement production, deforestation as well as the burning of fossil fuels like coal, oil and natural gas by man. The largest source of greenhouse gas emissions from human activities is from burning fossil fuels for electricity, heat, and transportation.

Effects

If individual efforts are not exercised to reduce carbon pollution from burning coal, oil, and gas, it will result to a legacy of extreme weather, poor air quality and less water in our water bodies. Additionally, it would cause increased plant and animal extinctions and extreme costs to future generations. The carbon released and trapped in our atmosphere causes global warming, which rapidly melts of the polar ice caps, raise the sea levels, disturb animals' natural habitats and originates so many negative effects on weather events that are dangerous for life on earth.

Carbon footprint calculation

Day-to-day activities of humans are mostly dependent on electricity, which is generally coming from coal-based power plants, diesel and petrol for vehicles and LPG for cooking in kitchen. All of the energy, used is derived from these fossil fuels, which are GHG concentrated. The following stepscan be used to calculate carbon footprint resulting from the use of electricity, petrol, diesel, and LPG.

Step 1:Data collection

Electricity: find the monthly-consumed power unit from the annual electricity billsissued by State Electricity Board/ Distribution/Collection companies. Multiply them by 12 for calculating yearly-consumed power units. In India, one power unit of electricity is equal to 1KWh of electricity.

Petrol/Diesel: Add the volume of petrol/diesel (litres) used in car/motorcycle in a year. If the exact value is not known, approximate average values can be added.

LPG: Generally, one LPG cylinder has around 14 kg of liquefied petroleum gas. Multiply the number of cylinders used in a year by 14 and add the resulted value in the calculation.

Step 2:Calculation

a) Electricity : Input value (KWh/Y) x 0.85 (Emission Factor*) = Output value (CO_2, Kg)
b) Petrol : Input Value (Litres/Y) x 2.296(Emission Factor*) = Output value (CO_2, Kg)
c) Diesel : Input Value (Litres/Y) x 2.653 (Emission Factor*) = Output value (CO_2, Kg)
d) LPG : Input Value (Kg/Y) x 2.983 (Emission Factor*) = Output value (CO_2, Kg)

Carbon Footprint: Add (a+b+c+d) = Output value (Kg of CO_2).

The final Carbon footprint should be in tons of CO_2 (tCO_2). Therefore, to calculate the total carbon footprint in terms of ton of CO_2, divide final value (a+b+c+d) with 1000.

* Emission factor is most typically measured as mass per unit of consumption e.g. Tonnes per MWh. It is a co-efficient to describe the rate of release of greenhouse gases (GHGs) into the atmosphere by a given activity.

How to reduce carbon footprint

Below mentioned are some quick and easy to implement means of reducing carbon footprint so that one can start living an eco-friendlier life in no time at all:

- Insulate home
- Switch to renewables
- Buy energy efficient products
- Use less water
- Turn off the lights
- Consume local and seasonal products
- Limit meat consumption
- Select fish from sustainable fishing.
- Bring reusable shopping bags and avoid products with excessive plastic packaging.
- Make sure to buy only what is needed, to avoid waste

General guidelines to reduce carbon footprint in day-to-day life

Going green in everyday life is a great way to conserve energy and help stop global warming. Many things can be done to live green, as follows:

- Stop buying plastic. Get a reusable material and use it all times. This will save money and the environment.
- Combine walking or biking to regular short-trip destinations. In most cases, a person can walk a mile in less than 20 minutes. This is also a great way to add exercise to the modern busy schedule.
- Turn off lights and unplug devices when not in use.
- Keeping the tires on car properly inflated and getting regular tune-ups. When the car's tires are low on pressure, it has to work harder to move, wasting gas and increasing emissions in the process.
- Eat more food that is grown or made locally.
- Use the cold-water cycle for washing clothes and do laundry in full loads. This will decrease the amount of water and energy used along with helping save time and money.
- Set the thermostat to 78 in summer and 67 in winterand turn-off the heater and AC whennot in use. It will also make a big difference in energy bill.
- Drive efficiently. Use the accelerator lightly, coast to red lights, stay near the speed limit, and park and go inside instead of wasting engine in a drive-thru.
- Keep stuff out of the landfill. Sell the items, which are no longer in, use to saving shops, have a yard sale, or donate them to charity. Recycle or repurpose everything that cannot get rid of.
- Use alternative transportation (bus, train, carpool, or bike) to get to work one day per week.
- Start own compost: Composting helps to reduce greenhouse gas emissions that come from the decomposition of organic matter that takes place in landfills. Composting can be done at home simply by collecting all of the food scraps. Composting the organic waste is a great way to supplement or replace synthetic fertilizers.
- Buy locally obtained food: By supporting the produce grown closest to the consumer, the emissions it takes to transport the food to grocery store can be reduced. A fair amount of the greenhouse gas emissions involved in the production of food come from its transportation.
- Start own garden: By growing own food, the emissions that come from the transportation of goods to local markets and grocery stores can be eliminated.
- Only buy groceries that are needed: While going for grocery shopping, buy the items needed for the whole week in order to minimize waste. Try not to buying in bulk unless it is possible to eat everything before it goes bad. By failing to use all of the food that is bought, carbon footprint is unnecessarily increased.
- Donate excess food: Try best to buy what is needed only; one can still end up with excess. If the food is in excess to that can be finished, make sure to donate it to someone in need,

especially if it will go bad within the next few days. This helps reduce someone else's carbon footprint and simultaneously helps to prevent unnecessary increase in carbon footprint at another end.

- Buy ethically sourced honey: A bee will only produce about a teaspoon of honey in its lifetime. This means the mass-produced honey stored in most grocery stores is made by artificially fertilizing bees to produce more honey. These inhumane practices with bees are causing their species to rapidly die out. So, buy it from a local farm whose practices are ethically sound. Buying honey from small-scale beekeepers will reduce carbon footprint.
- Eat at sustainable restaurants: Nowadays, many restaurants pride themselves on being "farm to table". This means they get their food directly from local farms. This cuts down on carbon emissions from transportation and from irresponsible farm practices. So, choose to go to places that serve sustainably sourced food.
- Store the food properly so it stays fresher for longer: By getting the longest shelf life out of the food, carbon footprint from transportation to the grocery store can be decreased.
- Buy seasonal food: Certain produce requires a lot more time and energy to get to the local grocery store. To help cut down on these emissions from transportation, consider shopping for seasonal food.
- Use a reusable water bottle: To reduce the plastic usage, buy a reusable water bottle that can be usedevery day. Plastic production leads to many carbon emissions so by using reusable bottles, the water and carbon footprint can be reduced.
- Use less hot water: Using cold water to wash dishes, the energy it takes to heat the water can be saved. By using hot water wisely, the leftover water from the kettle can be taken to wash the dishes.
- Recycle at home: Recycling and composting are two major ways to reduce the carbon footprint by recycling the wastes and putting the food scraps in a personal compost pile, environmental impact at home can be reduced instantly.
- Replace old light bulbs: Changing out any old or dimming light bulbs is an easy way to save energy in home. Old light bulbs and incandescent light bulbs take up excess energy, so replacing them with LED lights can conserve thousands of watts of energy each year.
- Insulate the home: Insulating the home will help control the indoor climate to keep home warm in the winter and cool in the summer without light up the energy bills. More energy and money can be saved in this manner from season to season.
- Unplug unused appliances: Without even realizing, the machines that are kept plugged-inlike refrigerator, washing machine etc. can drive up energy bill. Refrigerator, for example may be using excess energy to run its digital clock, so make sure to unplug or turn off any unused appliances while not in use.
- Monitor the temperature inside fridge and freezer: Make sure the fridge is between 35-38^{o}F to ensure it is not too cold or using too much energy. Also, check the fridge seal to make sure no cold air is escaping.

- Do not put hot objects immediately into the fridge or freezer: If leftovers are kept cool before putting them in the fridge, less energy is required from the fridge to keep them cold. Fridge is one of the biggest consumers of energy in home, so any added efficiency helps.
- Clean out the fridge more often: the energy a fridge uses can be decreased by cleaning out any food that are not going to be consumed. When unused food is in the fridge, it takes up space and energy. Clean out the fridge weekly, but do not leave it too empty, otherwise, it will not maintain its cold temperature.
- Install solar panels: Going solar is a great way to reduce the carbon footprint. By installing solar panels, 100% of the electricity usage can be offset, depending on how many panels are installed and how many kW use each month by the consumer.
- Check car tire pressure routinely: Flat or quickly flattening tires can really hurt the gas mileage. With new tires, kilometers covered per litre of fuel can be maximized along with reduced CO_2 release into the atmosphere.
- Carpool: One of the best ways to reduce the carbon footprint is by carpooling with others. While going outside together, try to use one vehicle to get there. Depending on how often carpooling is done and the distance is travelled, money spent on travelling and gas released, both can be decreased.
- Use public transportation: in areas where public transportation is easily accessible, use it. By using public transportation, one can drastically increase the energy savings and reduce the number of cars on the road.
- Drive Smart: Make the decision to drive on highways and higher speed roads so that starting and stopping of the vehicle can be minimized. Much better gas mileage can be achieved by moving at a constant speed and in an efficient gear.
- Travel by car or train instead of flying: travelling by car or train instead of flying on a plane should be opted for going to trips. By doing this, tons of non-renewable energy sources can be prevented from being released directly into the atmosphere during air travel.
- Recycle the old clothes: donate the old clothes to a charity or even brands that have a good return policy. H&M, for example, launched a garment-collecting program back in 2013 that lets drop off and recycle garments in any condition to any H&M store in the world.
- Reduce the carbon footprint in the community by planting a tree: This is a much larger task, but plating trees in the local parks by the community as a whole, one can make a huge impact.

5Rs of zero waste to reduce carbon footprint

Bea Johnson (2013) gave the world the Five-R (5Rs) of reduction of carbon footprint in her book: *Zero Waste Home-The Ultimate Guide to Simplifying Your Life by Reducing Your Waste*. They are, **R**efuse, **R**educe, **R**euse, **R**ecycle, and **R**ot. These 5Rs give us a new framework for dealing with waste in our lives, in part by helping us acknowledge the habits that lead to more waste and more trash. They are as follow:

Refuse: Refusing helps eliminate a lot of waste from the start. It is about saying “no” to free stuff that becomes instant waste. It takes a bit of practice and preparation to find and actively join

reusable alternatives into daily life. Some things that can be refused on a daily basis are single-use plastics like disposable coffee cups, utensils, and straws. Even bioplastic straws, which are theoretically compostable, often end up in the trash because municipal composting facilities cannot identify them as bioplastic. All are conditioned to say yes and accept swag bags, free coupons, magazines, leaflets, and anything else because it is free. Accept the things needed, and refuse the rest.

Reduce: Simply reduce what you are purchasing by being aware about what is needed. This goes hand-in-hand with refusing. Be realistic about what is actually needed. Before making purchases, make sure it is really needed. While price is a huge factor, try to find the best quality in budget. Well-made products will last longer, reducing the times needed to repurchase.

Another tip is to take care of possessions by following cleaning instructions and labels so everything lasts a long time. Personal care products that can be refilled or bought "pumpless" also help reduce packaging waste and often come with perks like free shipping and less-expensive product-per-ounce.

Reuse: Reusing and repairing go hand in hand. When an individual is deciding whether to toss something out and buy a new one, try to or find a way to reuse or repair it. This applies to clothing, furniture, and technology. If phone or laptop is broken, instead of immediately purchasing a new one, seek repair options first. Reusing also means selling or donating used items so they go to loving homes instead of the landfill. Have a yard sale, or ask friends and family if they have a need for things. One can also reuse by buying second hand. Shop at saving and antique stores and go to yard sales. One can save a lot of money and reuse something someone else did not want. Do not forget a library card, which is a great way to reuse books, music, and movies.

Recycle: One of the universal ways to reduce waste is to recycle, but recycling is far from perfect. The world went into a fall when China stopped accepting recycled waste in 2018 and recycling is not available or in active use everywhere on planet earth. This does not mean that you should not recycle—definitely recycle. But we need to look at strategies like avoiding plastic packaging (driving down demand for plastics) and making use of materials that can be composted (driving up demand for alternative packaging). When recycle, ensure that sorting and cleaning you are recycling according to local regulations.

Rot: "Rot "fun to say, fun to do. One might be thinking, "There is no way I have time to compost" and that might be true for an individual. If it is, that is fair and that is life. There are many different ways to compost now that make it a bit easier than it used to be. Vermiculture composting is a great option if have limited outdoor space. There are also composting pick-up services, and drop-off spots in many cities and towns. Collect food scraps in fridge or freezer so they do not smell, and then make drop weekly.

Conclusion:

The choices made in ordinary everyday life about home exercises, transport, food and the products purchased have impacts right across the world. The amount of goods created or consumed by a single person affects environmental change directly and it can be reflected at

global level. Overall, each of the individual footprints altogether swings the world's natural resources. Dangerous atmospheric deviations due to alarmingly increasing emission of carbon and water pollution are the major natural issues liable for quick environmental change. Going zero waste is an incredible advance towards battling environmental change. Thinking about the significance of the environmental changes, there is a need to provide the information and data with respect to carbon and water footprint among the Indian population. This chapter assembles the definition, computation and steps to lessen the carbon that may potentially assume a huge part in changing the mentality, standards, propensities or habits of the residents to safeguard the future climate.

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A PERSPECTIVE REVIEW PROBE ON FORMULATION OF NUTRI CHOCOLATE USING PUFFED LOTUS SEEDS

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Abstract:

Lotus is a member of a small family of *Nelumbonaceae* and is regarded as a sacred flower grown as a freshwater aquatic plant. All plant parts are utilized as food and medicine. Lotus seed (LS) is an important part of the lotus plant due to its nutritional and bioactive components. *Nelumbinis semen* is commonly known as lotus seeds that have been used as a vegetable, functional food, and medicine for 7,000 years. These are low caloric, a rich source of multiple nutrients and bioactive constituents, which make it a unique therapeutic food. *N. semen* plays an important part in the physiological functions of the body. Nowadays, people are more conscious about their health and desire to treat disease naturally with minimal side effects. So, functional foods are getting popularity due to a wide range of essential constituents, which are associated to decrease the risk of chronic diseases. Nutritional composition is considered crucial for evaluating new ingredients for food applications. The overall profile of the lotus seed makes them potential nutraceutical and bioactive food ingredients. These bioactive compounds from seeds are involved in anti-adipogenic, antioxidant, antitumor, cardiovascular, hepato-protective, anti-inflammatory, anti-fertility, anti- microbial, anti-viral, hypoglycemic, etc. Moreover, the relationship between functional compounds along with their mechanism of action in the body, their extraction from the seeds for further research would be of great interest. The article aimed to comprehensively collate some of the vital information published on lotus seeds incurred in the agri-food sector and critically discussing the potentiality of tapping bioactive compounds from lotus seeds considered as wastes and/or by-products in the entire agriculture-based food sector.

Keywords: bioactive compounds, extraction techniques, functional foods, lotus seeds, polyphenols, therapeutic potential

Introduction:

As well as being used in India and other parts of the world, lotus seeds are sometimes referred to as fox nuts, makhana, or water lily seeds. They are frequently used to make popped lotus seeds, also known as phool makhana, which have gained popularity outside of their country of origin, India. Popped lotus seeds are an Ayurvedic superfood that has been consumed for millennia in India and are now gaining popularity elsewhere. Studies have revealed that people have been gathering and eating water lily seeds for hundreds of thousands of years! Evidence implies that people in the Yangtze region of China consumed them all through the Neolithic era. The first known use of prickly water lily seeds dates back 750,000 years and was discovered in Israel when artifacts from the prehistoric Acheulean culture were found there!

The Indian lotus flower, also known as the prickly water lily, produces lotus seeds. They are a significant crop in northeast India, particularly in the Bihar region, which supplies 90% of the world's lotus seeds (our Plant Pops Popped Lotus Seeds are sourced directly from farmers in Bihar). The lotus flower *Nelumbo Nucifera*, which is harvested in China and frequently used in Chinese and Japanese cuisine, should not be confused with the lotus seeds we use because it is an entirely different plant. The seeds of the water lily plant are manually collected from the plants, which are found in tiny ponds, lakes, and rivers. Unlike other ingredients such as corn or soy, water lily plants don't need additional water to thrive, making them more sustainable to produce and harvest. A quick snack to have while watching a movie is lotus seed.

Kamal seed or lotus nut are other names for lotus seed. *Nelumbinis semen*, the scientific name for lotus seeds, is derived from the lotus plant, *Nelumba nucifera*. It has been used as a vegetable, medicinal, and functional food for nearly 7000 years. Dried lotus seeds come in two varieties: one has a white peel and the other has a brown peel. In India, Japan, and China, large-scale production and cultivation of lotus seeds has taken place. The People's Republic of China's Ministry of Health has authorized lotus food as both food and medicine. Lotus seeds are rich in nutrients and have fewer calories than other seeds, thus they are vital to the body's processes. Let's examine a few of lotus seeds' health advantages.

Nutritional composition of lotus seeds:

Proximate analysis

Because they contain a healthy balance of proteins, lipids, carbs, and minerals, lotus seeds are regarded as being very nutrient-dense. Table 1 lists the lotus seeds' nutritional profile per 100 g dry weight of seeds. It has been noted that different lotus types and growing conditions have distinct nutritional values. Nevertheless, the physiological traits and general makeup observed throughout investigations are remarkably similar (Shahzad *et al.,* 2020). Additionally abundant in vitamins, zinc, iron, calcium, and phosphorus are the seeds of the lotus.

Proteins

Between 16 and 28% of the protein in lotus seeds is protein that is soluble in water (albumin), salt (globulins), alkali (glutelin), and alcohol (prolamins), according to four solubility classifications (Pan *et al.,* 1993; Shin *et al.,* 1999; Wu *et al.,* 2007; Bhat and Sridhar, 2008; Mukherjee *et al.,* 2009; Luo et al) Table 1 lists the various protein fractions found in lotus seeds along with their varying degrees of solubility. By adding 0.1 to 0.5 M/L of NaCl to the lotus

seed, which is an effective protein solubilizer, the extraction of proteins can be improved. NaCl will aid in the extraction of both salt- and water-soluble proteins.

Conversely, the extraction procedure for the protein fractions that are acid-soluble, alkali-soluble, and prolamine uses 0.1 M HCl, 0.1 M NaOH, and 70% aqueous alcohol, respectively (Zeng *et al.,* 2013, Luo *et al.,* 2016, Su *et al.,* 2021). Albumin is the predominant protein in lotus seeds, accounting for 41.6% of the total amount, followed by globulin (26.6%), glutelin (18.0%), and prolamin (6.0%), respectively (Zeng *et al.,* 2013, Luo *et al.,* 2016). Compared to albumin and globulin, which have the lowest E/T ratios at 35.87% and 36.72%, respectively, prolamine has the largest proportion of essential amino acids to total amino acids in proteins (40.56%) (Zeng *et al.,* 2013, Vinayashree & Vasu, 2021).

Carbohydrates

Starch, oligosaccharides, and other polysaccharides make up the carbohydrates in lotus seeds (Tian *et al.,* 2012). Previous studies have used medium-pressure liquid chromatography when combined with ELSD and a diode array detector (DAD) to separate the oligosaccharides of lotus seeds. These studies reported the presence of five oligosaccharides in lotus seeds, with corresponding weights of 1.107, 0.554, 0.183, 0.443, and 0.243 g/100 g of lotus seeds (Lu *et al.,* 2017). Additionally, hot water extraction obtained 0.95% of the water-soluble polysaccharides from dried lotus seed, but ultrasound-assisted extraction obtained 1.51% (Tian *et al.,* 2012; Zheng *et al.,* 2016).

Vitamins and minerals

B1, B2, B6, C, and E are among the vitamins that are thought to be abundant in lotus seeds (Table 1). According to Zheng *et al.* (2003), Ling *et al.* (2005), Wu *et al.* (2007), Mukherjee *et al.* (2009), all of these vitamins have positive physiological effects, antioxidant activity, and immune-stimulating qualities. According to earlier studies (Guo, 2009; Mukherjee *et al.,* 2009), the lotus seed rhizome includes vitamins (B1, B2, B3, B6, and C). A vast number of minerals, including calcium (Ca), potassium (K), chromium (Cr), sodium (Na), copper (Cu), magnesium (Mg), iron (Fe), zinc (Zn), and manganese (Mn), are also present in significant quantities in lotus seeds.

Nutritional value of lotus seed:

Numerous bioactive substances, including alkaloids, glycosides, triterpenoids, flavonoids, polyphenols, polysaccharides, essential oils, and many more, may be present in lotus seeds.

Table 1: Nutritional value of lotus seed

Nutrients	**Values/100g**
Protein (g)	4.93
Total lipid (fat) (g)	0.63
Carbohydrate, by difference (g)	20.63
Energy (kcal)	106.24
Calcium, Ca (mg)	52.16

Iron, Fe (mg)	1.13
Magnesium, Mg (mg)	67.2
Phosphorus, P (mg)	200.32
Potassium, K (mg)	437.76
Sodium, Na (mg)	1.6
Zinc, Zn (mg)	0.34
Copper, Cu (mg)	0.11
Manganese, Mn (mg)	0.74
Vitamin A, IU (IU)	16
Thiamin (mg)	0.2
Riboflavin (mg)	0.05
Niacin (mg)	0.51
Pantothenic acid (mg)	0.27
Vitamin B-6 (mg)	0.2
Folate, total (mcg)	33.28
Folate, DFE (mcg_DFE)	33.28
Fatty acids, total saturated (g)	0.11
Fatty acids, total monounsaturated (g)	0.12
Fatty acids, total polyunsaturated (g)	0.37

Lotus seed properties:

According to several research, lotus seed may have the following properties:

- Lotus seeds may have antioxidant properties.
- It may provide pain relief.
- It may improve heart health.
- It may benefit liver health.
- It may strengthen the immune system.
- It may help with memory.
- It has the potential to lower blood sugar levels.

Lotus seed's potential health benefits

Some of the potential applications for lotus seed include:

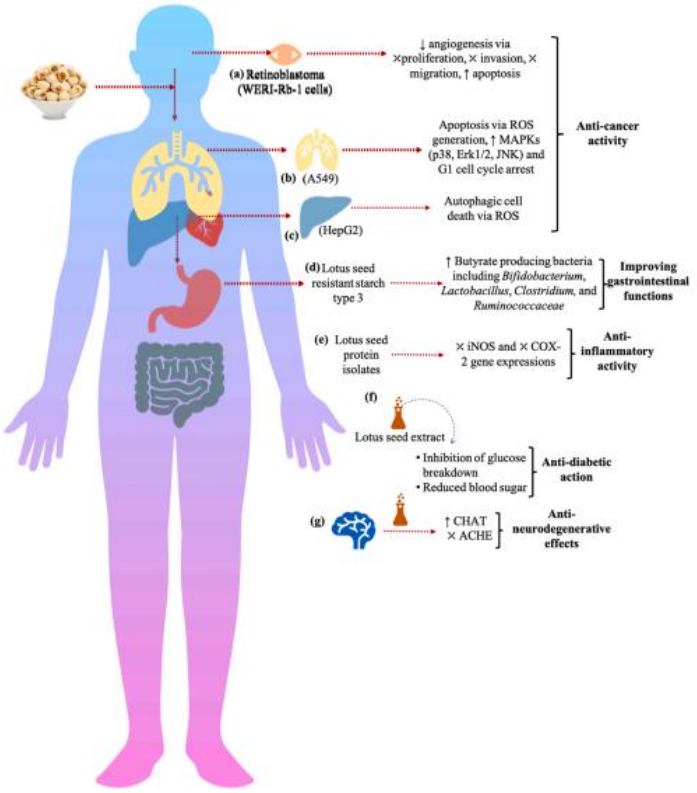

Fig. 1. A schematic diagram summarizing various biological activities of lotus seed (S. Punia Bangar *et al.*, 2022)

Management of weight

Adipocytes (fat cells) are responsible for excess weight in the body. According to a 2011 study by Achike *et al.,* lotus seeds may block the production of fat cells and lower the weight of fat tissues. Furthermore, polyphenols present in lotus seeds may enhance the body's lipid profile. However, further research is needed to determine whether lotus seeds can aid with weight management. As a result, before making any dietary adjustments, you should contact with your dietitian.

Alzheimer's disease

Alzheimer's disease is a neurodegenerative disorder which results in memory loss and other mental impairments. Lotus seeds may have neuro-protective effects i.e, it may protect the nerve cells from damage as in the case of Alzheimer's disease. A study by Kim *et al.* in 2014 showed that pro-anthocyanidins in lotus seeds may reduce brain ageing and cognitive impairment. In addition, lotus seeds may reduce the risk of Alzheimer's disease by decreasing the harmful free radicals and accumulation of unnecessary calcium. However, further studies are required to check if lotus seeds can be beneficial in improving the symptoms of Alzheimer's disease. You must consult your doctor if you suspect any symptoms of Alzheimer's disease.

Depression

A study by Sugimoto *et al.* in 2008 showed that bioactive compounds like saponins, flavonoids, alkaloids and tannins may help to reduce anxiety and depression. The alkaloids in lotus seeds may increase the time of sleep. Getting good sleep may help manage depression. However, these studies were conducted on mice.[1] Further studies on humans are required to check if lotus seeds may be beneficial for anxiety and depression.

Type I and II diabetes

A study by Mani *et al.* in 2010 showed that lotus seed might be used in the case of type 1 and type 2 diabetes. Various minerals present in lotus seeds may be beneficial for diabetes. For example, zinc in lotus seeds reduces oxidative stress in type 1 diabetes patients and transports glucose to the cells in type 2 diabetes patients. Chromium may increase the number of insulin receptors and glucose metabolism, thereby decreasing blood glucose levels in type 2 diabetes patients. However, further studies are required to check if lotus seeds can help to reduce blood glucose levels. You must check your blood sugar levels regularly and consult your doctor in case of abnormal blood sugar levels.

Cancer

A study by Poornima *et al.* in 2013 showed that lotus seeds might act against lung cancer. A bioactive compound, nepherine in lotus seed, may kill the cancer-causing cell (apoptosis) and inhibit their growth. However, further studies are required to check if lotus seeds may act against cancer. Therefore, you must consult your doctor if you suspect cancer instead of self-medicating.

Relieve pain

A study by Chakravarthi *et al.* in 2009 showed that the flavonoids in the lotus seeds might help to relieve pain. Lotus seeds may inhibit the cyclooxygenase enzyme, which is responsible for releasing pain mediators. However, further studies are required to check if lotus seeds can help relieve pain. Therefore, you must consult your doctor if you experience prolonged pain.

Antioxidant

A study by Rai *et al.* in 2006 showed that flavonoids in lotus seeds might be responsible for their antioxidant potential. The flavonoids may destabilise the harmful free radicals (molecules in the body) and reduce oxidative stress by removing the free radicals from the body.

This would reduce the risk of several diseases like diabetes, heart disease, liver disease, etc. However, more studies are required to check the antioxidant potential of lotus seeds.

Interactions with Other Drugs:

Lotus seeds may interact with diabetic medicines like insulin. You must consult your doctor before you consume lotus seeds if you are on diabetic medicines.

Chocolate

Food is required to give us energy, and chocolate is relatively energy dense, meaning it is high in calories for a small portion size. Because of this, it has often been included in the food supplies for polar explorers and lifeboat rations etc. It also contains the three essential components of food, i.e. protein, carbohydrate and fat (although not in ideal proportions), together with some vitamins and several minerals. Eating a limited amount (up to a standard bar per day) of chocolate has been shown not to have a significant effect on migraines, acne or tooth decay. On the other hand, cocoa has been found to contain compounds that have positive effects in the prevention of heart disease and possibly some cancers, whilst the possible psychological effects have had a lot of media attention.

Chocolate is a semi-solid suspension of fine solid particles of sugar, cocoa, and milk powder (depending on the type), making about 70% total, in a continuous fat phase, consisting mostly of cocoa butter (Afoakwa, 2014). Chocolate shelf-life is limited by the changes in the polymorphic state of cocoa butter or by rancidity development in solid chocolate. The resistance of chocolate to fat bloom is closely related to the crystallization of cocoa butter (Fernandes *et al.,* 2013). At least six crystal forms (I-IV) of cocoa butter can be distinguished, however, form V is most desirable. This form can be achieved by performing proper tempering procedures (Fernandes *et al.,* 2013; Quast *et al.,* 2013).

Conclusion:

Lotus is widely cultivated in the Asian continent (mainly China) as one of the important economically important crops for its rhizome and seeds. In the last decade, seeds from lotus seeds have emerged as one of the important sources of major nutrients and phytochemical compounds. It has been highlighted in the review that the superior quality of lotus seed proteins (or its hydrolysate), polysaccharides (starch), and lipids can be a useful candidate for the preparation of functional foods. The presence of numerous secondary metabolites (phenolics, terpenoids, and alkaloids) imparted a spectrum of healthful properties to the lotus seed. *In vitro* and *in vivo* findings from the researchers suggests that lotus seed extracts have important anti-cancer, anti-proliferation, anti-diabetic, anti-inflammatory, neuroprotective, antioxidant, and immune modulatory activities. Further, considering the nutraceutical properties, lotus seed flour could prepare innovative food products. The interdisciplinary research approach has established lotus seed as one of the important nutraceutical and pharmaceutical ingredients. However, further investigations should be aimed at the safety and efficacy of lotus seeds for application in the food and pharma industry. A more detailed study is needed to decipher the molecular mechanism behind each biological activity. Further, the bio-accessibility and bioavailability of lotus seed compounds in the human body need to be investigated. Furthermore, clinical trials of beneficial

components of lotus seed will also improve the applicability of the lotus seed in the pharmaceutical and food industries.

Storage stability:

The shelf-life of composite chocolate products may be limited, especially if they are subjected to unfavorable storage temperatures (Couzens & Wille, 1997). Keeping storage temperatures below the melting point of confectionery fat is not sufficient to prevent bloom formation because of the narrow melting range of confectionery fats and the significant impact of slight changes in temperature on solid content. Keeping chocolate products under 18°C is, generally, recommended to minimize bloom formation (Widlak & Hartel, 2012). Storage at low temperature (T < 18°C) generally minimizes bloom formation. Storage in the 18–30°C temperature range is below the melting point of Beta V crystals of cocoa butter and bloom occurs more quickly with an increase in temperature. When the temperature reaches 32°C, the cocoa butter is partially melted. Upon subsequent cooling, the cocoa butter crystallizes uncontrollably into unstable polymorphic forms, causing bloom to occur very quickly (Ali *et al.,* 2001; Wooton *et al.,* 1970). Chocolate shelf-life may be prolonged if chocolate is seeded with a large amount of stable crystals in form V. It can be expected that the amount of these crystals may be increased by keeping the chocolate below the melting point of the stable cocoa butter crystals (retemperation). These temperature conditions made the removal of latent heat and the creation of a crystal network of the V form possible. Hence the aim of the study was to evaluate the impact of keeping chocolate bars immediately after production for 24 hr at 24°C (retemperation) on the changes occurring during a 6-month storage period. The effect of different storage conditions (6°C, 12°C, 20°C, and 30°C) on texture, color, and sensory characteristics was also evaluated. Dark and milk chocolate bars with different fillings were involved in this study to compare the impact of selected factors on different products.

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OHMIC HEATING: CONCEPT, DESIGN AND DEVELOPMENT

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Abstract:

A recent interest in rapid, environmental friendly, simple and energy efficient methods of food processing has resulted in considerable attention towards ohmic heating as novel thermal technology. The available literature on different aspects of ohmic heating of foods like principle and concept, design and development of ohmic heating systems, electrical conductivities of foods, ohmic heating behavior, various food applications of ohmic heating etc are presented under various sub headings. The rate of heat generation mainly depends on two factors in ohmic heating one is electric field strength and other is electrical conductivity. This modern food processing method has wide range of applications in food processing and various researchers had used it for sterilization, pasteurization, extraction, blanching, dehydration, evaporation, juice extraction, peeling, thawing, fermentation and rice bran stabilization**.** Its advantages over conventional heating include volumetric, faster and uniform heating, higher energy efficiency, technically simple.

Introduction:

Ohmic heating is a novel thermal food processing technology now a day's getting popularize as "green technology". It is known by various names like Joule heating, electro conductive heating, electro heating and electrical resistance heating (Pereira and Vicente, 2010). The basic principle of this method is very simple and based on the Ohm's law of electricity. The passage of alternating electric current through electricity conductive food material such as a liquid, liquid-particulates or pumpable foods obeys Ohm's law and heat is generated due to electrical resistance of the material (Patel and Singh, 2018; Sawant *et al.,* 2018). The food materials which contain sufficient amount of water and electrolytes (mineral salts) can act as electric charge carriers and allows electric current to pass through them resulting generation of internal heat due to electrical resistance of food (Joshi, 2018). In conventional methods of heating, heat transfer occurs from a heated surface to the food material by means of convection and conduction, while in case of ohmic heating it occurs volumetrically from inside the food. The rate of heat generation mainly depends on two factors in ohmic heating one is electric field strength and other is electrical conductivity. It avoids thermal damage to nutritional components of food material, such as vitamins and pigments, (Sastry and Barach, 2000; Loypimai *et al.,* 2015) and prevents overheating. This modern food processing method has wide range of applications in food processing and various researchers had used it for sterilization, pasteurization, extraction, blanching, dehydration, evaporation, juice extraction, peeling, thawing, fermentation and rice bran stabilization**.** Its advantages over conventional heating include volumetric, faster and uniform heating, higher energy efficiency, technically simple, low

capital cost and maintenance. (Stancl and Zitny, 2010)**.** reviewed ohmic heating and mentioned that the first application of ohmic heating in food processing was in 1919 for pasteurization of. Foods can be subjected to various pretreatments including blanching, salting, thermal, enzymatic or chemical pretreatments prior to ohmic heating. Several advantages of this method have been reported by various researchers such as uniform and rapid heating; continuous process; higher sterilization and pasteurization at comparatively lower temperatures; good product quality and processing of foods with high solid fractions. Ohmic heating possesses many other advantages when compared with conventional methods of heating for example, direct contact with heat transfer surfaces is not involved in it as in case of conventional pasteurization and sterilization which can lead to prevent the problem of surface fouling and nutritive loss of food product. The food particulates are handled gently without any mechanical equipment or phenomenon. It is simple and easy to monitor the processing time. It direct method of processing the food and hence provides rapid cooking, less clumping, and energy efficient.

The fundamentals of ohmic heating

The principles of ohmic heating are very simple as illustrated in Fig.1 & Fig. 2. Ohmic heating is based on the passage of alternating electrical current (AC) through a body such as a liquid-particulate food system which serves as an electrical resistance in which heat is generated (Sawant *et al.,* 2018)**.** AC voltage is applied to the electrodes at both ends of the product body. The rate of heating is directly proportional to the square of the electric field strength and the electrical conductivity. The electric field strength can be varied by adjusting the electrode gap or the applied voltage. However, the most important factor is the electrical conductivity of the product and its temperature dependence (Kumari *et al.,* 2016)**.** If the product has more than one phase such as in the case of a mixture of liquid and particulates, the electrical conductivity of all the phases has to be considered. The electrical conductivity increases with rising temperature, suggesting that ohmic heating becomes more effective as temperature increases, which could theoretically result in runaway heating. A difference in the electrical resistance and its temperature dependence between the two phases can make the heating characteristics of the system very complicated. Since electrical conductivity is influenced by ionic content, it is possible to adjust the electrical conductivity of the product (both phases) with ion (e.g. salts) levels to achieve effective ohmic heating. A mild electroporation mechanism may occur during ohmic heating operating at low frequency (50–60 Hz) which allows electrical charges to build up and form pores across cell walls (Ayoub *et al.* 2017). The equations which describe conventional and electrical heating are different since the processes which govern heating are different [3]. The heat generated by the passage of current at any point in the system is given by

$$Q = \sigma E^2 \quad \ldots (1)$$

which is equivalent to the more familiar I^2R. Here σ is the local electrical conductivity (S/m) and E is the electric field strength (V/m). The voltage distribution is given by

$$\nabla(\sigma \nabla E) = 0 \quad \ldots (2)$$

and thus depends on the distribution of electrical conductivity within the medium as well as the system geometry. Equation (2) differs from the usual form of Laplace's equation:

$$\nabla^2 E=0 \quad \ldots (3)$$

because it deals with a medium in which the electrical conductivity is a function of both position and temperature. The most important parameter in the applicability of ohmic heating is the electrical conductivity of the material. Most food stuffs, which contain water in excess of 30% and dissolved ionic salts have been found to conduct sufficiently well for ohmic heating to be applied. Non-ionized materials such as fats, oils, sugar and syrups are not suitable; heir conductivity is too low.

Design and development of ohmic heating apparatus

From review of literature, it was observed that number of possible combination and configurations to design the ohmic heating system have been reported (Pare *et al.,* 2009). However, before going for the actual design of any kind of ohmic heating system, several factors and key elements need to be taken in to consideration The important design variables considered to attention in developing an ohmic heating system include a power source, electrode material and dimension, current density, applied voltage, heating chamber, wiring, personnel and equipment safety. The arrangement of these design variables is also important for proper functioning of ohmic heating system. The electrodes made from food grade materials must be in direct contact with the food material to be heated. The distance between two electrodes can vary depending on the size of the system, but by increasing or decreasing this gap, the applied voltage gradient (V/m) may change accordingly. The thermocouple, to measure the temperature during ohmic heating, should be placed at central positions. The heating chamber should be made up with food grade and chemically inert, electrically nonconductive material, it should withstand higher temperature and it should be of light weight. In addition to the variables related to ohmic heating system, the food material to be heated, its moisture content and properties especially electrical conductivity, specific heat and heating rate are also to be taken into consideration for the development of an ohmic heater (Lima, 2007).

A T-shaped cylindrical geometry was adopted for construction of ohmic heating chamber in which black cumin seed slurry was heated at desired temperature. The ohmic heating chamber, 8 cm in diameter, 18 cm in length and 5 mm in thickness was made of borrosil glass material for a capacity of 600 g (100 g powder: 500 ml water) black cumin seed slurry. On the basis of volume and density of the slurry, the dimensions and capacity of the heating chamber were finalized made with 1:5 slurry ratio (Pereira and Vicente, 2016). A circular geometry avoids the leakage of slurry from the heating chamber due to the absence of any joint around it. The glass state material is nontoxic, corrosion resistant, non-adhesive and has good insulation property. The inner and outer surfaces of heating chamber were coated with teflon liquid by using a cotton cloth so that it can withstand higher temperatures (up to 100 ºC for 20 min holding time).

Electrodes in ohmic heating play an important role by conveying the current uniformly into the heating medium. In this study, the stainless steel (SS) electrode was used as it has been reported to be the most electrochemically active material during ohmic heating at all the pH values (Stancl and Zitny, 2010). Electrodes were connected to the power supply through a control panel. The diameter and thickness of disc shape electrodes were 50 mm and 3 mm

respectively. The internally threaded holes (4 mm diameter) were provided for compression fittings at the centre of both the electrodes. Both the electrodes were fitted with insulator caps to make the system leak proof.

To make the ohmic heating apparatus electrically safe and to avoid accidents during experimentation, three insulator caps, made of wood were fixed at all the three ends of T-shaped ohmic heating chamber. Two insulator caps were permanently fixed by internal threads for tight fitting around the electrodes to cover them at both horizontal ends of the chamber. However, a cap was kept removable at vertical end of chamber to fill slurry for ohmic heating treatment and was removed it after the treatment. The control panel, with temperature controller (Make: Multispan, Model No.: DTC 1901, Range: 0-800 ºC) with cut-off system, ammeter (Make: Multispan, Range: 0-60A), voltmeter (Make: Multispan, Range: 0-220V), AC variable and three digital displays to measure and control the ohmic heating variables like temperature, time and voltage gradient were fixed on 25 cm long, 14 cm wide and 16 cm tall wooden body. The ohmic heating chamber was fixed at the center of control panel by using a 16 cm tall wooden stand. The stand was fixed with an arrangement of loosening and removing the chamber by simply opening the screw for cleaning and washing of chamber after each experiment. The entire ohmic heating apparatus with its control panel was placed and fixed on a 40 cm long, 50 cm wide, 30 cm tall and 5 cm thick wooden base platform.

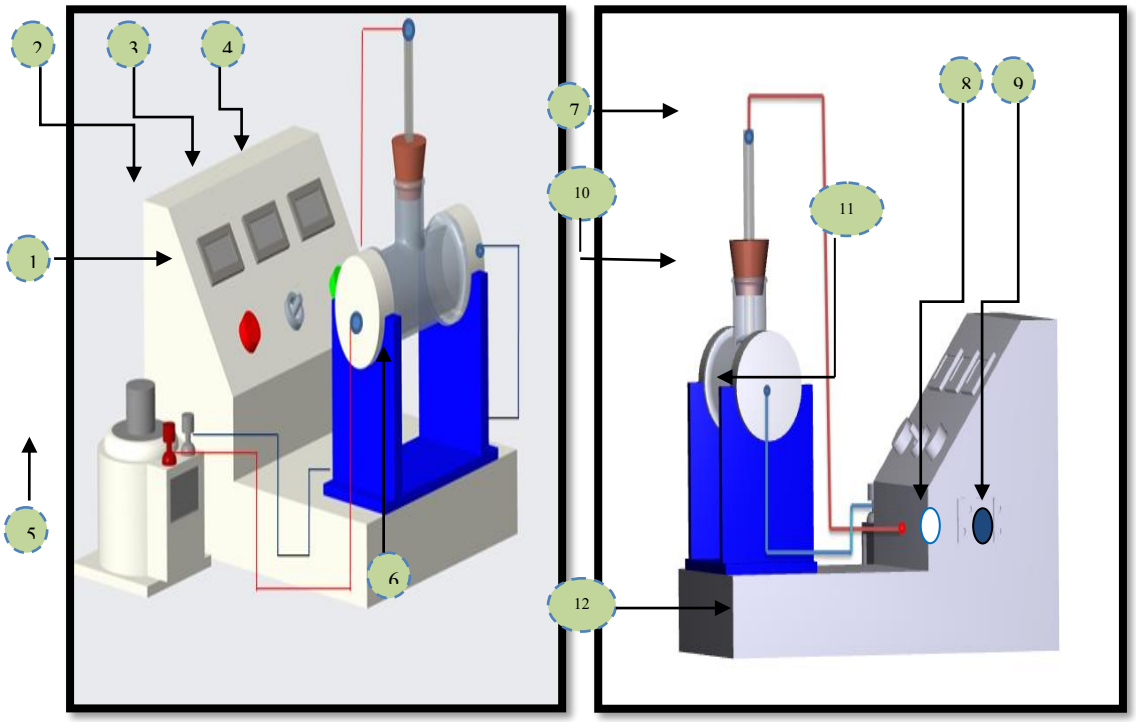

Fig. 1: Schematic diagram of laboratory ohmic heating apparatus

(1-Start button, 2-Ammeter, 3- Voltmeter, 4- Temperature display, 5-Dimmerstat, 6- Wooden Stand, 7-Thermocouple, 8- Main switch, 9- Dimmerstat connection, 10- Rubber cap, 11- Electrodes, 12- Base)

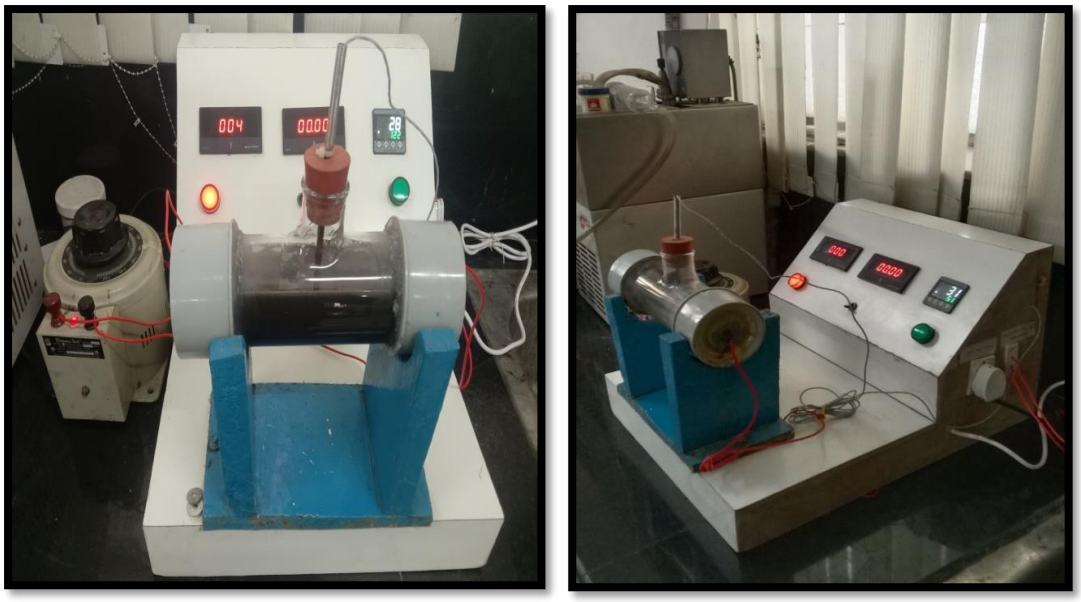

Fig. 2: Developed laboratory ohmic heating apparatus

Table 1: Different components of ohmic heating system and their function

Sr. No	Name of component	Feature	Material of construction	Dimension	Functions
1	Ohmic heating chamber	T-shape Geometry	Glass with Teflon and anabond 666 paste coated to make it thermally stable	Length= 18 cm Diameter = 8 cm Thickness = 0.5 cm	Hold and heat slurry during experiment
2	Electrode	Disc shape	Stainless steel (SS-316)	Diameter = 5 cm Thickness = 0.3 cm	Transfers electric current from power source to the product to be heated
3	Insulator cap	Circular/disc shape with internal thread for tight fitting	Wood	Diameter = 7.5 cm Thickness = 0.03 cm	Makes ohmic heating apparatus electrically safe and prevents heat loss and leakage from the apparatus,

4	Control panel	Power supply to electrode and monitoring and controlling ohmic heating variables	Wooden box	Length = 40 cm Width = 15 cm Height = 21 cm	Supply power to the electrodes and monitor and control ohmic heating variables (temperature, voltage and time)
5	Base	Entire ohmic heating setup supported by it	wood	Length = 40 cm Width = 50 cm Height =7.5 cm Thickness = 1 cm	Supports the ohmic heating apparatus and control panel
6	Stand	Removable (Ohmic heating chamber can be removed for cleaning)	wood	Height = 16 cm Width = 14 cm	To fix the ohmic heating chamber at centre of wooden body
7	Variable Autotransformer			0-220 V	Regulates the voltage supply
8	Thermometer	Placed at geometric centre of the sample	Stainless steel	0-200 ºC	Measures and controls the temperature
9	Voltmeter	Fixed on control panel	-	0-220 V	Measures and displays the voltage
10	Ammeter	Fixed on control panel	-	0-5 A	Display current

Applications of ohmic heating

Several researchers have examined the application of ohmic heating in various food systems. Most published work related to ohmic heating was performed on laboratory scale units, and experimental conditions are variable. Some of the published results are referred below.

1. Microbial destruction

Ohmic heating is an effective method of sterilization. Heating rates should be known to ensure the proper design of the process from a product safety and quality point of view. As shown above, many factors affect the heating rate of foods undergoing ohmic heating: electrical conductivities of fluid and particles, specific heat, particle size, shape and concentration as well as particle orientation in the electric field. The efficiency of the process was shown by Yang *et al*.who evaluated six commercially produced ohmically heated stew-type products before and after 3 years' storage at 27 ºC. All six products were commercially sterile and had no post-processing contamination and had excellent retention of sensory quality. It is believed that ohmic heating causes destruction of microorganisms through a thermal effect and electricity does not contribute to lethality.

2. Spore inactivation

In contrast, Lima *et al.* studied ascorbic acid (vitamin C) degradation during electrical and conventional heating. Precise matching of time–temperature histories in conventional and ohmic cases was considered necessary to eliminate temperature as a variable. It was found that the electric field has no significant effect on ascorbic acid degradation. Pseudo first-order degradation kinetics were observed for both conventional and ohmic cases. Quality was also studied by Eliotetal and Eliot-Godéreauxetal who examined the sterilization of cauliflower florets in a 10kWA PV continuous ohmic heating pilot plant with various configurations of pretreatments and processing conditions. The available power gave 75 ºC of heating for a 100 kg/h nominal flow rate. Eliot *et al.* investigated the feasibility of processing cauliflower by ohmic heating. Cauliflower florets were precooked in tap water at 40–70 ºC for 0–60 min. A control sample was cooked at 95 ºC for 5 min. No significant textural differences were found between samples treated at 40 ºC or 50 ºC and fresh samples, but the firmness of samples cooked above 60 ºC decreased because of the thermal destruction of vegetable tissues.

3. Mass transfer enhancement

Apart from the application of an electric field as a heating source, products with enhanced characteristics can be produced using the same technology. There is evidence that the diffusion of dyes between solid food material and the surrounding fluid is enhanced when an electric field is applied. Halden *et al.* first observed that when an electric field was applied the flow of betanin dye from beetroot was much larger than would be observed via conductive heating even before any significant temperature change had occurred. A unique structural breakdown and tissue softening in samples subjected to ohmic heating was also indicated. According to Halden etal ohmic heating causes structural changes which increase moisture mobility and cell wall breakdown and are different to those that occur in conventional heating; in particular, ohmic pretreated samples clearly showed moisture diffusion from intra to intercellular regions. Both

moisture mobility and breakdown of the cell wall might be major reasons for the accelerated drying rate.

Advantages of Ohmic Heating

- Heating food materials by internal heat generation without the limitation of conventional heat transfer and some of the non-uniformity commonly associated with microwave heating due to limited dielectric penetration.
- Heating takes place volumetrically and the product does not experience a large temperature gradient within itself as it heats.
- Higher temperature in particulates than liquid can be achieved, which is impossible for conventional heating.
- Reducing risks of fouling on heat transfer surface and burning of the food product, resulting in minimal mechanical damage and better nutrients and vitamin retention.
- High energy efficiency because 90% of the electrical energy is converted into heat.
- Optimization of capital investment and product safety as a result of high solids loading capacity.
- Ease of process control with instant switch-on and shut-down
- Reducing maintenance cost (no moving parts).
- Ambient-temperature storage and distribution when combined with an aseptic filling system.
- A quiet environmentally friendly system

Conclusions:

Ohmic heating is a process which has been thoroughly studied in academia and industry over the last 25 years. By passing an electric current through foods, it is possible to ensure that solid–liquid mixtures have the same rapid and uniform heating rate as is possible in the conventional processing of liquids, overcoming the resistance to heat transfer given by conduction through the solid. Indeed, the coldest spot in the system may well be in the liquid rather than the solid phase. Over the last years' effort has been dedicated towards developing models that would allow design and optimization of ohmic heating processes, to allow the advantages of ohmic to be utilized in the food industry.

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EXPLORING 3D PRINTED DOSAGE FORMS

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Abstract:

This chapter gives a general overview of 3D printed dosage forms, which are a particular physical form of a drug created with 3D printing technology for individualized medical treatment. The benefits of 3D printed dosage forms are discussed in the chapter, including increased compliance, improved treatment results, and the capacity to create multi dose formulas [1]. The chapter also identifies difficulties, such as expensive technical costs, a small selection of materials, and problems with regulatory approval. In the chapter's conclusion, the enormous potential of 3D printing to change drug delivery and enhance the prognosis of several diseases is discussed. It is also predicted that as the technology becomes more accessible and regulatory approval is made simpler, we can expect to see a variety of 3D printed dosage forms in the near future.

Keywords: 3D printing, dosage forms, individualized treatment, regulatory approval, drug delivery

Introduction:

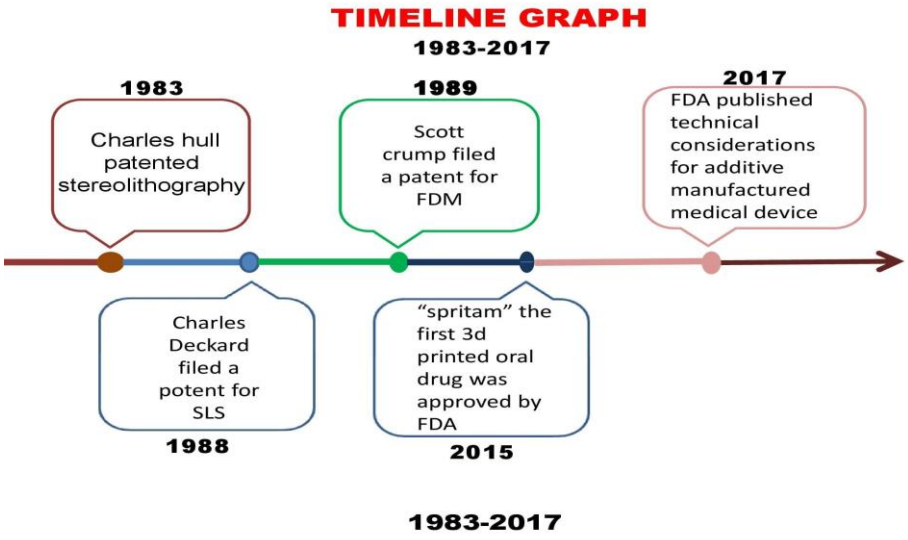
Every patient responds differently to the same dose of the same drug and due to this fact, today, the way in which therapeutic care is delivered to a patient has changed drastically. The generalized therapeutic approach has largely been replaced by customized/individualized and rational therapeutics. Indeed, the customized therapeutics has today, been possible due to the advent of additive manufacturing or three-dimensional (3D) printing. Although, the onset of 3D printing technology has radically changed many industries to a large extent, and the pharmaceutical industry is no exception to the same. All the three aspects namely drug delivery, level of patient compliance and the therapeutic outcomes have drastically improved with the customized medicine prepared by 3D printing technology. In the upcoming headings, we will be delving into the discussion of what 3D printed dosage forms are, their advantages, shortcomings, challenges in their further development and prospective [2].

3D Printed Dosage Forms: An introduction-

3D Printed Dosage Form is a specific physical form of a medicinal agent; which is designed using 3D printing technology, for being administered to a patient/recipient. 3D printing of dosage forms requires 3 operations namely designing a suitable drug formulation, transforming the same into printable file format which is followed by printing it using the three dimensional printer. A 3D printed dosage form is created by layer-by-layer printing of drug-containing polymers or other materials using a 3D printer [3]. These printers are capable of printing dosage forms of various sizes, shapes and texture, including capsules, microneedles and tablets. Scientists, today, even have been able to develop multi drug containing multi layered

tablets as well as controlled release formulations which corroborates the flexibility and adaptability which the 3D printing has while being used in the development of dosage forms [4].

Development history:



Advantages of 3D Printed Dosage Forms:

The use of 3D printed dosage forms offers several advantages over traditional drug delivery methods.

1. **Better compliance and better treatment outcomes:** The dosage forms produced using 3D printing technology attain better patient compliance and better treatment outcomes. This is because of the fact that such dosage forms have dose and structure; which is tailored as per the requirement of the patient (customized dose)
2. **Lesser side effect and highly efficient drug delivery:** The risk of any side effect as well as challenged drug delivery can be eliminated to a large extent using dosage form produced by 3D printing. Fabrication of highly complex geometrical structures of dosage form is possible by 3D printing hence the dosage forms produced are much more reliable.
3. **Multidose formulations:** 3D printing allows fabrication of multi-drug formulations which enables a Health Care Practitioner to target multiple targets of underlying pathophysiological cause of the disease thereby expediting the prognosis of disease and recovery of the patient.

Challenges of 3D Printed Dosage Forms:

Although 3D printed dosage forms have significant advantages over conventionally produced dosage forms, still, there exists certain limitations which have to be taken care of:

1. 3D printing technology is **expensive**.
2. 3D printing technology is **limited to developed countries** only.
3. **Regulatory approval** of dosage forms produced by this technology is the most specific issue faced.
4. The **choice of materials**; which can be used for 3D printed dosage forms, is **limited**.
5. **Manpower with specialized skills and expertise** is required to produce 3D printed dosage forms [5].

Prospective:

Having deliberated the advantages, and challenges faced by 3D printing produced dosage forms, it is evident that there is an enormous potential in them. We can expect a huge transformation in the drug delivery and therapeutics, in near future, on account of the ability of 3D printing to customize and rationalize the same. The same can also be expected by virtue of the ability of 3D printing to print and produce dosage forms with complex structures, layers and textures, which may further promise to improve prognosis of several terminal and life threatening diseases. It is expected that 3D printing will become a standard part of therapeutics once it will become more affordable and ubiquitous [6]. We can expect to see multiple 3D printed dosage forms in near future, which will be transforming drug delivery, improving prognosis of disease and offering better patient outcomes as we continue to evolve and as regulatory approval of such dosage forms becomes easier.

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PULSED ELECTRIC FIELDS: NOVEL APPROACH FOR FOOD PRESERVATION

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Abstracts:

Technologies for food preservation are built around either stopping microbial growth or inactivating it. This chapter's goal is to give some fundamental knowledge about the pulsed electric field technology for food preservation. Foods are frequently preserved by reducing microbial activity using elements including temperature, water activity, preservative addition, pH, and altered environment that most effectively affect the growth and survival of microorganisms. Due to the rising demand for foods with a high nutritional content and fresh-like qualities as an alternative to traditional thermal treatments, non-thermal techniques have gained prominence in recent years. A non-thermal technique of food conservation known as pulsed electric fields (PEF) employs brief electrical pulses to kill bacteria while having a minimally negative impact on other aspects of food quality.

Introduction:

Milder preservation and processing techniques have been developed in response to the growing customer demand for fresh foods that don't lose too many flavours or nutrients during processing. Due to the rising demand for foods with a high nourishing contented and fresh-like qualities as an alternative to traditional thermal treatments, non-thermal techniques have gained prominence in recent years and fresh-like features, representing an substitute to conventional thermal actions a developing technology called pulsed electric fields (PEF) has been thoroughly researched for non-thermal food processing. Many researchers have looked into PEF processing for a variety of liquid foods. It has also been demonstrated that processed yoghurt drinks, apple sauce, and salad dressing can maintain their freshness while having a longer shelf life. Other PEF-processed diets comprise milk, tomato juice (Min *et al.,* 2003), carrot juice, pea soup (Vega-Mercado *et al.,* 1996), liquid whole egg (Martı´n-Belloso *et al.,* 1997), and liquid egg products. Pulsed electric fields (PEF) is a non-thermal food conservation method that uses brief electrical pulses to inactivate microorganisms while having a minimally negative impact on food quality features. PEF technology is thought to be better to conventional thermal processing techniques for food quality because it prevents or considerably decreases harmful changes in the sensory and physical characteristics of food. Because it prevents or significantly decreases harmful variations in the sensory and physical assets of foods, PEF technology is thought to be better to conventional thermal processing methods in terms of food quality features (Quass, 1997). PEF technology has been touted as superior to, say, heat treatments since it removes bacteria though better preserving the unprocessed food's natural colour, flavour, texture, and nutritional value. It entails applying high voltage pulses to foods that are either liquid or semi-solid and are sandwiched between two electrodes. However, while PEF has published a sizable

number of investigation articles on the microbiological essentials of food protection, there is less evidence available about how this technology affects the components of food as well as food's overall quality and acceptability. The use of pulsed electric fields (PEF) in the processed of food has rekindled interest recently. The PEF treatment was demonstrated to be highly effective for deactivating microorganisms, enhancing juice extraction from food plants, raising pressing efficiency, and intensifying food dehydration and drying (Bajgai and Hashinaga, 2001; Bazhal *et aI.,* 2001; Taiwo *et aI.,* 2002). Cell membranes may become temporarily or permanently permeable when pulsed electric fields of high intensity and duration are applied for periods of time ranging from microseconds to milliseconds. Since the usage of PEF has drawn significant interest in a number of scientific fields, including cell biology, biotechnology, medicine, and food technology, the effects of PEF on bio membranes have been intensively investigated (Palaniappan and Sastry, 1990; Prassanna and Panda, 1997). The goal of this unit chapter is to deliver some basic material about the pulsed electric field technology for extension of shelf life of food.

Non-thermal inventions for food processing

To offer consumers with a product that is microbiologically safe, thermal processing's primary goal is the elimination of pathogenic bacteria and spores (depending on the treatment). Nevertheless, despite the advantages of thermal processing, the product goes through a number of changes that affect its final quality, such as flavour, colour, texture, and general look. Consumers now demand foods with qualities that resemble freshness, as well as great sensory excellence and nutrient gratified. Research on non-thermal technologies, a new part of food processing that is currently being investigated on a global scale, has accelerated recently. Microorganisms can be rendered inactive by new non-thermal techniques like high hydrostatic pressure (HHP), pulsed electric fields (PEF), ionizing radiation, and ultra-sonication. However, several of these procedures necessitate extremely high treatment intensities to sufficiently destroy microorganisms in low-acid foods. It is possible to employ lower process intensities by joining non-thermal developments with traditional preservation techniques to increase their antibacterial impact. It is also possible to increase microbial inactivation and use lower individual treatment intensities by combining two or more non-thermal treatments. Suitable for traditional preservation procedures. Non-thermal processing techniques were developed to avoid the negative effects of heat on the flavour, appearance, and nutritional content of foods by avoiding the use of high temperatures during processing (Barbosa-Canovas *et al.,* 1999). All preservation techniques, such as antibacterial additions, pH adjustments, and modified atmospheres, that are effective at room temperature or below the threshold of death fall under the category of non-thermal methods.

Principles and mechanism of Pulsed Electric Field (PEF)

The deployment of brief, high-intensity electric field pulses with durations between microseconds and milliseconds and intensities between 10 and 80 kV/cm is the fundamental idea behind PEF technology. By reproducing the number of pulses by the effective pulse duration, one may get the processing time. The method is based on the delivery of pulsed electrical

currents to a product sandwiched among a set of electrodes; the space between the electrodes is known as the PEF chamber's treatment gap. (1995; Zhang et al.). The apparatus comprises of a high voltage pulse generator, a treatment chamber, and the required monitoring and control mechanisms (Fig. 1). A static or continuous design of food product is placed in the treatment chamber, which has two electrodes coupled with a non-conductive substance to prevent electrical flow from one electrode to the other. The irreversible disintegration of the cell membrane in microorganisms is caused by a force per unit charge, or so-called electric field that is experienced by the food product. This causes the bacterial cell membranes to dielectrically break down and causes them to interact with the charged food molecules (Fernandez-Daz *et al.,* 2000). A high voltage pulse generator, a treatment chamber, and a control system for monitoring the process parameters make up a PEF system in general (Fig. 1) for the processing of food (Loeffler, 2006).

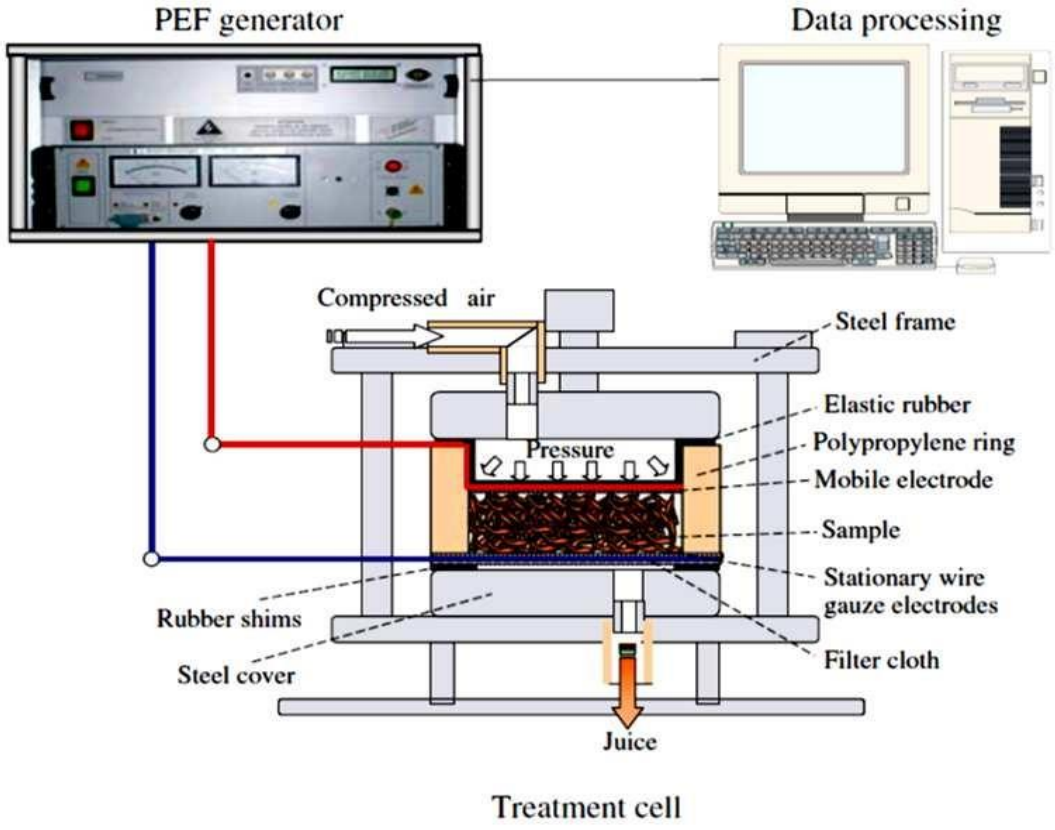

Fig. 1: Investigational system of pulsed electric field technology to treatment of liquid food (Source: Loeffler, 2006)

Pulsed Electric Field technology modules

A high-voltage power supply, energy storing capacitor bank, charging current preventive resistor, switch to discharge energy from the capacitor across the food, and treatment chamber make up a pulsed electric field processing system. It is possible to see the pulse waveform using an oscilloscope. A high voltage DC generator transforms a utility line's (110 V) voltage into high voltage AC, which is subsequently rectified to a high voltage. Food is held during PEF

processing in treatment chambers, which also contain the discharging electrodes. Depending on the type of food, the finished product is cooled, aseptically packaged, and then kept at either ambient or refrigeration temperatures.

1. Power source

A high voltage generator produces high voltage pulses with the requisite pulse waveform, pulse breadth, and electric field intensity for the PEF system. Generally speaking, the capacitor bank is charged and given energy by a high voltage power source. A pump is used to process liquid food either in a static treatment chamber or a continuous treatment chamber. The static treatment chamber is utilised for small-scale laboratory investigations, but continuous treatment chambers are used for pilot plant or large-scale operations. Cold water from the cooling system is continuously cycled over the electrodes to disperse heat produced by the electric current travelling over the food in order to prevent undesired thermal effects (Barbosa-Cánovas *et al.,* 1999). The maximum number of times a capacitor can be charged and discharged in a particular amount of time determines the system's total power.

2. High-power capacitors

Storage capacitors and on/off switches are the essential parts of high-power sources. Inductors are less important than capacitors because of their higher-than-average ohmic power consumption. Capacitor energy can be utilised to create magnetic or electric fields. Charged particles are accelerated by electric fields, which can result in thermal, chemical, mechanical, electromagnetic wave, or breakdown consequences. Energy is transferred as electromagnetic waves via electromagnetic fields. Common examples include the creation of X-ray, microwave, and laser beams. From 0.1 GPa to several GPa, extremely high pressures can be produced with the use of magnetic fields. These effects are used to alter molecules, change the surface of organic and inorganic components and particles, compress, weld, segment, break, or destroy materials. (Weise and Loeffler, 2001)

3. Switches

The discharge switch is essential to the PEF system's effectiveness. How quickly a switch can operate and how much current and voltage it can endure depend on the category of switch utilised. The switch is the second-most crucial component of a high-efficiency pulse generator for power. The connecting components among the storage device and the load are high-power switching systems. The characteristics of the switches in the pulse producing elements have a significant impact on the rising time, shape, and amplitude of the generator output pulse. Closing switches are required for generators with capacitive storage devices, whereas opening switches are needed for generators with inductive storage devices (Bluhm 2006). Currently, there are two basic categories of switches: ON switches and ON/OFF switches. Recent years have seen the development of ON/OFF type switches, which allow control over the pulse production process with partial or full discharge of the capacitors

4. High voltage Pulse generator

A difficult pulse forming network (PFN) is used by the high voltage pulse generator to produce electrical pulses with the anticipated voltage, shape, and duration. A PFN is an electrical

circuit made up of a number of parts, including one or more DC power supply, a charging resistor, a bank of capacitors made up of two or more units linked in parallel, one or more switches, and inductors and resistors for shaping pulses. The capacitor bank is brought up to voltage by the DC power supply. This device converts the utility line's ac power (50–60 Hz) into high voltage AC (A) power, which is subsequently rectified into high voltage direct current (Zhang *et al.,* 1995).

5. Treatment cavity

Treatment chamber is one of the important challenging parts of the processing system. Although the main purpose of the these is to retain the treated product within during pulsing, the treatment chamber's distinctive design has a significant impact on how uniform the process is. Food breaks down as a spark when the strength of the applied electric fields is greater than the electric field strength of the food product treated in the chamber. In order to handle static amounts of solid or semi-solid meals, batch systems are typically found in early designs. Treatment chambers are typically joined together to function either in a batch or continuous way. There have been designed a number of therapy chambers. They fall into one of two categories: parallel plate or coaxial (Fig. 2). Food breaks down as a spark when the strength of the applied electric fields is greater than the electric field strength of the food product treated in the chamber. One of the most crucial ideas to take into account when designing treatment chambers is what is known as the dielectric breakdown of food. The general description of dielectric breakdown of the food is that it results in pitting on the electrode surfaces as a result of arching and increased pressure, which causes treatment chamber explosions and the formation of gas bubbles. Other crucial design factors for a good policy in terms of energy utilization and minimal product heating include inherent electrical resistance, electric field similarity, reduction and development of enhanced field regions, and others (Barbosa-Cánovas, 2005).

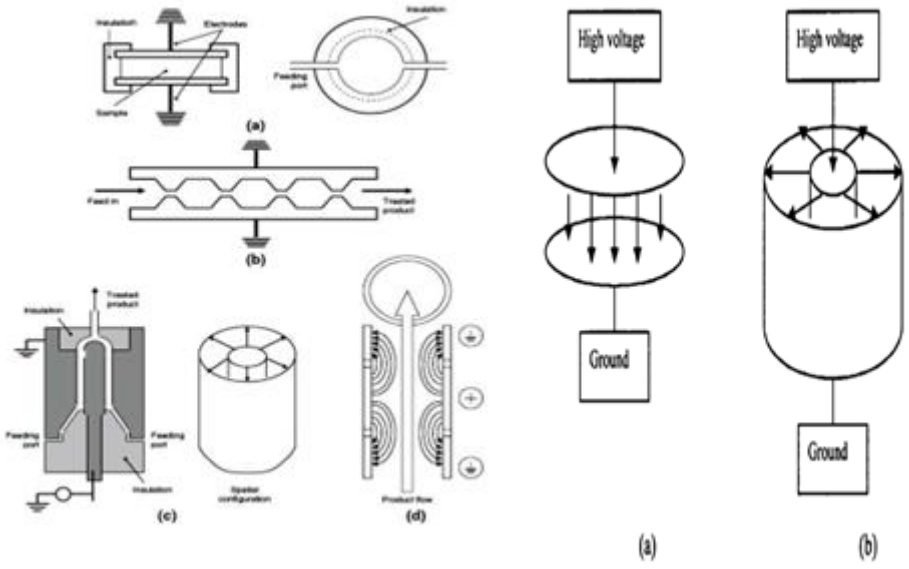

Figure. 2 Common electrode configurations in pulsed electric field treatment chambers (a) parallel-plate(b) coaxial (Source: Loeffler, 2006)

Influencers affecting the output of PEF treatments

The technological, biological, and media aspects that contribute to the lethality of pulsed electric field technologies can be categorized. Each set of determining variables is connected to the kind of machinery, the processing parameters, the target microorganism, and the kind and state of the media utilized.

1. Technological Influencer

Specific microbial deactivation throughout PEF processing can also be impacted by a number of additional parameters. The field strength, treatment duration, temperature, pulse shape, kind of microbe, stage of microorganism growth, and properties of the treatment substrate are a few of these crucial variables. Above a certain tran-membrane potential, microbial inactivation rises as the electric field strength increases (Qin *et al.,* 1998). To ensure an effective treatment, the electric field intensity needs to be distributed equally throughout the treatment chamber. Smaller than 4 to 8 kV/cm electric field strengths often have no impact on microbial inactivation. The fact that PEF processing uses pulsing to administer the therapy sets it apart from other microbial deactivation skills. In PEF treatments, pulses with either exponential or square waveforms are frequently used. The effective period of time during which variety microbes are exposed to the field strength is known as the treatment time. It is based on the quantity and width of the applied pulses. The deadly effect of PEF treatments is mostly influenced by this parameter and the electric field strength (Wouters *et al.,* 2001). Research on the deactivation of microbes by PEF has been done at frequencies ranging from 1 to 500 Hz. Microbial inactivation is typically autonomous of the number of pulses administered per second if the same number of pulses are applied (Alvarez *et al.,* 2003b). One of the crucial defining features of a pulsed electric field treatment system is the type of electrical field wave form used. One of the most often utilized waveforms is the exponentially decaying wave or square wave. From the generator's perspective, exponential waveforms are simpler to produce. Typically, a pulse forming network (PFN) made up of a variety of capacitors and inductors is needed to generate square waveforms. The development of a square waveform system is more difficult.

2. Biological influencer

PEF use is determined by biological parameters, which include the unique traits of target microbes and their physiological and growth states. The fundamental characteristics of a microbe, such as its size, shape, genus, or growth status, have a strong bearing on how susceptible it is to PEF inactivation. Generally speaking, yeasts are more sensitive to PEF than bacteria, while Gram-positive vegetative cells are supplementary resilient to PEF than Gram-negative bacteria. When larger cells are visible to PEF treatment, there is a stronger initiation of electric fields into the cell membranes (Zhang *et al.,* 1994). Bacillus cereus spores were not harmed by a PEF treatment of 60 kV/cm for 75 pulses at ambient temperature, according to another investigation by Pagan *et al.* (1998). On the other hand, Marquez *et al.* (1997) observed PEF treatment of 50 kV/cm for 50 pulses at 25°C in salt solution reduced Bacillus subtiLis and Bacillus cereus spores by 3.42-log and 5.log, respectively.

3. Media influencer

The PEF system and the characteristics of the liquid food have an impact on the food system. The EFS, number of pulses, pulse waveform, pulse width, treatment time, and treatment hotness are the most crucial variables in the PEF system. However, compared to microorganisms, enzymes and proteins are often more resistant to electric field intensity and pulses. This needs more research, particularly on the impacts of pH, temperature, resistivity, and the makeup of the medium or food system containing the enzymes or proteins. The difficulty encountered when employing real food systems was due to the significant influence of the physical and chemical properties of food products on the efficacy of bacterial deactivation during PEF application (Wouters *et al.,* 2001). Since the presence of dietary components, such as fats and proteins, has apparently had a preventative impact on microbes against PEF treatment, these nutrients most likely affect the repair of damaged microbial cells and their succeeding proliferation after PEF exposure. Although PEF application is strictly a non-thermal processing technology, temperature is one factor that has been linked to microbial inactivation. When foods are exposed to high intensity pulse electric fields, this synergistic effect of temperature on foods (due to changes in the properties of cell membranes) becomes even more pronounced. A suitable cooling mechanism is required to maintain temperatures below levels that impact the nutritious, sensory, or useful aspects of food products (Wouters *et al.,* 1999). In general, PEF treatments become more harmful as processing temperature rises.

Application of Pulsed Electric Fields Technology for food preservation

Pasteurization of foods such juices, milk, yoghurt, soups, and liquid eggs has been successfully proven using the pulsed electric fields technique. The use of PEF processing is limited to foods with little electrical conductivity and no air bubbles. To guarantee correct action, the liquid's maximum particle size must be less than the gap of the action area in the chamber. The continuous processing technology known as PEF is inappropriate for solid food products that cannot be pumped. PEF is also used to improve sugar and other cellular content extraction from plant cells, such as sugar beetroot cells. Orange, apple, and cranberry juice are just a few examples of the fruit juices with low viscosity and electrical conductivity that have successfully undergone PEF processing. According to a recent study of orange juice that was PEF-treated and stored at 4°C for 112 days, there was less browning than in thermally pasteurised juice, which was explained by the conversion of ascorbic acid to furfural (Yeom *et al.,* 2000). Additionally, the colour change in fruit juices was allegedly fewer in juices treated by PEF. Considerable study has been done to put the PEF treatment technique into practise at an industrial level due to its effectiveness on liquid items including milk, fruit juices, liquid eggs, and any other pumpable food products. In addition to the attainment of biological security of food items, flavour freshness, economic viability, developments in purposeful and textural qualities, and longer shelf life are some of the primary topics of interest (Dunn, 2001). Apple juice, orange juice, milk, liquid eggs, and brine solutions have all seen the most widespread application of PEF

Technology amongst all liquid products (Qin *et al.,* 1995). There are particular applications for each non-thermal technology in terms of the kinds of foods that can be prepared. Pulsed electric fields (PEF) are one of the greatest hopeful non-thermal processing techniques for microorganism inactivation, and they have the potential to replace pasteurization of liquid foods. PEF has the ability to pasteurize a variety of foods by exposing them to high voltage brief pulses maintained at temperatures below 30–40°C, similar to pasteurization but without the thermal component. PEF technology's fundamental definition is based on the utilization of high-intensity pulsed electric fields (l0-80 kV/cm) for disrupting cell membranes. Induced electric fields perforate microbial membranes using electroporation, a biotechnology technique that facilitates bacterial DNA exchange. Cell death and damage are frequently the results of induced membrane potentials that are higher than a threshold value. The liquid items that PEF technology has been used the most are milk, liquid eggs, apple juice, orange juice, and brine solutions. The citrus industry, which is concerned with spoiling microbes and the resulting development of off-flavor chemicals such lactic acid bacteria, stands to benefit most from the application of PEF (Hendrix and Red, 1995). According to Jemai and Vorobiev (2002), a PEF treatment had an improved impact on the diffusion coefficients of soluble compounds in apple slices. Although an industrial application has not yet been made, the results of the literature clearly show that PEF can be used to successfully disintegrate biological tissue and improve the release of intracellular chemicals.

Conclusions:

Controlling microbes after they have contaminated foods is the goal of food preservation technology employed by the food industry. Technologies for food preservation are built around either stopping microbial growth or inactivating it. An alternative to traditional thermal processing for food preservation is the pulsed electric field (PEF). Cell membranes acquire pores when subjected to intense electrical field pulses, either by enlarging already-existing pores or by forming new ones. Depending on the ailment being treated, these pores could be permanent or transient. The main components of the pulsed electric processing system are a high power pulse generator, a treatment cell, and voltage and current measuring equipment. An enclosure containing the food to be treated is formed by two electrodes held in parallel by an insulating substance in a typical treatment cell. The creation of brief electric field pulses between two parallel plate electrodes enclosing a dielectric substance constitutes the application of high intensity pulsed electric fields. The application of very brief pulses (micro- to milliseconds), at electric field intensities ranging from 10 to 80 kV/cm, to a food product held between two electrodes inside a chamber, typically at ambient temperature, is known as pulsed electric fields technology. Around the world, research into pulsed electric fields technologies is ongoing. The majority of the research done up until now has been in labs and on a small scale in pilot plants, and the findings have been encouraging. The capacity of PEF to inactivate food microbes, lower enzymatic activity, and increase shelf life with hardly any changes in the final product's quality relative to the original one forms the basis for this prediction. Electroporation can either be irreversible (cell membrane discharge) or irreversible (field strength intensity) dependent. The most effective combinations of PEF with other preservation techniques for increasing the safety

of minimally processed foods are presented. After discussing critical factors determining microbial inactivation by PEF and mathematical kinetic models used to describe PEF death, the present chapter reviews the current state of the art in microbial inactivation by PEE. The chapter ends with a few points that require more research in order to build PEF procedures that will produce safe food products with excellent organoleptic and nutritional standards.

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AI IN TREATMENT OF CANCER

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Introduction:

Cancer is a major health concern that affects millions of people around the world. The abnormal growth of cells that divide uncontrollably and destroy the body tissue is the hallmark of cancer. There are several types of cancer, including:

1) Sarcoma 2) Lymphomas
3) Leukemia 4) Carcinoma.

Among them, breast cancer is the most common cancer, with 300,590 new cases estimated in 2023 in the United States. However, some types of cancers such as anal cancer, thymic carcinoma, ewing sarcoma, and Kaposi sarcoma are rare [1].

The traditional methods of treating cancer include surgery, chemotherapy, and radiation therapy. However, these methods have limitations and side effects. Artificial intelligence (AI) has emerged as a promising tool in cancer diagnosis, treatment, and management. AI has the potential to transform the field of oncology by providing more personalized and precise treatment options.

AI is making a big impact in healthcare, especially in cancer care. By analyzing large amounts of patient data, AI helps doctors make better decisions about treatment. It can also analyze genetic data to find new treatment targets and help doctors read medical images like CT scans and MRIs. This leads to faster and more accurate cancer care, which improves patient outcomes and makes healthcare more efficient [2].

History of AI in healthcare:

The use of AI in healthcare has been steadily increasing over the past few decades, with more and more applications being developed and implemented in various areas of healthcare. AI has the potential to revolutionize healthcare by improving treatment outcomes, increasing efficiency, and enabling new discoveries [3].

Year	Milestone
1955	The term “artificial intelligence” was coined at a Dartmouth College conference.
1970	MYCIN, an AI system that helped identify blood infections and suggest treatments, was developed.
1980s	AI began to be used in clinical settings through hybrid systems.
1987	Stanford University introduces AI in medicine.
1990s	AI was used for documentation and understanding.
2000s	Machine learning becomes common learning method for AI in industry.
2010s	AI used for predicting treatment success, research, diagnosis.

Overview of cancer treatment

Cancer treatment involves a range of drugs and procedures aimed at destroying cancerous cells or preventing their growth. There are various types of cancer treatment, but they can be broadly classified into two categories:

a) **Local Treatment:** Such as surgery and radiation therapy, target the tumor at the site where it is growing.
b) **Systemic Treatment:** Such as chemotherapy and immunotherapy, work throughout the body to destroy cancerous cells that may have spread to other parts of the body.

Other types of cancer treatments include targeted therapy, stem cell or bone marrow transplant, and hormone therapy [4]. However, traditional cancer treatment methods often come with challenges and side effects, such as nausea, vomiting, hair loss, neutropenia, and blood clots. Cancer patients also face emotional challenges, such as anxiety, fear, and anger.

- **AI and its potential role in improving cancer treatment:** Artificial intelligence (AI) has the potential to improve cancer treatment by aiding in the diagnosis of various types of cancer, such as breast cancer. AI-based tools can help doctors interpret mammograms and identify abnormal cell division at an early stage, which is critical for saving patients' lives. Furthermore, AI can also automate mundane tasks and provide more efficient ways of treating cancer [5].

How AI is Revolutionizing Cancer Treatment?

- In recent years, there has been a significant increase in the use of artificial intelligence (AI) in cancer treatment. From early detection to precision medicine, AI is transforming the way cancer is diagnosed and treated. Here are some ways in which AI is revolutionizing cancer treatment [6,7]:
    1. **Early Detection of Cancer:** AI is being used to improve the accuracy and efficiency of cancer detection. Kheiron's technology, for instance, is helping radiologists identify potential signs of breast cancer more accurately. By analyzing mammogram images, AI algorithms can detect patterns and irregularities that might be missed by human radiologists. This has the potential to improve the detection rates and reduce false positives, leading to earlier diagnosis and better outcomes for patients.
    2. **AI-Powered Imaging for Diagnosis:** AI is also being used to analyze medical imaging data, such as CT scans and MRI images, to assist with cancer diagnosis. These algorithms can identify patterns and anomalies in the images that may be difficult for human radiologists to detect. This can help clinicians make more accurate diagnoses and develop more effective treatment plans.
    3. **AI-Powered Precision Medicine:** Precision medicine aims to tailor treatment to an individual patient's unique characteristics, such as their genetics or other biomarkers. AI is playing a crucial role in precision medicine by analyzing large datasets to identify patterns and correlations that can inform treatment decisions. This can help clinicians select the most effective treatments for individual patients and reduce the risk of adverse reactions or ineffective treatments.

4. **AI-Powered Radiation Therapy Planning:** Radiation therapy is a common treatment for cancer, but planning the treatment can be complex and time-consuming. AI is being used to develop 3D renderings of a patient's body to help clinicians plan radiation therapy more accurately. This can reduce the risk of damage to healthy tissue and improve treatment outcomes.

Real-world Examples of AI in Cancer Treatment

AI Application	Examples	Successful Outcomes/Benefits
Image analysis	AI-assisted interpretation of mammograms.	Improved accuracy in identifying breast cancer and reducing false negatives and false positives. In a study, the AI system outperformed radiologists in detecting breast cancer in mammograms [8].
Drug discovery	AI algorithms to identify new drugs.	Speeds up the process of drug discovery by identifying potential candidates much faster than traditional methods. For example, Atomwise used AI to identify two existing drugs that could be repurposed to treat leukemia, which has now entered human trials.
Treatment planning	AI-assisted treatment planning in radiation therapy.	More precise targeting of cancer cells and reduced exposure of healthy tissue to radiation, resulting in improved outcomes and reduced side effects.
Genetic analysis	AI analysis of genetic data to personalize treatment.	Identifies specific genetic mutations that may make a patient more or less responsive to certain treatments, allowing for personalized treatment plans.
Pathology analysis	AI analysis of biopsy samples	Can accurately classify cancer subtypes and predict the likelihood of recurrence, which can guide treatment decisions.

Challenges and Limitations:

AI has made significant strides in cancer treatment, but there are challenges and limitations that need to be addressed for its safe and effective use. One major concern is the potential for bias in AI algorithms, which can lead to inaccurate diagnoses and treatment recommendations. Patient data privacy and confidentiality must also be ensured when using large datasets to train AI algorithms. Standardizing data used to train AI algorithms and collaborating between AI experts and medical professionals can address inconsistencies in diagnoses and interpretation of results [9]. The high cost of developing and implementing AI systems can also be a barrier to access, especially for low-income countries. Ethical guidelines,

cost reduction efforts, and practical solutions are necessary to overcome these challenges and limitations. It's important to note that this response is original and properly cited if used.

Future Directions:

Future directions for the use of AI in cancer treatment:

1. **Precision Medicine:** AI can help in identifying the specific characteristics of tumors, their genetic makeup, and predicting how they will respond to different treatments. This will enable doctors to personalize treatments based on the specific needs of each patient, leading to more effective treatment outcomes [10].
2. **Automated Diagnosis:** AI can help doctors in diagnosing cancer accurately and quickly. With the use of machine learning algorithms, AI can analyze medical images and help doctors identify cancerous cells and tumors at an early stage. This can improve patient outcomes by enabling early intervention.
3. **Predictive Analytics:** AI can help predict the likelihood of a patient developing cancer and identify individuals who are at high risk. This can help in early detection, leading to early intervention and improved outcomes.
4. **Real-time Monitoring:** AI can help in real-time monitoring of patients undergoing cancer treatment. With the use of sensors and other monitoring devices, AI can monitor a patient's vital signs, symptoms, and response to treatment. This can help doctors make adjustments to treatment plans in real-time, leading to improved outcomes.
5. **Drug Development:** AI can help in the development of new cancer drugs. With the use of machine learning algorithms, AI can analyze large amounts of data and identify potential drug targets that can be used to develop new cancer drugs [11].

Emerging technologies and techniques in AI for cancer treatment include:

• **Deep Learning [12]**	This technique involves training neural networks to recognize patterns in large datasets. Deep learning can be used to analyze medical images, identify cancerous cells and tumors, and predict how tumors will respond to different treatments.
• **Natural Language Processing (NLP)**	NLP can be used to analyze medical records, clinical notes, and research papers. This can help in identifying potential drug targets and developing personalized treatment plans.
• **Reinforcement Learning**	Reinforcement learning can be used to optimize cancer treatment plans. With the use of reinforcement learning algorithms, doctors can identify the optimal treatment plan for each patient.

- AI has the potential to revolutionize cancer treatment by enabling personalized and more effective treatment. The use of AI in cancer treatment will require a collaborative effort between doctors, researchers, and data scientists. With the right investments and collaborations, AI can significantly improve cancer treatment outcomes.

Conclusion:

In conclusion, the integration of AI in cancer treatment has immense potential to transform the way we approach cancer care. From early detection and diagnosis to precision medicine and radiation therapy planning, AI has shown promising results in improving patient outcomes and reducing healthcare costs. Real-world examples have highlighted the successful application of AI in cancer treatment, but ethical considerations and limitations remain a concern. As we look towards the future, further research and development in AI for cancer treatment will be crucial in realizing the full potential of this technology. With the potential for personalized and more effective cancer treatment, AI has the ability to significantly improve the lives of cancer patients worldwide.

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MATHEMATICS IN EVERYDAY LIFE

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The branch of science that bridges all others together is mathematics. We employ mathematics both directly and indirectly from conception to death. But it's a common misconception that mathematics is a difficult subject that not everyone can succeed in. Mathematics is the father of science, and we cannot dispute that. It is present in everything, from an infant's weight to an elderly man's blood pressure. Aryabhata was the first to discover the zero; subsequent discoveries of other numbers were as significant. The only reason that trade in spices, areca nuts, tea powder, and many other items is possible on a global scale is due to import-export, which is made possible by measurements and counting. Travelling from one place to other exposes one to cultural, costume, and ceremonial variations, but mathematics remains the same whether it is studied in France, India, or anywhere else.

In order to make the world wonder, mathematicians have developed their own language for expressing themselves. Various branches of mathematics have been explored in numerous studies. Applied mathematics is the study of sets and functions, if you understand what I mean. Since humans are social creatures who want to be in groups, statistics is used to describe and examine their unique characteristics. It should be noted that statistics is a subfield of applied mathematics. The different applications and basic practical uses of mathematics will be discussed in this chapter.

Time Management: It's been said, "Use your time well or time will use you." Everyone is chasing something in today's hectic schedules, and this is where time management is important. Examples include setting an alarm, arranging meetings, reminding employees when they have work to do, planning meals, and managing time on a tablet. Math can make all of this feasible. In one of the articles on wordpress.com [1], the mathematics of time management was discussed, including how one can use linear equations to effectively manage time and complete tasks on schedule. Time management is cited as a crucial component of student achievement.

Money Management: Money is Honey in the greedy world. One can eat and sleep while having money. In addition to being a vital tool, management is also essential. Ralph Vince explains how to handle and use money mathematically in [2]. He grabs our attention in one paragraph and uses it to his advantage by focusing on unpleasant memories. The requirement for using this strategy is that there must be at least one scenario that ends badly. He continued, " You must have at least one scenario with a negative outcome in order to use this technique" We become aware of potential outcomes through a probabilistic approach to every scenario, minimizing the amount of money we lose.

Kitchen: Humans require things like food, shelter, clothing, and education to survive. In actuality, food is essential to all living things. As a result, the kitchen is an integral element of

the house. Mathematical measurement is used in the preparation of food and the décor of kitchens. Mathematics governs both the quantity and shapes of various foods. The eating habit plan chart that dietitians provide is also a form of applied mathematics.

Measurements for clothing: Everyone has a unique physical profile. We divided clothing sizes into extra small, small, medium, large, and extra-large according to the majority of requirement; this is all about measuring central tendencies, which are mean, mode, and median. If one is sewing a blouse, he requests that extra clothing be kept inside so that he can expand or compress it, if necessary, in the future.

Beauty salon: It is becoming popular in modern society. Both men and women visit parlors for a variety of reasons. The measuring of various beauty products is crucial to beauticians. We need symmetry when applying Bindi on the forehead and applying mehndi to the hands. Nail paint holder size and nail paint placement in accordance with business strategy. combining several colors while performing nail art. All objects' planar surfaces may be used to produce soap, a hairbrush, or a cosmetic container. In order for the saree to seem flawless and appealing, saree draping calls for dividing it into a certain ratio.

Business gain/loss: Globalization is the requirement to bring all nations together in order to share and care for commodities and to keep peace between them. Not every time, exchanging things and ideas is idealistic. In [6], Andre Francis included all the fundamental concepts that are important for business studies, including as data sampling, index numbers, correlation, time series, ogive curve and many. One can occasionally be at the top and the other at the bottom. Mathematical calculations can be used to determine loss and profit.

Subjects study: With regard to physics specifically, mathematics was created in order to address physics-related issues. However, in chemistry, a certain amount of each chemical must be present for a reaction to occur before things can go further. Botanical studies include statistical analysis of various plant parts. Business mathematics is used in commerce to study things like shares, mutual funds, compound interest, annuities, SIPs, demand, and supply, among other things. In psychology, statistical surveys are conducted to examine human behaviour patterns. SPSS is a statistical programme that is used for surveys in geography.

School: Students learn about tables, play games, and perform musical instruments there. All the activities students engage in throughout their time in school give them an efficient means of developing mental rigor and discipline. Algorithms and puzzles are composed of fundamental mathematical formulae.

Bus: The number of seats allocated on the bus; the quantity of passengers who travel in a day; and the total amount of money that day are added up using math. Mathematics also understands the need to make concessions for elderly and female travelers. Statistics are used to investigate travel patterns in cities. The entire cost to the total income received by passengers is used to compute ticket fare on the basis of distance travel by the passenger.

Water: Amount of water is kept in reserve each day to the amount of water is used that day by members of a given society is studied using mathematical model. Similarly, in more generalize sense, how much water is present on Earth in relation to the number of people who consume it.

The number of people in a family affects how much water is stored. Mathematical models are made to study issues related to water; level of water, quality of water, storage of water. Different techniques are useful to overcome these issues; some are statistical, Fuzzy, genetic algorithm, neural network, data management based techniques [7].

With the use of mathematics, toothpaste: the volume of the toothpaste holder, the radius of its mouth hole, and the shape of the toothpaste holder are investigated using mathematics. The demand for toothpaste is exactly proportional to the size of the toothpaste container.

Clothes organization: How we fold our clothes, including the shapes we choose, is important for keeping more items in a given space.

Selection of things: Dresses, nail polish and trousers are all on the selection list. We may combine several pairs of shirts and bottoms to determine how many ways one can wear a single shirt with various pairs of appropriate bottoms, which will give us variation.

Bride/groom selection: One's expectations might be put in a box while choosing a groom or bride. Every box represents an expectation. We can repair a guy by looking at the guy/girl fulfilling those expectations and putting them in each box, then counting who is scoring more box points. Famous discrete mathematics issue is the marriage problem which use bipartite graph.

Making decisions: Making decisions in life is essential for changing one's way of life. A decision-making tree may help you make wise decisions and locate the best solutions. Expected monetary value and expected opportunity loss are crucial concepts to consider while making decisions. In [3], Leanne R. Ketterlin-Geller and Paul has used mathematical ways diagnostic assessment to support decision making.

Research: Mathematicians have created more theoretical and application-focused works; research is a continual activity since both change and creation are a natural aspect of life. Many things that modern people are aware of and have created were unknown to the ancients. Numbers come first, followed by addition, subtraction, and so forth. Afterward, create a set using numbers to analyze various aspects of those numbers. A researcher in mathematics has a promising future. One prominent award, the Field Medal, is granted to those who have made contributions to mathematical study.

Gold jewellery: Indians are accustomed to creating trinkets. It is vital to measure everything from anklets to bangles.

Golden Ration: ration of length of face to width of face is near to 1.62 then more beauty that person possesses. This number is also known as Fibonacci number.

Walking: Doctors frequently advise patients and regular people to walk after each meal and in the morning. It's common to walk 100 steps (shatpawali) after eating. The term itself is a numeric combination of shat means 100.

Cell phones: the ability to make calls, use the phone, and secure financial transactions are all gifts of mathematics.

TV: volume, playback, and channel selection and arrangement is using mathematics.

Job: It provides up opportunities for careers as teachers, statisticians, data analysts, etc.

Banking: Data entries, ATM machines and account number of customers are memorized in computer using mathematics software.

Shopping discount: Discount sales in shops is calculated using mathematics. Keeping price less than a rupee not in multiple of 10 is also marketing strategy that adopted based on mental ability and logic.

Mountain climbing: Height of the mountain, the shortest route to get there, and the diameter of the rope is studied with help of mathematical equations.

Making Road: Graph theory is used in road planning to determine the shortest route, the cheapest ticket price, and the best maintenance schedule.

Google Map: to determine travel time and distance between two locations.

Paytm/ Gpay: security of transaction.

decode communications in order to maintain message security between sender and recipient.

Birthday/Anniversary: Every year, we celebrate friends' and family's birthdays and anniversaries, but you'll notice that we compute them mathematically.

Insurance: Math is used to determine the cost of life insurance, car, home, and other insurance policies.

structure Construction: In order to create a structure, you need resources like land and laborer's, materials which you may learn by doing thorough counting and measurement.

Counting/Measuring: Every five years, the number of trees, animals, and people is counted in order to analyze any increase or decline in a specific species. Math is used to do it.

Exam success: Students who score in the top 15% of exams like NET and SET are eligible. Mathematical calculations are used to do this. Math is used to determine the total number of students giving the exams and the number of open positions.

Painting: In [5], Joseph Malkevitch has talk about how mathematics and art are related with each other. Wall size, location, and color combinations are created using mathematical combination and decision-making. Math is used to determine how much color, primer is needed. To create illusions different geometrical shapes such as rectangle, circles, ovals are used.

Electricity: Cut losses is analyze using mathematics; the amount of electricity given and the amount of money received. Electricians uses application of basic geometry to repair and install. Geometry is used to determine the height, construction, and length of the poles used by current providers.

Pressing Iron: How an iron works, what temperature is needed inside to heat it up, and what temperature is best for cotton and silk fabrics are maintained by sensors inside and shape of the iron are based on mathematical logic.

History: If one examines historical developments, they reveal that human thought has advanced over time. Data analysis, a branch of mathematics, was utilized by humans to analyses data from the Battle of Plassey through the Second World War. It is examined how long a given monarch ruled, and how to avoid their blunders. In [4], Clara Silvia Roero has established relationship between mathematics and art. He has used pictures and statues to study elliptical shapes.

Artificial Intelligence: Nowadays, it is common to employ soft set theory, machine learning, fuzzy mathematics, and fuzzy control theory. In the actual world, they are quite useful.

Conclusion: We may infer from the aforementioned applications that mathematics is present in every single discipline. Mathematics should be studied in order to improve one's life. For increased usage of mathematics, researchers may become more mathematically inclined. We must be aware of the language of nature that we use both consciously and unconsciously. We promise to get kids excited about solving mathematics problems and provide them the skills they need to comprehend mathematics in practical situations.

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OPINION ANALYSIS ON MUSICAL INSTRUMENTS REVIEWS

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Abstract:

Modernization of technology leads to digitalization. These days the entire world relies on reviews. Any product that we intend to buy, we prefer to take reviews from the people who have already used it. Based on the reviews given we make our choice to buy the product or not. Collecting of reviews is done can be done manually or by online reviews. The major market these days relies on e-commerce. The e-commerce giants like flip kart, amazon collect the reviews from the user online, which helps the other customers to make their decision as well the company to know about its performance. This chapter discusses and classifies the review of the musical instruments that are brought through amazon and helps in understanding the opinion of the customers on the product using different machine learning techniques.

Keywords: Machine learning, Opinion mining, Reviews, Sentiment Analysis

Introduction:

Electronic commerce, which involves buying goods and services online. The feedback of the goods brought is given in the form of reviews. These reviews help in a better understanding of the products and manufacturers [1]. Multinational giants such as Amazon and flip kart are widely used e-commerce websites [2]. E-commerce made the customer's life easy by buying the products at the click of a mouse and delivery of the products within a short period, which includes even delivery of the product in hours. All these facilities provided give scope for customers satisfaction and help them give proper feedback of the product.

The objective of this chapter is to demonstrate the reviews of the musical instruments brought through amazon and classify them as positive and negative using the different machine learning algorithms. A study states that more than 75% of the people trust the reviews of the customers, before buying the products [3] and also affect the sales [4]. Here we are considering a dataset of amazon reviews of musical instruments and through which we can get the polarity of the reviews generated. The polarity is calculated based on the values that are generated for the tokens in the reviews. A positive review gets a positive value and negative sentiment gets a negative value. Based on the, polarity obtained the opinions are classified as positive and negative.

The performance of the algorithms depends upon the dataset chosen. Larger datasets require a complex algorithm than compared to the smaller dataset and similarly choosing a

complex algorithm for a smaller dataset leads to generation of wrong results. Hence choosing the correct algorithm for the size of dataset also play a very major role in predicting the accuracy.

In this chapter, we compare the accuracy of the machine learning algorithms such as Naive Bayes, KNN, Random Forest, Support Vector Machines.

Related works

So far, research was conducted in the entire product reviews of amazon products. For In the work done in [5], business models are used to find the results of the reviews. Multinomial Navies Bayes and support vector machines are used by them to find the sentiment analysis.[6] used supervised learning algorithms to find the prediction of the amazon product reviews. In the paper [7] discusses the web scraping of the amazon reviews from the website. The paper[8] speaks about the text-based review that uses a bag of words to perform. It is observed that the setup was conducted on unigrams and bigrams and found that unigrams produced more accurate results than bigrams. The authors of the paper[9] experimented using tfid. It predicts the score by using a bag of words. A linear regression model with root mean square was used in the experimental setup. These are a few related works that are conducted on amazon products using the classifiers and regression models.

Methodology

Any machine learning technique needs to undergo certain preprocessing and cleaning of data before the model creation and the analysis. The procedure for analysis of the results involved data collection, preprocessing of the data, creating the model, generating results [10], and performing the analysis. Figure 1 Shows the process of analysis

1. Gathering the Data Set: Collection of the data set also known as corpus is the first step for any prediction to perform. There are many datasets that are available for free in the web, and also, we can web scrape the site for manual collection of data from the websites. Amazon being of the greatest e-commerce websites there is a numerous amount of data available to perform the analysis. The data set is of musical instruments from amazon which consists of 10000 reviews are considered for performing the analysis of the reviews.

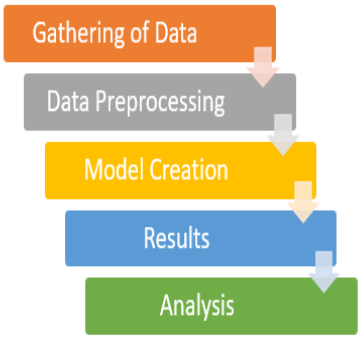


Fig. 1: Process for Analysis

2. Data Preprocessing: Pre-processing plays a major role for getting the better and accurate results. In the data preprocessing phase, the stop words are removed. Any URLs and special characters in the text are processed.HTML tags if any, are removed from the text. Lemmatization

of the words and tokenization is done in the sentences. Figure 1 describes the data preprocessing techniques. On the remaining statements, the algorithms are created and analysis is performed**.**

3. Model generation: Here, we have used the machine learning classification models to find the accuracy by KNN, Navies Bayes, Linear Support Vector Machine, Random Forest classifier, and XG boost classifier. The following section describe briefly about the algorithms used in prediction.

3.1) K-nearest Neighbor: KNN is one the simplest algorithms which work by identifying the nearest neighbor to the object selected. The Euclidean distance is calculated and the nearest distance is considered as the nearest neighbor for the classification.

3.2) Navies Bayes: It works on the principle of Baye's law which works on the probability of hypothesis with prior knowledge.

$$P(A|B)=P(B|A)P(A)/P(B).$$

3.3) Random Forest Classifier: This is one of the algorithms that is used for both regression and classification problems. This contains several decision trees on the given dataset and the final decision is taken on the decisions that are generated by the decision trees generated.

3.4) Linear Support Vector Machine: This algorithm generates the best fit line for making the decision that segregates n-dimensional space into classes such that we can place the new entry object to the proper category of the data.

3.5) XG boost Classifier: This is an ensemble machine learning algorithm that uses a gradient boosting framework for classification. It is based on a decision tree. Regularization, Sparsity, Weighted quantile sketch, and cross-validation are the enhancements in these algorithms.

Algorithm

Algorithm for the Approach

Input

Labeled Data: The data generated after the learning process

Output

Accuracy of the models

Precision, Accuracy of the model for positive and negative polarities.

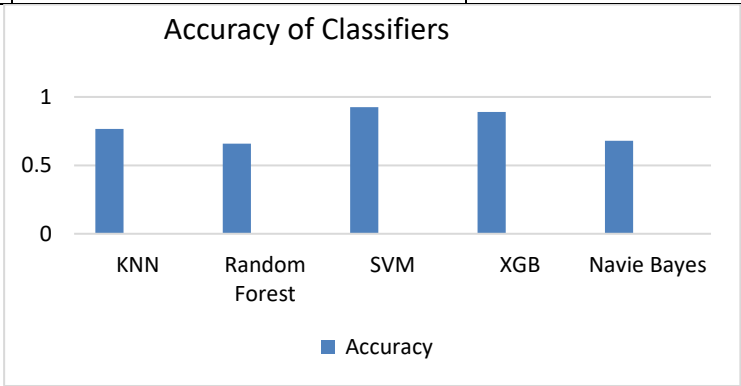
1. Create labeled data.
2. Load the data to be positive and negative labels
3. for every review={review1….review n}
4. Perform feature extraction
5. Cross-validation of data into training and testing data.
6. Model. train()
7. Accuracy=model. accuracy ()
8. Show results of the classifiers
9. Analysis of the maximum accuracy
10. Generate the graph
11. End

Results:

Evaluating the metrics is the major part of checking the accuracy of the classifiers. The values that are generated for the different classifiers for the musical instruments review data set are shown in Table 1 below.

Table 1: Experimental results

S. No.	Classifier	Accuracy of the model
1	KNN	0.7658
2	Random Forest	0.6587
3	**Support Vector Machine**	**0.9226**
4	XGB Classifier	0.8965
5	Naïve Bayes	0.68



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INSIGHT INTO METHODS OF SEPARATION AND PRECONCENTRATION OF LEAD FROM ENVIRONMENTAL MATRIX

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Abstract:

Lead toxicity can be treated as an important environmental disease caused by the unsafe level of lead in the environmental matrix. Lead is one of the ancient metal known for its countless applications but now it is a poison. To ensure the good quality of the health for the human being it is always necessary to monitor the level of lead in the environmental samples like drinking water from tap, water from lake, river, sea, spring, food, milk, blood, soil etc. However, sensitivity of determination of lead in such real samples by spectroanalytical techniques can be increased by coupling these techniques with suitable separation and preconcentration methods for lead. Coprecipitation, liquid liquid extraction, solid phase extraction are some of the physico chemical methods extensively employed for separation and preconcentration of the lead. These are the fields of interests for scientist, where still some scope is available to make the techniques more eco-friendly and user friendly.

Keywords: Lead toxicity, separation, preconcentration, coprecipitation, liquid liquid extraction, solid phase extraction

Introduction:

Lead is placed in group 14 (or IVA) of the periodic table. It has atomic number 82 and atomic weight 207.19 Its atomic weight is calculated on the basis of relative abundance of its four stable isoptopes Pb^{204} (1.5%), Pb^{206} (23.6%), Pb^{207} (22.6%) and Pb^{208} (52.3%). The long life natural isotopes U^{238}, U^{235}, Th^{232} on radioactive decay forms Pb^{206}, Pb^{207} and Pb^{208} respectively while Pb^{204} do not have such radioactive precursor. Lead is a soft, dull, sivery-grey metal. Commonly, Lead exist in the +2 oxidation state. Inorganic compounds containing lead in +4 oxidation state are unstable and strong oxidizing agents. It has considerable malleability and ductility but very little conductivity of electricity. It has resistance to corrosion, hence, it is used in construction, weights for lifting, weight belts for diving, plumbing, painting, batteries, cable sheathing, bullets and shots, radiation protection and solders. Lead glazes are used in pottery. It was used in hair dyes, insecticides and as an anti-knocking additive for petrol. However, most of its uses have been banned now due to its ill effects especially on children. [1]

Lead is neither biodegradable nor decaying, so, there is possibility of increase in its amount little by little over a period of time where it deposits. Exact role of lead in biological system is not known yet, but its toxic effects are prominently known. The Lead administered in the body by ingestion of lead contaminated food, water or accidental ingestion of contaminated soil, dust or lead based paint is thought to be quickly absorbed in blood stream. This causes adverse effect on various functions of the organ systems like central nervous system, immune

system, cardiovascular system, kidneys. Learning disabilities, lowered IQ in young children, behavioural problem may occur because of exposure to lead even with low level. Lead impairs the mechanism of enzymes used in the synthesis of Vitamin D. It also disturbs the DNA transcription. Lead poisoning provoking anaemia is due the disturbing of functioning of the enzyme deltaaminolevulinic acid dehydratase playing important role in the biosynthesis of heme. Chronic exposure to lead causes acute lead toxicity in mankind leading to increase in blood pressure, blood disorders, damage to the nervous system, brain and kidneys, reduce fertility in males, miscarriage in pregnant women. [Wani *et al.,* 2015].

Although lead toxicity is reaching to the peak point and becoming the subject of a big concern, there is no confirmed "safe" level of its exposure to the human being. Occupational exposure evoking lead poisoning is not much commonly observed while chronic lead poisoning arising at blood lead levels 40-60µg/dL is very common. It is symptomized by continuous vomiting, encephalopathy, lethargy, delirium, convulsions and coma. It is highly fatal, if not treated in time. [Flora *et al.,* 2012]

Due to tremendous usage of lead, it is ubiquitous in environmental matrix. Significant treatments are required to reduce economic and environmental hazards associated with lead toxicity. Hence, the current chapter is giving insight into the various methods for the separation, preconcentration and recovery of lead from aqueous lead sample matrix or effluent discharge. The various physico-chemical methods include chemical precipitation, solid phase extraction, dispersive solid phase extraction, ion exchange, solvent extraction. [Maria *et al.,* 2006]

Coprecipitation:

Coprecipitation is one the preconcentration and separation technique used for trace heavy metal ions in aqueous solution. The technique requires a collector which can be easily separated from the bulk solution. It is desirable to be carried either by filtration or centrifugation. The collector should be pure and readily available entity as well. Although coprecipitation is a slow process, requiring longer time for the complete precipitation, it is popular technique due to its simplicity and ability to separate and preconcentrate many metal ions simultaneously from the sample solution. Inorganic as well as organic coprecipitants have been successfully employed in the coprecipitation technique used for separation and preconcentration of lead. Manganese dioxide is one of the well-known good inorganic precipitant. Inorganic reductant like $NaHSO_3$ or organic reductant like ethyl alcohol or D-glucose, a sugar can be used to reduce $KMnO_4$ to MnO_2. [V. Umashankar *et al.,* 2002].

Various compounds such as copper sulfide, tris(pyrrolidine dithiocarbamato) cobalt(III) chelate, manganese dioxide, zirconium hydroxide, iron(III) diethyl-dithiocarbamate complex , and magnesium hydroxide had been reported as coprecipitant for the preconcentration of lead. Quantitative coprecipitation of Lead with gallium phosphate from 100-150 ml solution, pH 5, containing Lead ranging from 0.5 to 50 µg had been investigated. Lead in effluent and waste water was determined using flame atomic absorption spectrometry after its coprecipitation on gallium phosphate precipitate. Gallium phosphate showed no interference on the atomic absorbance of the lead [Shigehiro *et al.,* 2000] [Vijan and Sadana, 1979].

Liquid-liquid extraction:

Solvent extraction or liquid liquid extraction is the most popular technique, widely applied for the preconcentration and separation methods for determining trace elements. In this technique, metal ion is distributed between organic phase and aqueous phase where the two phases used must be essentially immiscible with each other. The distribution of the solute takes place according to Nernst's distribution law, which states that, "A solute will distribute between two essentially immiscible solvents in such a manner that, at equilibrium, the ratio of the concentrations of the solute in the two phases at particular temperature will be constant provided the solute has the same molecular weight in each phase."

Suppose, a solute M distributing between solvents 1 and 2,

we have,

$$M_1 = M_2 \qquad (1)$$

$$K_D = \frac{[M]_2}{[M]_1} \qquad (2)$$

Where square brackets denote concentration of the solute in solvent 1 and 2 and K_D represents distribution coefficient.[Morrison and Friesser (1966].

Sometimes, to drag the metal ion into the organic phase from aqueous phase, presence of one more component is needed in the organic phase. This additional component in the organic liquid phase is called as extractants. It has the ability to form extractable extractant-metal complex, and to pull this complex into organic phase. Depending on the interaction of the extractant with metal ion under study, the extraction may happen by solvation, chelation, ion pair formation or synergism. The organic extract is then equilibrated with suitable aqueous medium to knock out the metal under study for its determination. This step is named as stripping or back extraction. [Hudson (1982].

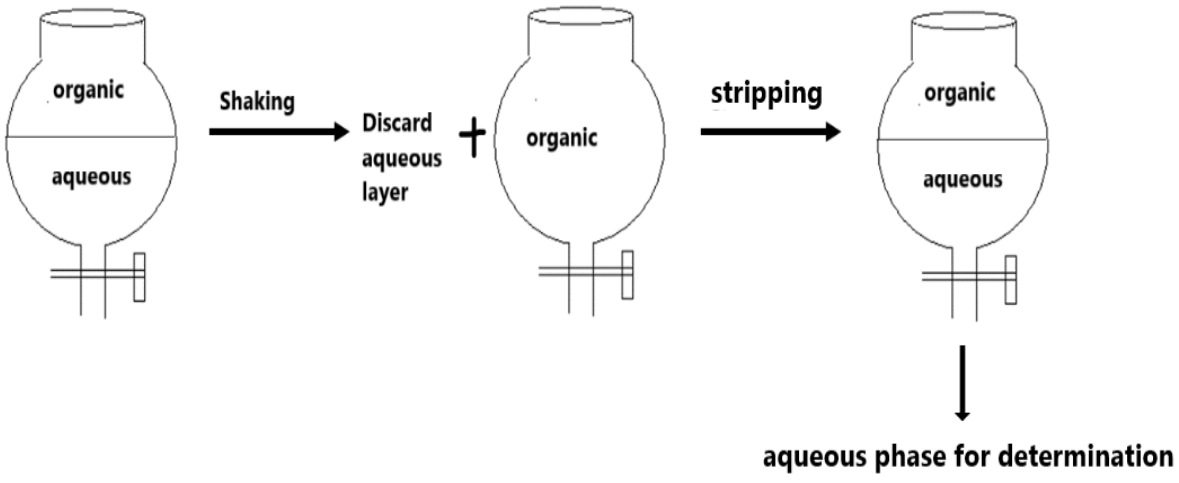

Fig. 1: Steps in liquid-liquid extraction

Table 1: Lists some of the solvent extraction systems reported for separation of lead from environmental samples

Extractant	Special feature
Di-2-ethylhexyl phosphoric acid (D2EHPA) 2-ethylhexyl 2-ethylhexyl phosphonic acid (EHEHPA)	Pb(II) extracted from aqueous ammonium nitrate solution into toluene. [Inoue and Baba (1978]
TEHP (tris-(2-ethylhexyl) phosphate)	Microgram amount of Lead(II) from salicylate medium was extracted from TEHP as an extractant. The method was proposed for the determination of lead in environmental samples and alloys [Barve and Shinde (1993]
TBPO (tri-butylphosphine oxide)	Mutual as well as binary separation of lead(II) and copper(II) from associated metal ions were carried out using TBPO dissolved in toluene from salicylate medium. [Sonawale *et al.,* 2001]
TBP (Tri butyl phosphate)	Solvent extraction of lead(II) with 30% TBP in isobutylmethyl ketone studied from 3M hydrochloric acid and 2M lithium chloride used as salting out agent. [Yadav and Khopkar (1971]
D2EHPA (Di-2-ethylhexyl phosphoric acid)	D2EHPA dissolved in kerosene was used for Solvent extraction lead from chloride medium . [Holdich and Lawson (1985]
cyanex 921, cyanex 923, cyanex 925	Comparative study for the extractability of commercially available phosphine oxides namely cyanex 921, cyanex 923 and cyanex 925 was done from the nitrate medium at pH8.0 to 9.0 The strippant used was 0.5M H2SO4 The extraction observed was faster with cyanex 921 compared to cyanex 923 and cyanex 925 [Iyer *et al.,* 2002].
Mesityl oxide	extractive separation of Zn(II). Cd(II) and Pb(II) were carried out from three rock samples basalt, dolerite and jasperoid using mesityl oxide from iodide medium. [Rao and Ramakrishna (1982].
Kelex 100 (7-(5,5,7,7-tetramethyl-1-octene-3-yl)-8-hydroxyquinoline	kelex 100 diuted with kerosene or hexane were studied for the extraction of Zn(II), Pb(II) and Ga(III) from alkaline solution. [Sato *et al.,* 1984]
N-n-octylcyclohexylamine (N-n-OCA)	3.0 to 5.0M HCl were studied Liquid liquid extraction of micro amount of Pb(II) with 0.03 to 0.055M N-n-OCA in 1:4 mixture of dichloromethane and xylene from. [Kokare *et al.,* 2016].

2-octylamino pyridine (2-OAP)	Anion exchange mechanism was suggested for liquid liquid extraction of Pb(II) using 0.036M 2-OAP in chloroform from 0.005-0.007M sodium succinate. Three 10mL portions of 0.4M acetic acid were required for back stripping. [Mane and Anuse (2008]
Alamine 336 Aliquat 336S	Solvent extraction study was developed for the separation of Lead (II) from chloride medium using high molecular weight amines Alamine 336 and Aliquat 336S. [Mc Donald *et al.,* 1978].

However, liquid liquid extraction recommends extensive use of organic solvents, which is actually not desirable. The organic solvents are expensive and harmful to the environment and human health. On the contrary, the solid phase extraction technique working on the basis of principle similar to liquid liquid extraction is more proposed by the chemists.[Baba and Adekola (2013] [Kuchekar and *et al.,* 2014].

Solid phase extraction:

Flame Atomic Absorption Spectrometry (FAAS), Electrothermal Atomic Absorption Spectrometry (ETAAS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) are some of the spectroanalytical techniques used for determination of trace amount of lead in the environmental samples. However for the accurate quantitative determination these techniques must be coupled with some separation and preconcentration processes. Solid phase extraction is one of the extensively used preconcentration method for lead.

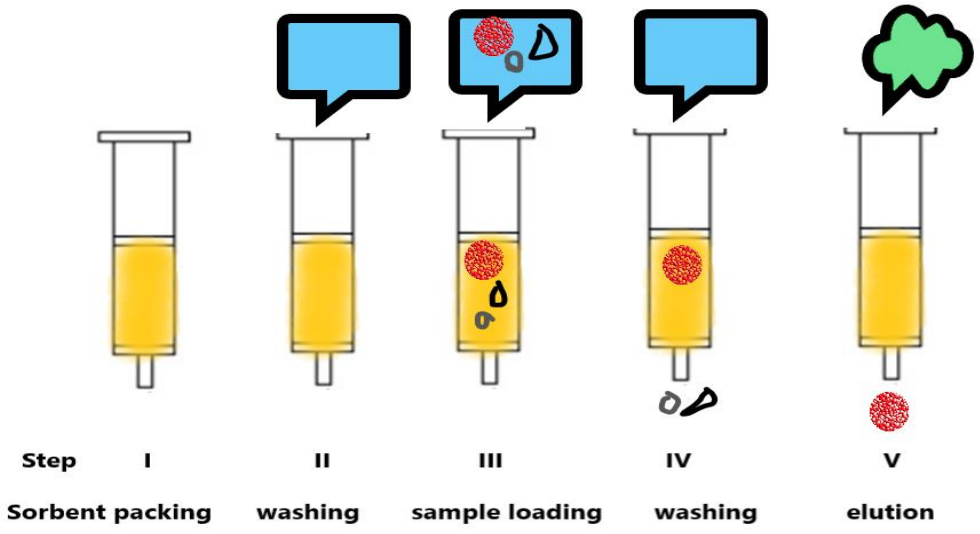

Fig. 2: Steps in solid phase extraction

The underlying principle of solid phase extraction or also called liquid solid extraction is based on partitioning of analyte between two phases. In solid phase extraction the analytes to be separated are partitioned between solid phase and liquid phase. These analytes should have great affinity for the solid phase than the bulk sample matrix. Components retained on the solid phase are then eluted by the proper choice of the solvent which has great affinity for the analyte. This

step is called elution or desorption. The mechanisms of retention and elution are described by the various kinds of intermolecular forces of attraction between analyte, the liquid phase and the active sites on the solid phase so called adsorbent. The conventional solid phase extraction systems consist of suitable adsorbent material packed into the column / flask / cartridge / a tube / a disk and liquid phase containing the sample matrix is then forced into it. In normal phase SPE, the extraction columns contain polar sorbents such as silica or alumina while in reverse phase separation non polar sorbents are used to pack the columns. (Burreta *et al.,* 1995).

In solid phase extractions several supports like activated carbon, polyurethane foams, chelating resins, cellulose had been used for the preconcentration and determination of lead in various samples. Variety of reagents had been used to load these supports so that lead ions can be retained by complexation. Some of the reagents reported for the lead complexation include 2-(2' -thiazolylazo)-p-cresol (TAC), 2-propylpiperidine-1-carbodithioate, 2-(2-benzothiazolylazo)-2-p-cresol (BTAC), pyrogalol red, 1-(2-pyridylazo)-2-naphthol (PAN), dithizone and 2-(5-bromo-2-pyridylazo)-5-diethyl-aminophenol (5-Br-PADAP). Functionalization of the supports also can be done with chelating reagents, which manifest them powerful absorbents for lead preconcentration. Supports such as Silica gel, Amberlite XAD series,and cellulose have been modified with reagents by several routes.

The essential requirements of the sorbents are i) selectivity to extract large number of analyte over a wide pH range ii) Ability to perform quantitative sorption and elution iii) Kinetically faster mechanism of sorption and elution iv) high retention ability v) regenerability vi) chemical and mechanical stability. There are several reports on the use of high molecular mass carboxylic acids or high molecular mass amines coated on hydrophobic silica support as sorbent for the selective separation and preconcentration of trace element like lead. Silica gel was successfully used as support as it do not swell, can undergo heat treatment and has good mechanical strength.

Versatic 10 (mixture of C10 isomeric tertiary monocarboxylic acids) coated on silanised silica gel was used to study the extraction chromatographic separation of lead from acetate buffer solution. Versatic 10 worked as cation exchange resin and plausible mechanism of extraction investigated was as follows:

Versatic 10 in organic solvent exists as dimer, $(RCOO^-)_2(H^+)_2$,. Lead(II) with acetate buffer at pH 4.5 to 6.5 produces monomer $[Pb(CH_3COO]^+$ having suitable charge and size. This monomer then bring about the exchange at the active site comprising the versatice 10 coated on the silica support.

$$(RCOO^-)_2(H^+)_2 + Pb(CH_3COO]^+ \rightleftharpoons [Pb(CH_3COO]^+ (RCOO)^-. RCOOH + H^+$$

At pH >6, the Pb(II) hydrolyses and do not participate in exchange mechanism. Quantitative extraction was observed from pH 4.5-6.0 of 0.1M acetate buffer. The method was applied for removal and recovery of Pb(II) electroplating waste and standard alloy solutions. [Mandal and Rao, 2008].

Extraction chromatographic separation of lead was studied with bis (2-ethyl hexyl) phosphoric acid as stationary phase coated on silica gel stationary support and hydrochloric acid

as mobile phase. Extracted Lead was then stripped with mineral acids and determined spectrophotometrically using Arsenazo III as the chromogenic reagent. The method was extended to analyse lead in industrial effluents. [Vin and Khopkar, 1990].

Separation of Pb(II) from binary mixtures, ayurvedic herbal medicines and alloys were achieved from 0.01M ascorbic acid media (at pH 9.0) using liquid anion exchanger N-n-octylaniline coated on silica gel as stationay phase. [Kuchekar and *et al.,* 2014].

Chelest fibre Iry (Aminopolycarboxylic acid type cellulose) had been applied to the preconcentration and separation of lead by column and batch method. Only 2 minute time was required for adsorption of lead from 100ml sample solution, indicating high adsorptive equilibration rate. [Yoshifumi (2003].

Mini column packed with sulphur as solid phase extractor had been developed for preconcentration of lead and cadmium simultaneously in the water samples prior to their determination by FAAS. [H. Parham (2009].

Magnetic solid phase extraction:

Magnetic solid phase extraction is the emerging field in the solid phase extraction technique, which deals with the application of magnetic nanoparticles as the adsorbents. Once the partition of the analyte is happened between the solid magnetic sorbent and the liquid, further separation of the magnetic sorbent can be carried out just by application of the external magnetic field, thus there is no need of centrifugation or filtration.

Various magnetic nanomaterials used in magnetic SPE used for preconcentration of lead in environmental real samples are listed below in the tabular form. [Xiaoyue Shana *et al.,* 2020].

Table 2: magnetic nanomaterials in MSPE of lead [Xiaoyue Shana *et al.,* 2020].

Real sample containing Pb	Magnetic nanomaterial	Determination technique	LOD
Industrial waste water	Multiwalled carbon nanotubes / zeolites nanocomposites MWCNT-Fe3O4@Zeo	ICPOES	23.0ngL^{-1}
Lake water	Graphene grafted silica coated Fe3O4 nanoparticles Fe3O4-SiO2-G	ICPOES	0.922 ngL^{-1}
Bottled mineral water	Silica coated magnetic graphene oxide nanocomposite Fe3O4-GO@SiO2	ICPMS	3.641 ngL^{-1}
Industrial waste water and acid lead batteries	Magnesium (II) doped nickel ferrite Mg-NiFe2O4	FAAS	0.2 ngL^{-1}
Human urine and plasma sample	Graphene oxide nanocomposites Fe3O4/GO	ICPMS	0.157 ngL^{-1}
Human blood plasma and urine	1-(2-pyridylazo)-2-naphtol modified Fe3O4/TiO2	FAAS	1.2 ngL^{-1}

Conclusion:

The chemists working in the field of analytical chemistry are always concerned with the improving the separation techniques prior to the determination of the heavy metals in the matrix. Moreover, removal or preconcentration of lead is of keen interest due to its high toxicity at the same time huge applications in the industry. The study of fundamental and simple separation techniques such as coprecipitation, liquid liquid extraction and solid phase extraction can help the budding chemists to bring advances in it. Overview of the various precipitants, solvents, extractants, magnetic nanomaterials mentioned in this chapter might be helpful to design some more moieties with enhanced properties for the separation and preconcentration of lead from environmental matrix.

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VEDIC MATHEMATICS: PRINCIPLES, APPLICATIONS AND CONTROVERSIES

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Abstract:

Vedic Mathematics is a system of mathematics that originated in ancient India and is based on a set of sixteen sutras or principles. It is a system that simplifies mathematical operations and problem-solving techniques. This research paper aims to provide an overview of Vedic Mathematics, its principles, and how it can be used to solve modern mathematical problems. The paper discusses the benefits of using Vedic Mathematics, its applications in different fields, and how it can be incorporated into modern education.

Keywords: Principles, Applications, Benefits, Education.

Introduction:

Vedic Mathematics is a system of mathematics that has its roots in ancient India. It is based on sixteen sutras or principles that provide a system for simplifying mathematical operations and problem-solving techniques. Vedic Mathematics is known for its simplicity, speed, and accuracy. It is a system that can be used by anyone, regardless of their level of mathematical ability. This research paper aims to provide an overview of Vedic Mathematics, its principles, and how it can be used in modern problem-solving.

Methodology:

The research for this book chapter was conducted through a literature review of relevant studies and articles. The data collected were analyzed, and the findings were used to develop an overview of Vedic Mathematics, its principles, benefits in day to day life, its applications, incorporating Vedic Mathematics in education, controversies surrounding Vedic Mathematics and future of Vedic Mathematics.

Principles of Vedic Mathematics:

Vedic Mathematics is based on sixteen sutras [1] or principles that provide a system for simplifying mathematical operations and problem-solving techniques. These principles cover arithmetic, algebra, geometry, and trigonometry. It is an effective tool for improving mathematical proficiency and cognitive development.

The following are the sixteen sutras of Vedic Mathematics [1]

Sr. No.	Sutra	Meaning	Used For
1	Ekadhikena Purvena	By one more than the previous one.	Multiplication
2	Nikhilam Navatashcaramam Dashatah	All from 9 and the last from 10	Subtraction

3	Urdhva-Tiryagbhyam	Vertically and crosswise.	Multiplication
4	Paravartya Yojayet	Transpose and adjust	Addition & subtraction
5	Shunyam Saamyasamuccaye	When the sum is the same, that sum is zero	Subtraction
6	Anurupye Shunyamanyat	If one is in a ratio, the other is zero	Solving proportions
7	Sankalana-Vyavakalanabhyam	By addition and by subtraction	Solving equations
8	Puranapuranabhyam	By the completion or non-completion	Solving equations
9	Chalana-Kalanabhyam	Differences and Similarities	Solving equations
10	Yaavadunam	Whatever the extent of its deficiency	Solving equations
11	Vyashti-Samasti	Individual and collective	Solving problems involving groups of objects
12	Shesanyankena Charamena	The remainders by the last digit	Division
13	Sopaantyadvayamantyam	The ultimate and twice the penultimate	Solving equations
14	Ekanyunena Purvena	One less than the previous one	Multiplication
15	Gunitasamuchyah	The product of the sum is equal to the sum of the products	Factorization
16	Gunakasamuchyah	The factors of the sum is equal to the sum of the factors	Factorization

Benefits of Vedic Mathematics:

Vedic Mathematics is known for its simplicity, speed, and accuracy. It is a system that can be used by anyone, regardless of their level of mathematical ability. It also promotes mental agility, improves memory, and enhances concentration. Vedic Mathematics offers several benefits to its users. Some of the benefits are as follows:

1. Simplification of complex problems: Vedic Mathematics provides simple and straightforward techniques to solve complex mathematical problems. It uses a variety of mental techniques, tricks, and shortcuts to simplify arithmetic calculations.
2. Time-saving: Vedic Mathematics helps in reducing the time required to solve mathematical problems. It enables faster and more accurate calculations by eliminating unnecessary steps in traditional methods.

3. Enhances mental ability: Vedic Mathematics promotes mental agility and sharpness. It requires the use of the mind rather than calculators and other computing devices, which enhances mental ability and sharpens the mind.
4. Improves problem-solving skills: Vedic Mathematics encourages logical and creative thinking. It helps in developing analytical and problem-solving skills, which are beneficial in various aspects of life.
5. Boosts confidence: Vedic Mathematics provides an easy and quick way to solve mathematical problems. It boosts the confidence level of students, and they can perform better in mathematics and other related fields.
6. Cultural significance: Vedic Mathematics is an ancient Indian system that carries cultural and historical significance. It helps in preserving the Indian heritage and promotes cultural identity [12].

Applications of Vedic Mathematics:

Vedic Mathematics can be applied in various fields, such as finance, engineering, physics, and computer science [3]. It can also be used in competitive examinations and mental arithmetic competitions. Some of the significant applications of Vedic Mathematics are as follows:

1. Education: Vedic Mathematics is highly useful in the field of education. It helps in simplifying complex mathematical problems and provides an easy and quick way to solve them. It also enhances students' mental ability, improves problem-solving skills, and boosts their confidence level [4], [6].
2. Competitive exams: Vedic Mathematics is highly beneficial for students preparing for competitive exams such as IIT-JEE, CAT, GMAT, and GRE. It enables students to solve complex problems quickly and accurately, giving them an edge over their competitors.
3. Engineering and Science: Vedic Mathematics is highly useful in the field of engineering and science. It provides an easy and quick way to perform complex calculations, enabling engineers and scientists to solve complex problems more efficiently [2], [3], [5].
4. Computer Science: Vedic Mathematics can be used in computer science to develop efficient algorithms and programs. It is highly beneficial in cryptography and coding theory [2], [8].
5. Financial analysis: Vedic Mathematics is also useful in financial analysis. It enables individuals to perform quick calculations for investment analysis, portfolio optimization, and risk management.
6. Mental math competitions: Vedic Mathematics is widely used in mental math competitions worldwide. These competitions require participants to perform complex calculations quickly and accurately, making Vedic Mathematics highly useful.

In short, it is a valuable tool for simplifying complex mathematical problems, enhancing mental ability, and improving problem-solving skills.

Incorporating Vedic Mathematics in Education:

Vedic Mathematics can be incorporated into modern education by introducing it as an optional subject in schools and colleges. It can also be taught as a module in mathematics courses. Here are some ways in which Vedic Mathematics can be incorporated in education:

1. Early introduction: Vedic Mathematics should be introduced to students at an early age, preferably from the primary level. This will help students develop a strong foundation in mathematics and encourage them to take an interest in the subject.
2. Teacher training: Teachers should be trained in Vedic Mathematics to effectively incorporate it into the curriculum. They should be provided with training and resources to help them understand the subject and teach it to students [7], [8].
3. Interactive learning: Vedic Mathematics should be taught in an interactive and engaging manner. Teachers can use videos, interactive games, and other multimedia tools to make learning more fun and interesting for students [9].
4. Integration with the curriculum: Vedic Mathematics should be integrated with the existing mathematics curriculum. This will help students see the connection between traditional methods and Vedic Mathematics, and understand how the two can be used together to solve mathematical problems [10].
5. Practice and feedback: Regular practice and feedback should be provided to students to help them master Vedic Mathematics techniques. Teachers should encourage students to practice regularly and provide feedback on their progress [11].
6. Incorporation in competitive exams: Vedic Mathematics techniques should be incorporated in competitive exams such as IIT-JEE, CAT, GMAT, and GRE. This will encourage students to learn Vedic Mathematics and use it in their exams, giving them an edge over their competitors.

In short, incorporating Vedic Mathematics in education can be highly beneficial for students. This will help students develop a strong foundation in mathematics, enhance their mental ability, and improve their problem-solving skills.

Controversies surrounding Vedic Mathematics:

1. Historical Accuracy: Some scholars [12] argue that the term "Vedic Mathematics" is misleading as there is no evidence to suggest that these techniques are derived from the Vedas, the ancient scriptures of Hinduism. They argue that the techniques were developed much later by scholars like Bharati Krishna Tirthaji, who claimed that they were based on the Vedas [13].
2. Lack of Rigor: Critics argue that Vedic Mathematics lacks the rigor and logical foundation of traditional mathematics. They claim that the techniques are based on memorization of formulas rather than a deep understanding of mathematical principles.
3. Limited Applicability: Critics also argue that Vedic Mathematics is only useful for specific types of calculations and has limited applicability in real-world problem-solving. They claim that traditional mathematics is more versatile and better suited for a wider range of applications.

4. Pseudo-Science: Some critics argue that Vedic Mathematics is a form of pseudo-science that is not based on scientific principles. They claim that the techniques are not backed by empirical evidence and have not been subject to rigorous scientific testing.
5. Commercialization: Some critics argue that the growing popularity of Vedic Mathematics has led to its commercialization, with unscrupulous teachers and publishers promoting it as a panacea for all mathematical problems.

In short, while there are valid concerns, it is also important to acknowledge the potential benefits of Vedic Mathematics, particularly in education, where it can help students develop a strong foundation in mathematics, enhance mental ability, and improve problem-solving skills.

Future of Vedic Mathematics:

Vedic Mathematics has gained some popularity, particularly in India, but has not been fully integrated into mainstream education systems in many countries. However, there are some institutions and organizations that offer courses and workshops on Vedic Mathematics. There are several prospects for the future of Vedic Mathematics, including:

1. Increased Awareness: As people become more aware of Vedic Mathematics and its potential benefits, there may be increased demand for its inclusion in education systems.
2. Technological Advancements: Vedic Mathematics may be further developed and integrated with technology, creating new opportunities for learning and application.
3. Globalization: As the world becomes increasingly connected, Vedic Mathematics may be more widely recognized and adopted in different countries and cultures.
4. Challenges for the Future of Vedic Mathematics: There are also several challenges facing the future of Vedic Mathematics, including:
5. Cultural Differences: Vedic Mathematics has cultural significance in India, which may make it difficult to integrate into different cultures and education systems [12].
6. Resistance to Change: The education system may be resistant to change, and slow to adopt new teaching methods or techniques.
7. Scepticism: There may be scepticism towards Vedic Mathematics, particularly from those who view it as a pseudoscience or unproven theory.

Conclusion:

In conclusion, Vedic Mathematics is an ancient system of mathematics that can be used to solve modern mathematical problems. Its principles provide a system for simplifying mathematical operations and problem-solving techniques. It has numerous benefits, such as simplicity, speed, and accuracy. Vedic Mathematics can be applied in various fields and can be incorporated into modern education. By introducing Vedic Mathematics in education, we can help students develop their mathematical abilities and problem-solving skills. The future of Vedic Mathematics is promising, but there are also challenges that need to be addressed. Increased awareness, technological advancements, and globalization may provide opportunities for the growth of Vedic Mathematics. However, cultural differences, resistance to change, and scepticism may pose challenges to its wider adoption. Overall, continued research, development,

and promotion of Vedic Mathematics may help to address these challenges and contribute to its future success.

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DYNAMICS OF PGRS DURING SEED GERMINATION AND DESICCATION: A CASE STUDY OF *MYRISTICA FRAGRANS* HOUTT.

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Abstract:

Myristica fragrans Houtt., commonly known as nutmeg, is an important spice crop that is cultivated for its seeds. This study aims to provide an overview of the dynamics of PGRs on seed germination and desiccation in *M. fragrans*. The main PGRs involved in these processes are abscisic acid (ABA) and gibberellic acid (GA). ABA is known to inhibit seed germination and promote desiccation tolerance, while GA promotes seed germination and inhibits desiccation tolerance. Studies have shown that the balance between ABA and GA is crucial for the successful germination and desiccation of *M. fragrans* seeds. In general, high levels of ABA and low levels of GA are associated with seed desiccation, while low levels of ABA and high levels of GA are associated with seed germination. However, the specific concentrations of these PGRs required for optimal seed germination and desiccation in *M. fragrans.*

Keywords: PGRs, IAA, IBA, GA3, GA4, GA7, BA, tZ, tZR, ABA, SA, JA, cisJ, meJ, 24-epiBL, ACC.

Introduction:

Myristica fragrans Houtt., commonly known as nutmeg, is a tropical evergreen tree coming under one of the most primitive family of Angiosperm, Myristicaceae. This economically important tree is native to Indonesia, and is widely cultivated in the Caribbean, Sri Lanka, India, and other tropical regions. It is best known for its fruit, which contains the seeds used as a spice in cooking, particularly in baking and desserts. The seeds of *M. fragrans*, commonly known as nutmeg, have a recalcitrant nature, which means that they are difficult to store for long periods without losing their viability. Any delay in planting may lead to a significant decrease in the seeds' viability, and the germination rate could rapidly decline over time. PGRs (plant growth regulators) like auxins, gibberlins, cytokinins and cytokinins play a critical role in regulating various plant growth and developmental processes, including seed germination. In the case of *M. fragrans* also PGRs, have been shown to have significant effects on the germination process.

Materials and Method:

Seed collection

Mature fruits of *M. fragrans* were collected from the Myristica plantations from Thrissur District of Kerala. (N 10°29'56"L, E 76°14'01"L. Altitude - 40 m). The collected fruits were brought to the laboratory in sealed polythene bags. The seeds were removed from the fruits and dearillated. Seeds did not have any apparent physical damage or infestation by insects were selected the seed surface was sterilized with 1% sodium hypochlorite for 30 minutes and washed thoroughly with sterilized distilled water for five times.

Plant growth regulators (PGR)

The simultaneous hormone profiling included various plant growth regulators (PGRs), such as two types of auxins: indole 3-acetic acid (IAA) and indole 3-butyric acid (IBA); three types of gibberellins: GA3, GA4, and GA7; three types of cytokinins: benzyl aminopurine (BA), trans Zeatin (tZ), and trans Zeatin riboside (tZR); abscisic acid (ABA); salicylic acid (SA); three types of jasmonates: jasmonic acid (JA), cis-jasmone (cisJ), and methyl jasmone (meJ); as well as epibrasinolide (24-epiBL) and 1-amino cyclopropane-1-carboxylic acid (ACC).

Hormone extraction and LC-MS/MS

The extraction of different plant growth regulators was followed as per the protocol suggested by Pan *et al.* (2008). To begin, 100mg of dry tissue was frozen in liquid nitrogen and homogenized in a mixture of 1-propanol, water, and concentrated hydrochloric acid (2:1:0.002, v/v/v). The resulting mixture was sonicated for 30 minutes and left overnight at 40°C. For the liquid endosperm of tender nuts, the first step was to pass it through a sep-pac cartridge (C18 column) to remove impurities. Then, 5mL of the sample was homogenized using the same mixture of 1-propanol, water, and concentrated hydrochloric acid, sonicated for 30 minutes, and left overnight at 40°C. Next, dichloromethane was added to the homogenate, sonicated for 30 minutes, and centrifuged at 12,000rpm for 10 minutes. After centrifugation, the bottom layer was transferred to a conical flask containing sodium sulfate to remove any remaining water traces. Finally, the sample was evaporated using a flash evaporator and 1mL of mobile phase was added to the dried sample. The solvent was then filtered using a nylon filter paper and injected into LC-MS/MS for hormone analysis.

The mobile phase consisted of two solvent systems, A and B. Solvent A was composed of a mixture of water, acetonitrile, and acetic acid in the ratio of 95:5:0.05 (v/v/v), while solvent B was composed of acetonitrile, water, and acetic acid in the ratio of 95:5:0.05 (v/v/v). A gradient program was employed in the analysis, with the following sequence: 85% solvent A and 15% solvent B for 1 minute, followed by 15% solvent A and 85% solvent B for 1 minute, and then a linear gradient leading to 85% solvent A and 15% solvent B for 4 minutes. The flow rate was set at 0.20 mL/min, and the runtime was 20 minutes. The quantification of analytes was performed using the multiple reaction monitoring (MRM) detection method. The analytical column utilized was a 2.1 x 50 mm UPLC BEH C-18 column with 1.7 µm particles, protected by a vanguard 2.1 x 5 mm BEH C-18 with 1.7 µm. The guard column was sourced from Waters (USA), and the column temperature was maintained at 25°C. The samples were extracted three

times, and calibration curves were generated using chemical standards of hormones procured from Sigma-Aldrich (USA). The eluted hormones were quantified using a chromatographic column TQD-MS/MS (Waters – Acquity, USA).

Result and Discussion:

The profiling of various 15 plant growth regulators (PGRs), such as two types of auxins: indole 3-acetic acid (IAA) and indole 3-butyric acid (IBA); three types of gibberellins: GA3, GA4, and GA7; three types of cytokinins: benzyl aminopurine (BA), trans Zeatin (tZ), and trans Zeatin riboside (tZR); abscisic acid (ABA); salicylic acid (SA); three types of jasmonates: jasmonic acid (JA), cis-jasmone (cisJ), and methyl jasmone (meJ); as well as epibrasinolide (24-epiBL) and 1-amino cyclopropane-1-carboxylic acid (ACC) at seed germination and desiccation and the results were done and compared with the PGR level of seeds from freshly harvested fruits. The result with statistical analysis is as shown in Table 1.

Table 1: Profiling of plant growth regulators

Sl. No	**Plant Growth Regulators**		**Fresh Seed** ng g-1DW	**Germinating seed** ng g-1DW	**Desiccated seed** ng g-1DW
1	Auxins	3-Indole Acetic Acid (IAA)	14.34±0.00^{b}	29.13±1.09^{a}	8.34±0.00^{c}
2		3-Indole Butyric Acid (IBA)	3.40±0.01^{a}	5.96±0.58^{a}	0.86±0.01^{b}
3	ABA	Abscisic Acid (ABA)	305.59±0.09^{a}	21.36±0.53^{c}	191.06±0.74^{b}
4	Gibberellic Acids (GAs)	Gibberellic Acid 7 (GA7)	0.04±0.00^{a}	0.05±0.00^{a}	0.03±0.00^{a}
5		Gibberellic Acid 4 (GA4)	32.09±0.11^{b}	62.59±1.48^{a}	7.48±0.00^{c}
6		Gibberellic Acid 3 (GA3)	0.32±0.01^{a}	0.44±0.02^{a}	0.19±0.00^{a}
7	Epibrasinolide	Epibrasinolide	18.48±0.17^{a}	11.33±0.12^{b}	13.81±0.58^{b}
8	ACC	1-Amino Cyclopropane-1-Carboxylic acid	0.67±0.01^{a}	0.99±0.09^{a}	0.06±0.00^{b}
9	SA	Salicylic Acid (SA)	6292.14±1.79^{a}	4774.66±32.46^{c}	5408.18±4.07^{b}
10	Jasmonates	Jasmonic Acid (JA)	16.75± 0.16^{a}	8.08±0.04^{b}	19.58±0.15^{a}
11		Cis-Jasmone	32.76±0.13^{a}	19.12±0.54^{b}	22.20±0.12^{b}
12		Methyl Jasmonate	30.21±0.54^{b}	49.34±0.62^{a}	17.37±0.10^{c}

13	Cytokinins	Zeatin trans isomer (tZ)	5.45±0.19[a]	5.79±0.36[a]	1.45±0.06[b]
14		Trans zeatin Riboside (tZR)	0.07±0.01[b]	0.09±0.01[b]	0.14±0.02[a]
15		Benzyl aminopurine (BA)	0.58±0.00[b]	1.0±0.04[a]	0.51±0.01[b]

Values are Means of three replicates with Standard Error; Significant changes were indicated by letters in superscript. ±: Standard Error; ng: nanogram, g: gram, DW: Dry weight.

During the three stages studied (fresh, desiccated, and germination), the principal form of auxins in M. fragrans seeds was IAA. Its concentration was highest during seed germination (29.13±1.09 ng g-1DW), followed by the fresh stage (14.34±0.00 ng g-1DW), and the desiccated stage (8.34±0.00 ng g-1DW). On the other hand, the concentration of IBA was comparatively low in all three stages. There have been reports suggesting that IAA (indole-3-acetic acid) is the primary auxin present in the developing seeds of angiosperms, as stated by Bewley and Black in 1994. Additionally, evidence suggests that IAA plays a critical role in establishing embryonic polarity in both somatic and zygotic embryos, as noted by Michalczuk *et al.* in 1992 and Kong *et al.* in 1997.

Gibberellins are plant growth regulators that affect many physiological processes, including seed germination. M. fragrans seeds were assayed to determine the levels of the three principal forms of gibberellins - GA3, GA4, and GA7. The results showed that GA4 was the predominant form of gibberellins in both developing and germinating seeds, while GA3 and GA7 were present in significantly lower levels during seed maturation and germination. However, the level of GA4 in embryonal tissue was significantly affected by embryo desiccation. The GA4 content decreased drastically from 32.09±0.11 ng g-1DW (fresh seed) and 62.59±1.48 ng g-1DW (germinating seed) to 7.48±0.00 ng g-1DW. In contrast, the levels of GA3 and GA7 remained relatively unchanged during seed germination and desiccation. Recalcitrant seeds proceed directly to germination without maturation drying and dormancy. Therefore, Fennimore and Foley (1998) have reported that the level of GAs in these seeds at the fully mature stage is higher compared to orthodox seeds.

Cytokinins are important in promoting cell division and elongation in embryonic tissue, as well as providing positional information for embryo development. The cytokinins Benzyl amino purine (BA), Zeatin trans isomer (tZ), and Trans Zeatin Riboside (tZR) were analyzed to understand their role in seed germination and desiccation. During embryo development, tZ was present in much higher quantities than tZR and BA. Desiccation increased the concentration of tZR from 0.07±0.01 ng g-1DW in fresh seeds to 0.09±0.01 ng g-1DW in germinating seeds, and then to 0.14±0.02 ng g-1DW. A Cytokinins can enhance cell division by stimulating protein synthesis and shortening the mitotic and meiotic interphase period (Davies, 1995).

ABA is a crucial growth regulator that plays a significant role in preventing premature germination and enabling the acquisition of desiccation tolerance in seeds. The level of ABA is

higher in the fresh stage of embryonic tissue, and it declines during seed germination, which contributes to the delay in germination (Ali - Rachedi *et al.,* 2004). However, during desiccation, an elevated concentration of ABA (191.06±0.74 ng g-1DW) indicates a stressful condition that may result in a loss of seed viability.

According to Finkelstein *et al.,* 2008), the balance between gibberellins and ABA plays a crucial role in determining seed maturation and germination. Based on their findings, it can be inferred that seed germination occurs when the level of gibberellins is higher than that of ABA. Conversely, when the level of ABA exceeds that of gibberellins, seed germination is suppressed. Gibberellins and ABA are the primary regulators of the opposing processes of seed germination and dormancy, respectively (Groot *et al.,* 1992). ABA is mainly responsible for initiating seed dormancy, while gibberellins promote seed germination. Therefore, the balance between these two hormones is essential for regulating these antagonistic processes. Reports are that Ascorbic Acid (ASC) also plays a critical role in mediating the antagonistic effect of ABA and GA on seed germination in both recalcitrant and orthodox seeds (Tommasi, 1999).

Aside from the five classical phytohormones, there are many other plant growth regulators (PGRs) that have been identified. These include Salicylic Acid (SA), Jasmonates, Brassinosteroids (BRs) and ACC.

The plant growth regulator SA, which is known to have therapeutic effects on plants similar to those in mammals, has been found to have a significant impact on various physiological processes in plants, including seed germination. Throughout all stages of the study, SA was present in exceptionally high quantities. The reason for the higher quantity of SA at the fruit shedding stage was due to a substantial influx of SA from the maternal tissues (6292.14±1.79 ng g-1DW). However, the SA levels dropped during germination (4774.66±32.46 ng g-1DW). Interestingly, SA levels increased to 5408.18±4.07 ng g-1DW upon desiccation.) SA has the potential to alleviate abiotic stress (Ahmad *et al.,* 2011)

In this study, the role of jasmonates as plant growth regulators (PGRs) in various developmental processes including embryo development and seed germination was investigated. The study analyzed three forms of jasmonates - JA, Me J, and cis J. The results showed that desiccation helped maintain the level of cis J in the embryonal tissue, while JA increased in response to dehydration of embryonal tissue. Jasmonates play a dual role in seed germination, as they are inhibitory towards the germination of non-dormant seeds but promote the germination of dormant ones, as reported by Somata *et al.,* 2017). The impact of jasmonates on embryo development has also been studied and documented by Browse and Howe (2008) and Gfeller *et al.,* 2010). Jasmonates serve as cellular regulators and participate in a variety of developmental processes in plants, such as embryo development, seed germination, root growth, and seedling development. The involvement of JA in these biological processes is indirect, as it induces the biosynthesis of secondary metabolites such as anthocyanins, glucosinolates, terpenoids, or phenolics (Somata *et al.,* 2017). The study also assayed the predominant form of brassinosteroids, 24epi-BL, and found that it was present in higher concentrations. Furthermore, desiccation was found to enhance the production of this PGR, suggesting its role in defending

against desiccation stress. Kaur *et al.,* 2017) suggested that BRs (phytobiliary receptors) aid plants in coping with abiotic stress by impacting various types of osmoprotectants. In fact, when subjected to drought, Arabidopsis thaliana has been found to exhibit augmented accumulation of critical osmolytes that confer stress tolerance (Fàbregas *et al.,* 2018).This recalcitrant species was found to contain extremely low levels of 1-Aminocyclopropane-1-carboxylic acid (ACC), which is the direct precursor of the plant hormone ethylene. However, a significant increase in ACC was observed during seed germination, and it declined during seed desiccation.

The relative abundance of PGRs at the time of seed shed gives an idea about the hormonal status required to carry out germination process without delay. The relative amounts of hormones in the embryos at seed shedding stage is as shown below.

SA > ABA> cisJ> GA4 > Me J > 24epi-BL > JA > IAA >tZ > IBA > ACC > BA > GA3 > tZR > GA7.

However, the level of PGRs in embryo during seed germination and desiccation greatly altered and relative amount of these PGRs resulting in the loss of seed viability or germination of seed. The relative amounts of hormones in the embryos during seed germination and desiccation were as given below.

During seed germination: SA > GA4> Me J> IAA > ABA> cis J > 24epi-BL > JA > IBA > tZ > BA > ACC > GA3 > tZR > GA7.

During seed desiccation: SA > ABA> cis J> JA > Me J >24epi-BL > IAA > GA4 > tZ > IBA > BA > GA3 > tZR > ACC > GA7.

The results indicate that desiccation was affected the synthesis of the major growth regulating phytohormones like PGGA4, IAA, BA and ABA.

Conclusion:

Plant growth regulators play a crucial role in both seed germination and seed desiccation processes. These regulators, such as hormones and signaling molecules, influence various physiological and biochemical events that determine the fate of seeds. During germination, plant growth regulators facilitate the activation of metabolic processes, leading to the breaking of seed dormancy and initiation of growth. They regulate water uptake, nutrient mobilization, and enzymatic activities required for seedling establishment. Additionally, plant growth regulators help coordinate the delicate balance between seed desiccation and viability maintenance. They control the expression of genes involved in desiccation tolerance, membrane stability, and antioxidant defense systems, thus ensuring successful seed preservation during storage. Understanding the intricate roles of plant growth regulators in seed germination and desiccation provides valuable insights for improving seed quality, crop productivity, and conservation strategies. Future research in this field will continue to unravel the complexities of these regulators, opening new avenues for sustainable agriculture and ecological restoration.

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MULTI-OMICS: AN INTEGRATIVE APPROACH TO ENHANCE DISEASE RESISTANCE IN PLANTS

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Abstract:

In view of ever-growing population and decreasing natural resources, there is need to enhance food production that can possibly be achieved by improving upon qualitative and quantitative traits of crop plants by adopting new analytical tools and technologies. During the last decade or so rapid progress has been made in plant biology, especially with the introduction of high throughput 'omics' technologies. The three primary omics technologies-genomics, proteomics, and metabolomics involve the quantitative and characterisation of the genome, proteome, and metabolome, respectively, using incredibly quick, automated, and miniaturised techniques. These technologies strive to elucidate the overall expression of genes, proteins, and metabolites in a context that is functionally relevant, and they give information about the molecular underpinnings of numerous essential processes involved in plant growth and development. Advances in plant genomics research have opened up new perspectives and opportunities for improving crop plants and their productivity. Using genome sequences, collections of transcripts from stress-specific cells and tissues, transcript, protein, and metabolite profiles, and their relationships, the genomics technologies have been proven to be helpful in understanding the multigenicity of biotic and abiotic plant stress responses.

Keywords: Omics, genomics, transcriptomics, proteomics, metabolomics, bioinformatics.

Introduction

With the advent of high yielding crop types during the 20th century, especially after the first "green revolution" that assisted in keeping up with population expansion, food production has increased significantly. Changes in the genetic potential of crops and pertinent management techniques have been the driving forces behind the rise in output. The conventional plant breeding techniques based on phenotypic selection and statistical qualitative genetics principles have enhanced yield stability and continuously increased seed production (Wollenweber *et al.,* 2005, Hammer and Jordan, 2007). Though long term selection experiments have indicated sufficient potential for genetic improvement of qualitative traits over many generations (Dudley and Lambert, 1992), a decline in crop yield increase has become evident (Conway and Toenniessen, 1999; Mann, 1999) that may result in a gap between demand and supply due to high population growth rate (Wollenweber *et al.,* 2005).

The global population projections are grim reminder of the imperative need to increase food production and double it by the year 2040 from the present level. The future food security

for evergrowing population will depend on acceleration of yield gains per unit land and per unit of input for the major food crops at rates well above the historical trend of past 50 years. However, natural resources are decreasing rapidly for agriculture as a result of economic development, which is diverting these resources for non-agricultural uses. Today's top resource and environmental challenges for farmers worldwide include soil erosion and degradation, water scarcity, drought, and salinity, the co-evolution of pests, pathogens, and hosts, and the effects of climate change (Tilman *et al.,* 2001). Water scarcity is becoming an increasingly major barrier to the expansion of agricultural output in arid and semiarid regions of the world (Raskin *et al.,* 1998; Gleick, 2000). International Water Management Institute has projected that by the year 2025, most regions of the world will experience either absolute or severe water scarcity (Ruttan, 2005). Thus, drought has become the single most limiting constraint to crop production worldwide, and the need for a 'blue revolution' in which water use efficiency (WUE) of crop plants is improved, has been highlighted (Zhang and Yang, 2004).

The emergence of the novel 'omics' technologies, such as genomics, proteomics and metabolomics, is now permitting researchers to identify the genetic underpinnings of crop improvement, namely the genes, that contribute to the improved productivity and quality of modern crop varieties. These omics technologies enable a direct and unbiased monitoring of the factors affecting crop growth and yield formation, and provide the data that can be directly utilized to investigate the complex interplay between the plant, its metabolism, and also the stress represented by the environment or the biological threats by insects, fungi or other pathogens. These technologies also help in thorough investigation of the biology behind agronomic traits at the physiological, biochemical and molecular levels, and permit the elucidation of molecular circuitry of the crop plants, ultimately paving way for improved crop production (www.genedata.com). Indeed, the various- 'omics' have become a staple of Plant Physiology (Raikhel, 2005).

The term 'omics' refers to the comprehensive analysis of the biological system. Informally, the neologism omics has come to refer to a comprehensive study involving the acquisition of vast data sets. An omics approach can be considered to be large-scale data rich biology consisting of a heavy data mining or bioinformatics component. The modern concept of omics was initiated by Human Genome Project, which was launched in 1986 (Wheelock and Miyagawa, 2006). Omics has been driven more by emerging experimental technologies than by the novel hypothesis (Yin and Struik, 2007).

Genomics, proteomics and metabolomics are the three core omics technologies, which respectively deal with the analysis of genome, proteome and metabolome of cells and tissues of an organism. These technologies involve large data sets and high throughout methods. Most of the people encompass all the three disciplines and technologies under 'genomics' and use this term in a broader sense. For example, `genomics' includes everything from gene sequencing, annotation of function genes, and genome architecture to studying patterns of gene expression at transcriptome level (transcriptomics), proteome level (proteomics) and metabolite flux (metabolomics). Due to the magnitude and complexity of omic data, these disciplines are

underpinned by information technology support through bioinformatics. In the following text, we provide a comprehensive introduction to the basic aspects of genomics, proteomics and metabolomics, and an overview of the associated techniques as well as (potential) applications of these technologies in crop improvement strategies.

Genomics

Plant genomes are best described in terms of genome size, gene content, extent of repetive sequences and polyploidy/duplication events (Campos-de Quiroz, 2002). Genomics investigates how variation in genes affects protein structure and function throughout the life of a cell. When a cell senses changes in its environment, it responds by accessing different components of genome. It is a rapid and accurate method of gene mapping on the chromosomes than deletion and restriction mapping. The genomic techniques are highly powerful, efficient and effective in solving complex genetic problems.

Techniques to study genomics

1. Genome sequencing

Elucidate the pattern of arrangement of nucleotide bases in the entire genome.

The availability of whole genome resources coupled with Next-Generation Sequencing (NGS) technologies has now been applied routinely and cost effectively to rapidly generate plant and/or pathogen genome or transcriptome marker sequences associated with virulence phenotypes in the pathogen or resistance phenotypes in the plant, potentially leading to improvements in plant disease management.

Generation	**Methods**	**Read length and Run time per run**	**Principle**
		First Generation	
Sanger dideoxy Method	Sanger dideoxy Method (Radioactivity or Fluorescence; DNA amplification	700-900 bases 20 min-3 hours	Chain termination
		Next generation Sequencing	
Second Generation	454 pyrosequencing (Fluorescence DNA Amplification, Massively parallel)	400-500 bases 24 hrs	Synthesis
	Illumina/solexa method (Fluorescence DNA	50-100 bases 1-10 days	Synthesis

	Amplification, Massively parallel) Solid method (Fluorescence DNA Amplification, Massively parallel)	 50-100 bases 1-2 weeks	 Ligation
Third Generation	Heliscope single molecular sequencer (Fluorescence; Single molecule) Pacific Biosciences SMRT (Fluorescence; Single molecule)	Upto 55 bases Fossil DNA accuracy Greatly improved 2500-3000 bases 30 min-2hrs	Single molecule Synthesis Single molecule Synthesis
Fourth Generation	Ion torrent (electronic pH; DNA Amplification Oxford nanopore (electronic current, Single molecule, Real time)	100-200 bases 2hrs 1000 bases 0.5-10 hrs	Synthesis Strand sequencing

2. Genome assembly

Once the sequencing is done, the sequence must be assembled before they can be analysed. Assembly generates a genome suitable for annotation,

i. *De novo* genome assembly
ii. Reference based genome analysis

3. Genome annotation

Genome annotation refers to finding the potential genes and assigning functions of gene in a newly sequenced genome based on its similarity to the already available gene sequences in sequence databases, (GenBank) to them by using similarity detection tool (BLAST programme).

- ✓ Most of this is done *in silico*, i.e., with the aid of computer programs such as *Pridigal, Augustus, Genemark, Fgenesh and Marker-P.*
- ✓ Gene Ontology (GO) mapping gives the classification of genes into 3 components according to their assigned molecular function, biological process and cellular component.

Functional genomics (transcriptomics and proteomics)

Functional genomics is the branch of genomics which includes functions of the genes encoded in the genome. It focuses on the dynamic aspects like transcription, translation and protein-protein interaction at different developmental stages and environmental conditions.

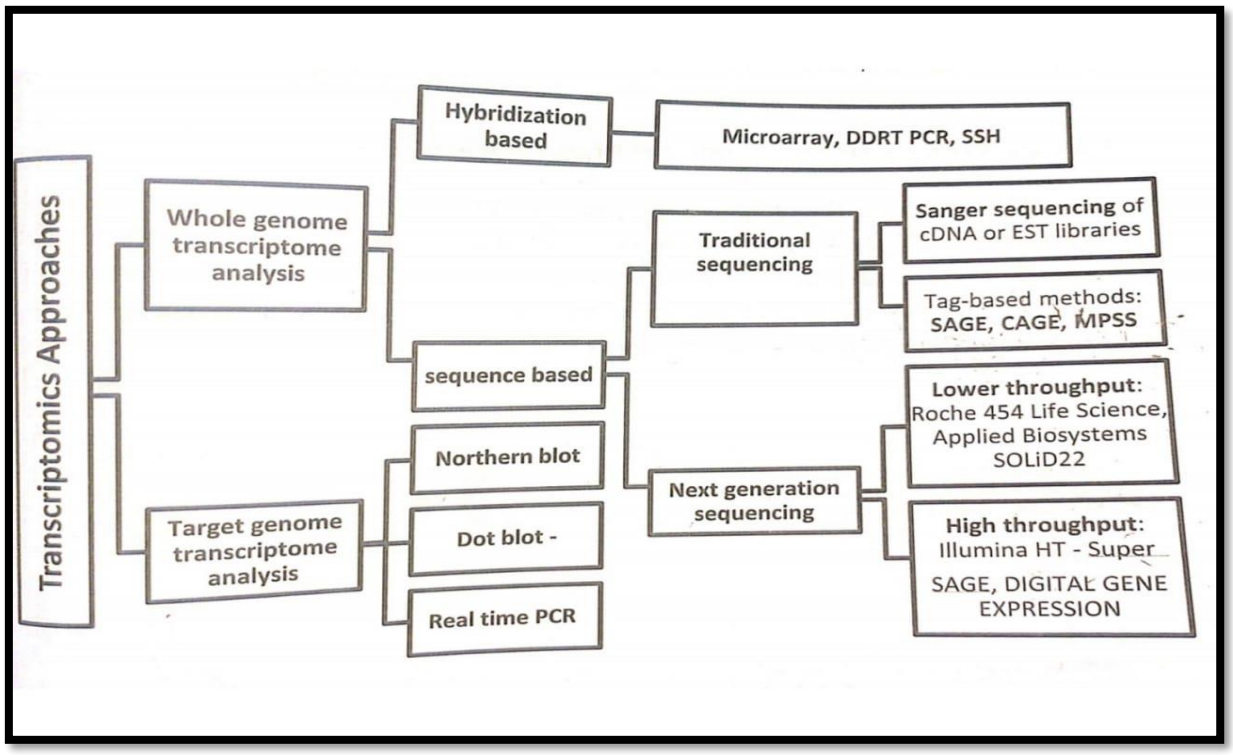
Thus functional genomics describe spatial and temporal characterization of genes from genome at transcriptome level called transcriptomics and at proteome level called proteomics.

Transcriptomics

Transcriptome: It is the whole set of RNAs (mRNA, rRNA, tRNA and other non-coding RNA) transcribed by the genome from a specific tissue or cell type at a developmental stages.

Transcriptomics: It is the comprehensive and high throughput analysis of gene expression. It is used to determine the transcriptional structure of genes, in terms of their start sites, 5' and 3' ends, splicing patterns and other post transcriptional modifications. It is also used

- To quantify spatial and temporal gene expression
- To predict gene function
- To study genome dynamics
- To discover cis-regulatory motifs
- To screen candidate genes



SAGE (Serial Analysis of Gene Expression)

SAGE is the library of long serial molecules prepared by, linking of a short sequence tag (10-14 bp) from unique positions of each transcript and sequenced by Sanger method. The resulting sequence are separated and mapped to a genome or set of ESTS to check expression of particular gene, total number of tags are counted. Quantification of presence of number of times a particular tag provides the expression level of the corresponding gene. SAGE can also help to

identify new genes expressing in particular tissue or condition. Sequencing cost is the main constraint in this method.

MPSS (Massively Parallel Signature Sequencing)

MPSS uses 17-20 bp signature sequence adjacent to the 3'-end of mRNA generated by restriction enzymes Sau3A or Dopll. Each signature sequence is first cloned on to microbeads (100,000 different sequences at a time) such that each bead consists of only one type of DNA sequence.

Steps:

1. Arraying of microbeads in flow cell for sequencing and quantification
2. Fluorescent labelling of signature sequences followed by hybridization
3. Detection of encoders with unique label by image acquisition
4. Cleavage and removal of set of four bases to reveal the next four bases for a new round of hybridization
5. Analysis of MPSS dataset by comparing with all other signatures and counting all identical signatures
6. The level of expression of any single gene is calculated by dividing total number of signatures for that gene present in the samples with all signature sequences identified

RNA-Seq (Whole transcriptome shotgun sequencing)

RNA-seq (high throughput sequencing of cDNA) is recently developed deep- sequencing technologies revolutionizing the study of the transcriptome which uses NGS to reveal the presence and quantity of RNA in a biological sample at a given moment. It is a first sequencing-based method that allows the entire transcriptome to be surveyed in a very high-throughput and quantitative manner.

- RNA-seq offer number of advantages over the microarray for differential expression analysis

Advantages

1. It is not limited to detecting transcripts that correspond to existing genomic sequence
2. It results low background signal
3. It does not have an upper limit for quantification
4. It has a large dynamic range of expression levels over which transcripts can be detected
5. It can be used for SNPs identification, paralogous genes and alternative splicing
6. It doesn't need hybridization

Disadvantages

1. It involves high cost
2. It requires high power computing facilities
3. It demands bioinformatics tools for analysis
4. It requires more skill to interpret the data

Proteomics

Proteome: It is the total set of protein species present in a biological unit (organelle, cell, tissue, organ, individual, species and ecosystem) at any developmental stage and under specific environmental conditions.

Proteomics: Systematic analysis and documentation of protein and post translational modification in an organism or organelle or in a cell or in a specific tissue at a given time. The term 'proteomics' was coined by Mark Wilkins (1994).

Secretomics: It is a subset of proteomics in which all of the secreted proteins of a cell, tissue, or organism are analysed.

Secretome: The global group of secreted proteins into the extracellular space by a cell, tissue, cell organ or organism at any given time and conditions through known and unknown secretory. The term 'secretome' was coined by Tjalsma *et al.* 2000.

Structural proteomics

For structural proteomics all protein sequences are first grouped into families on the basis of homology. Then representative proteins are selected from each family. Genome sequencing projects in second phase is mainly concentrated on proteome analysis, in which the detailed investigation of the functions, functional networks and Three-dimensional structures (3D) structures of proteins through NMR and X-ray crystallography and models are built for the remaining proteins via comparative modelling.

X-ray crystallography/X-ray diffractometer is a non-destructive technique to study for visualizing any crystalline structure of biomolecules like protein, antibiotics, fats, DNA, RNA using x-ray.

Proteomics tools

1. **Gel:** 2D-PAGE, DIGE (Differential in Gel Electrophoresis)
2. **Non-gel:** LC, LC/MS/LC-MS, MALDI-TOF, Mud PIT, Protein microarray, NMR, X-ray crystallography

Methodology

a. Sample fractionization and solubilization
b. Separation by 2D GE
c. Spot excision
d. Peptide digestion
e. MALDI TOF (Matrix-assisted laser desorption/ionization- time-of-flight)
f. Separate peptides
g. Data Base Search & Analysis
h. Functional Validation

List of main Proteome databases

a. SwissProt
b. TrEMBL (translated European Molecular Biology Lab)
c. Protein Information Resources (PIR)

These three databases recently merged to form UniProt, to search for information on desired protein.

Metabolomics

Metabolome: Complete set of small-molecule metabolites (such as metabolic intermediates, hormones and other signaling molecules, and secondary metabolites) to be found within a biological sample, such as a single organism

Metabolites: Intermediates and products of metabolism. Within the context of metabolomics, a metabolite is usually defined as any molecule less than 1 kDa in size. These include antibiotics, pigments, carbohydrates, fatty acids and amino acids

Metabolome analysis: It is an integrated approach by taking advantage of various instruments to characterize metabolites along with genes and proteins responsible for their generation

The systematic collection of metabolite profiles

It is performed with various instruments capable of high throughput and simultaneous measurement like,

- Gas chromatography mass spectrometry (GS-MS)
- Liquid chromatography mass spectrometry (LC-MS)
- Gas chromatography-time-of-flight mass spectroscopy (GC-TOF-MS)
- Capillary electrophoresis mass spectroscopy (CE-MS)
- High-performance liquid chromatography-ultraviolet-electrospray ionization mass spectrometry (HPLC-UV-ESI-MS)
- Fourier-transformed infrared spectroscopy
- NMR based methods

Statistical methods

Principal component analysis (PCA)

Batch-learning self-organizing mapping analysis (BL-SOM)

Partial least square (PLS)

Orthologus PLS (O-PLS)

Basic work flow

a. Sample collection, treatment and processing
b. Analytical platform
c. Separation techniques: GC, HPLC, UPLC, CE
d. Detection techniques: NMR&MS
e. Data analysis using multivariate analysisque: PCA, PLS, BL SOM
f. Validation followed by clinical application

Application of metabolomics

- To study of plant biochemistry secondary metabolites, chemicals such as asscents, flavours, alkaloids and pigments
- To understand how the plant combats infectious and non-infectious disease
- To identify key compounds important in host defenses

Conclusion:

Considerable progress has been made building infrastructure for applying knowledge and tools of genomics (and other ‘omics’) to allow the characteristics of crop plant to be altered for

improved actual and potential yields, increased resource use efficiency and enhanced crop system health. To develop ways to focus on problem- and process-oriented objectives leading to crop improvement, it is still necessary to link disciplines like structural genomics, transcriptomics, proteomics, and metabolomics with plant physiology, biochemistry, and plant breeding. This study is expected to advance into a highly computer-intensive field, allowing for in silico evaluations of crop responses to genetic fine-tuning under predetermined environmental scenarios and developing into a potent tool for aiding breeding for complex crop traits. It is impossible to overlook the genomics revolution's impact on plant breeding. In order to improve agricultural productivity and sustainability and use them for the benefit of the general population, it is essentially important to promote all novel approaches.

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UNDERSTANDING THE WATER FOOTPRINT: KEY TO PROTECT MOST PRECIOUS RESOURCE

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Introduction:

"Water is life's matter and matrix, mother and medium. There is no life without water"

Albert Szent-Gyorgyi

Every individual depends on water for livelihood. Therefore, conservation of water is very important to keep our water pure and away from pollution along with protecting the environment. Conservation means using the water supply wisely and responsibly. The traditional production-sector-based indicators of water-use do not indicate the water that is actually needed by the residents of a country in relation to their consumption pattern. Thus, with the shrinking water resources, the concept of water footprint has been introducedto create a consumption-based indicator of water utilization. Arjen Hoekstra (2002) introduced the concept of water footprint while working at the UNESCO-IHE Institute for Water Education, and expressed the water footprint as a metric to measure the amount of water consumed and polluted to produce goods and services along their full supply chain. The water footprint can be defined as "the volume of water needed for the production of thegoods and services consumed by the residents of a country". It can be internalor external. The internal component refers to the use of domestic water resources while the external component covers the use of water resources other than domestic. The water footprint shows the link that exists between daily consumption of goods by an individual and the problems of water depletion and pollution. The concept of water footprint is important because it assumes much more significance as we move towards a model of growth led by water and energy intensive industries, food products and consumer goods that not just put huge pressure on rarewater resources but contaminate freshwater also.

Thinking about the significance of the environmental footprint, there is a need to provide the information and data in regards to water footprint among the Indian populace. The definition, calculation, and steps to reduce water footprint are compiled in this chapter, which may have a significant impact on changing citizens' attitudes, habits, or manners to protect the environment.

Definition

Water footprint measures the amount of water used to produce each of the goods and services we use. It is a measure of humanity's appropriation of fresh water in volumes of water consumed or polluted. In other words, water footprint is the amount of water consumed in daily life, including the water used to grow and prepare the food eat, water consumed to produce the energy we use and for all of the products in daily life, for example car, furniture and clothes etc.

Classification of water footprint

- Blue water footprint- The blue component covers the use of groundwater and surface water during the production of a commodity.
- Green water footprint- The green component covers the use of rainwater for crop growth.

- Gray water footprint- The gray component covers the water required to dilute the water that is polluted during the production of the commodity.

Causes of increased water footprint

Proper understanding of the causes of increased water footprint is based on the use of freshwater systems by human and that problems of water shortages and pollution can be better understood and addressed by considering production and supply chains as a whole. The four major direct factors determining the water footprint of a country are volume of consumption, which is more or less related to the gross national income; consumption pattern; climatic conditions and agricultural practice adopted.

Effects

Freshwater is a limited natural resource but, its annual availability is limited and demand is growing every day. The water footprint of humanity has already exceeded sustainable levels at several places. Increased water usage/wastage/footprint has led to increased energy requirement to process and deliver it to homes, businesses, farms, and communities, which, in turn, has increased the pollution and depletion of fuel resources. Depleting groundwater and other water lakes can put water supplies, human health, and the atmosphere at great risk. Lower water levels due to removal of groundwater at a faster rate than recharge can occur, can contribute to higher concentrations of natural or human pollutants leading to further increased water footprints.

Water Footprint calculation

The water footprint of a product can be calculated by the adding the water footprints of the process steps taken to produce the product by considering the whole production and supply chain. Similarly, the water footprint of a consumer is calculated by summing the water footprints of all products consumed by the consumer. For example, water footprint of an individual is equal to the water required to produce the goods and services consumed by that individual. It can be calculated with the water footprint calculator developed by UNESCO-IHE. The calculations are based on the water requirements per unit of product.

Food consumption

Cereal products (wheat, rice, maize, etc.)-	Kg per week
Meat products	Kg per week
Dairy products	Kg per week
Eggs	Number per week
Vegetables	Kg per week
Fruits	Kg per week
Starchy roots (potatoes, cassava)	Kg per week
How many cups of coffee do you take per day?	Cup per day
How many cups of tea do you take per day?	Cup per day
How do you prefer to take your food?	
How is your sugar and sweets consumption?	

Domestic water used indoor

How many showers do you take each day?	Number per day
What is the average length of each shower?	Minute per shower
Do your showers have standard or low-flow showerheads?	Standard showerhead

	Lowflow showerhead
How many baths do you have each week?	Number per week
How many times per day do you brush your teeth, shave or wash your hand?	Number per day
Do you leave the tap running when brushing your teethand shaving?	Yes/No
How many loads of laundry do you do in an average week?	Times per week
Do you have a dual flush toilet?	Yes/No
If you wash, your dishes by hand then how many times are washed each day?	Number per day
How long does the water run during each wash?	Minute per wash
If you have a dishwasher, how many times is it used each week?	Number per week

Domestic water used outdoor

How many times per week do you wash a car?	Number per week
How many times do you water your garden each week?	Number per week
How long do you water your garden each time?	Minute per watering
How long per week, do you spend rinsing equipment?	
Driveways or sidewalks each week?	Minute per week
If you have a swimming pool what is its capacity?	Cubic meter
How many times per year do you empty your swimming pool?	Number per year

Industrial goods consumption

What is your gross yearly income?

Part of income, which is consumed by you	US$ per year.

How to reduce water footprint

Reduction in the water footprint has become utmost important because the limited freshwater resources are being consumed and polluted. Following are some important tips that can be considered for minimizing the water footprint:

- Use a low flow showerhead and faucet aerators.
- Install a dual flush or low flow toilet.
- Install a rain barrel for outdoor watering.
- Take shorter showers. A typical shower uses five to ten gallons of water a minute.
- Take baths. A partially filled tub uses less water than all but the shortest showers.
- Turn off the water while brushing your teeth. Before brushing, wet brush and fill a glass for rinsing mouth.
- Turn off the water while shaving. Fill the bottom of the sink with a few inches of warm water in which to rinse your razor.
- Only run full loads of laundry and dishes.
- Fix leaky taps to reduce water loss.
- Use a watering can rather than tube to water plants.

General guidelines to reduce water footprint in day-to-day life

- Animal products have a much higher footprint than fruit, vegetables, grains, etc. Therefore, cutting down the consumption of meat and other animal products like cheese is a great way to reduce the water footprint.
- White meat (poultry and fish meat) has a much lower footprint than red meat (beef and mutton).
- Only buy what one can eat: throwing perfectly good food away is a huge waste of water, so make a shopping list and stick to it.
- Just one cotton t-shirt can require 2,700 litres of water. Fast fashion means buying more clothes than ever before, and this habit should be squeezed.
- Buying second-hand or sustainably produced clothes reduces the water footprint.
- Regularly check the toilet for leaks.
- Put a plastic bottle in the toilet tank. Put an inch of sand or two pebbles in the bottom of a one-liter bottle to weigh it down. Fill the rest of the bottle with water and put it in the toilet tank, safely away from the operating mechanism. In an average home, the bottle may save five gallons or more of water every day without harming the efficiency of the toilet. If the tank is big enough, you may even be able to put in two bottles.
- Use a dishwasher instead of hand washing dishes: When the dishes are hand washed, 2 gallons of water every minute is used, whereas a dishwasher only uses 4-6 gallons per cycle. This means, for washing almost 4 times the number of dishes half the amount of water will be used by switching over.
- Use the automatic washing machine only for full loads: The automatic washer uses 30 to 35 gallons per cycle.
- Do not let the faucet run while cleaning the vegetables. Rinse the vegetables instead in a bowl or sink full of clean water.
- While washing the dishes by hand, do not leave the water running for rinsing. If having two sinks, fill one with rinse water. If having only one sink, first gather all washed dishes in a dish rack, and then rinse them quickly with a pan of water.
- Water the lawn only when it needs it: Watering on a regular schedule does not allow for cool spells or rainfall, which reduces the need for watering.
- Deep-soak the lawn: while watering the lawn, water it long enough for water to soak down to the roots where it is needed. A light sprinkling on the surface will simply evaporate and waste.
- Water during the cool parts of the day: Early morning is better than evening since it helps preventing the growth of fungus.
- Do not water the gutter: Sprinklers should be positioned in such a way so that water lands on the lawn or garden, not in areas where it does no good. Also, avoid watering on windy days when much of the water may be carried off to the sidewalks.
- Plant drought-resistant trees and plants: Many beautiful trees and plants bloom without irrigation.
- Put a layer of covering around trees and plants: Covering slows the evaporation of moisture.

- Use a broom to clean driveways, sidewalks and steps: Using a tube wastes hundreds of gallons of water.
- Do not run the tube while washing the car: Soap down the car from a bucket of soapy water. Use a tube only to rinse it off.
- Tell the children not to play with the water tube and sprinklers: Children love to play under a tube or sprinkler on a hot day. Unfortunately, this practice is extremely wasteful of precious water and should be discouraged.
- Check for leaks in pipes, lines, faucets and connections: Leaks outside the house are easier to ignore since they do not mess up the floor. However, they can be even more wasteful than inside water leaks especially when they occur on the main water line.
- Take shorter showers: Considering a shower uses about 2-5 gallons per minute, cutting the shower time down by 5 to 10 minutes, 12 and 25 gallons per shower pumping water can be saved. In addition, a reasonable amount of energy can be saved which was to be used for heating it. Thus, carbon footprint can be reduced by eliminating unnecessary water pumping and heating.
- Buy plants that require less water: Make sure that the plants are not overwatered. Water them manually and when the plants actually need it rather than having automated sprinklers. Even better, buy and upkeep plants that do not require much watering at all.

Conclusion:

The choices we make in ordinary everyday life about exercise at home, transportation, food, and the products we buy have effects all over the world. A person's individual production and consumption pattern directly affect the global environmental and can be seen worldwide. In other words, every individual footprint affects the world's natural resources. Dangerous atmospheric anomalies resulting from alarmingly increasing global temperature and water pollution are the most important natural factors causing rapid environmental changes. Thus, comprehensive information and data on the water footprint of the Indian population is required. This chapter defines; provide calculation measures and actions to reduce water footprint, which can be an important part of changing the attitude of people toward protecting climate for sustainable development.

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MATHEMATICAL CORRELATION OF VISCOSITY OF ASSOCIATED POLYMERIC SOLUTION AT DIFFERENT TEMPERATURES

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Abstract:

Viscosity of binary liquid mixture have been computed for 2-propanol and benzyl alcohol from McAllister three body (Mc3), four body (Mc4) based on Eyring's theory absolute reaction rate. Which correlate various thermodynamic properties of liquids using the concept of additivity and Jouyban Acree (JA) model based on least square regression analysis method at different temperatures and compared with the measured work of Ching-Ta, and Chein-Hsiun Tu. Standard deviation was the criterion of the success of results. Jouyban Acree (JA) model found to be more consistent with experimental findings.

Keywords: McAllister, Jouyban Acree, Correlation models, benzyl alcohol.

Introduction:

In past few years viscosity of pure liquids, binary and multi component liquid mixtures has become subject of deep interest for chemists in pharmaceuticals Chimankar et al., 2021) and industrial application such as process simulation, equipment design and molecular dynamics Mchaweh *et al.,* 2004) and Wang *et al.* (2005). sometimes experimental determination of transport and other thermodynamic properties of liquid mixtures are very difficult. In that case theoretical models play a significant role in the determination of various thermodynamic and transport properties. To fulfill this requirement various researchers Shukla *et al.,* 2017) have formulated theoretical models to determine the theoretical viscosity in the absence of experimental results. In the continuation of previously published work Awasthi *et al.,* 2019) and Awasthi (2021), this book chapter deals with a comparative study of two correlation models such as McAllister multi body interaction model McAllister (1960) and Jouyban Acree model Jouyban *et al.,* 2005) and (2020) at different temperatures to determine the theoretical values of viscosity and compared with the measured values Ching-Ta *et al.,* 2007). The main aim of this work is to compare and test the validity and applicability, these models at different temperatures for associated polymeric solutions.

Correlation models

McAllister multi body interaction model

McAllister multi body interaction model based on Eyring's theory of absolute reaction rate correlate the various thermodynamic properties of liquids using the concept of additivity. Eyring proposed an empirical equation to relate viscosity of liquid with temperature:

$$\partial = \frac{hN}{M} e^{\Delta G^*/RT^{\#}} \qquad (1)$$

Where $\partial =$ Kinematic viscosity

h = Planck constant

R = Gas constant

M = Molar mass

N = Avogadro number

$T^{\#}$ = Absolute temperature

ΔG^{*} = Movement of molecule between two layers of liquid

McAllister -3-body model

On the basis above assumption proposed by Eyring, McAllister considered three bodied planar encounters in molecules type (1) and (2) and revealed that total free energy of activation depends on fraction of total occurrences and free energy of activation of individual interactions.

$$\Delta G^{*} = X_1^3\Delta G_1^{*} + X_1^2X_2\Delta G_{121}^{*} + 2X_1^2X_2\Delta G_{112}^{*} + X_1X_2^2\Delta G_{212}^{*} + 2X_1X_2^2\Delta G_{123}^{*} + X_2^3\Delta G_2^{*} \quad (2)$$

$$\Delta G_{112}^{*} = \Delta G_{121}^{*} = \Delta G_{12}^{*} \text{ And } \Delta G_{121}^{*} = \Delta G_{122}^{*} = \Delta G_{21}^{*} \quad (3)$$

$$\text{Also, } \Delta G_{12}^{*} = \frac{\Delta G_{121}^{*} + 2\Delta G_{112}^{*}}{3} \text{ and } \Delta G_{21}^{*} = \frac{\Delta G_{212}^{*} + 2\Delta G_{122}^{*}}{3} \quad (4)$$

So on the basis of above assumption equation (2) may be written as:

$$\Delta G^{*} = X_1^3\Delta G_1^{*} + 3X_1^2X_2\Delta G_{12}^{*} + 3X_1X_2^2\Delta G_{21}^{*} + X_2^3\Delta G_2^{*} \quad (5)$$

Now applying equation (1) each set of interactions (111,221,121,112,211,122,212 and 222) and take the logarithms of equation, we get

$$\begin{aligned}\ln\eta = {} & x_1^3\ln\eta_1 + 3x_1^2x_2\ln\eta_{12} + 3x_1x_2\ln\eta_{21} + x_2^3\ln\eta_2 - \ln[x_1 + x_2M_2/M_1] \\ & + 3x_1^2x_2\ln[(2 + M_2/M_1)/3] + 3x_1x_2^2\ln[(1 + 2M_2/M_1)/3] \\ & + x_2^3\ln[M_2/M_1] \quad (6)\end{aligned}$$

McAllister -4-body model

If there is a big size difference between different interacting component molecules and their fraction of total occurrence then total free energy of activation can be computed by equation (7) using free energy of activation of individual interactions and fraction of total occurrences:

$$\Delta G^{*} = X_1^4\Delta G_1^{*} + 4X_1^3X_2\Delta G_{1112}^{*} + 6X_1^2X_2^2\Delta G_{112}^{*} + 4X_1X_2^3\Delta G_{2221}^{*} + X_2^4\Delta G_2^{*} \quad (7)$$

So on the basis of above discussion equation for 4-body interaction is obtained:

$$\begin{aligned}\ln\eta = {} & x_1^4\ln\eta_1 + 4x_1^3x_2\ln\eta_{1112} + 6x_1^2x_2^2\ln\eta_{1122} + 4x_1x_2^2\ln\eta_{222} + x_2^4\ln\eta_2 \\ & - \ln[(x_1 + x_2M_2/M_1)] + 4x_1^3x_2\ln[(3 + M_2/M_1)/4] \\ & + 6x_1^2x_2^2\ln[(1 + M_2/M_1)/2] + 4x_1x_2^3\ln[(1 + 3M_2/M_1)/4] \\ & + x_2^4\ln(M_2/M_1) \quad (8)\end{aligned}$$

M_1 and M_2 are molecular weight of corresponding liquids.

Jouyban Acree model

Jouyban Acree model based on no intercept regression method is one of the most useful correlation model.

$$\ln\eta = X_A.\ln\eta_1 + X_B.\ln\eta_2 + J_0\left[\frac{X_A.X_B}{T}\right] + J_1\left[\frac{X_A.X_B.(X_A - X_B)}{T}\right] + J_2\left[\frac{X_A.X_B.(X_A - X_B)^2}{T}\right] \quad (9)$$

Where J_0, J_1, J_2 are correlation coefficient computed from least square regression analysis.

Result and Discussion:

Table1 represents reduced volume, density of binary liquid mixture, experimental viscosity and viscosity computed by McAllistere-3-body, McAllister-4-body and Jouyban Acree models and their corresponding percentage deviations (%Δ) from 298.15-318.15K. Table2 represent standard deviation of considered correlation models to analyze the relative success of aforementioned models at different temperatures. From a close observation of table 1, it is observed that density of binary liquid mixture decreases as mole fraction (X_1) increases for a particular temperature but as the temperature increases density becomes low, which confirm the breaking of weak hydrogen bonds present between the components of binary liquid mixture. Viscosity computed from McAllister-3-body and McAllister-4-body model are very close to experimental values at initial mole fraction but as mole fraction increases both the models show deviations from experimental values as shown in figure 1. Jouyban Acree models found to be more consistent with experimental values for entire range of mole fraction from 298.15-318.15K.

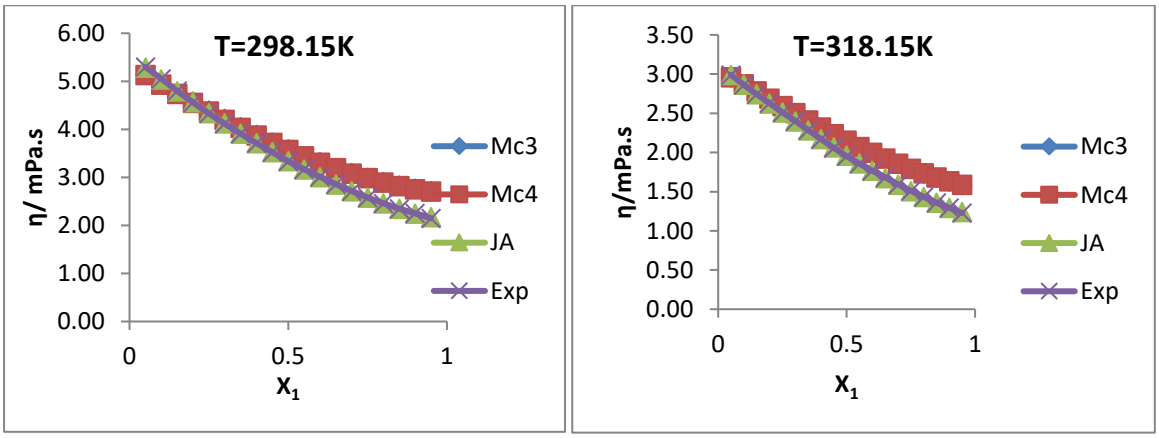

Fig. 1: Variation of viscosity with mole fraction at different temperatures

Standard deviations (σ) for McAllister-3, McAllister-4 and Jouyban Acree model varies from (0.028-0.018), (0.011-0.024) and (0.008-0.005) from 298.15-318.15k as shown in table 2. This variation confirms that Jouyban Acree model deals affair agreement with experimental values in comparison to McAllister -3 and McAllister-4-body models for polar liquid mixture over the entire range of composition at different temperatures.

Table 1: % deviation of McAllister-3-body, 4-body and Jouyban Acree at three different temperatures

X_1	V ~	ρ_{mix}	η_{exp}	η_{Mc3}	η_{Mc4}	η_{JAC}	$\%\Delta_{Mc3}$	$\%\Delta_{Mc4}$	$\%\Delta_{JAC}$
T=298.15K									
0.05	1.2534	1.0328	5.30	5.14	5.13	5.29	2.94	3.16	0.22
0.1	1.2545	1.0237	5.05	4.95	4.93	5.04	2.00	2.36	0.34
0.15	1.2558	1.0143	4.80	4.76	4.74	4.80	0.77	1.20	0.08
0.2	1.2573	1.0044	4.56	4.58	4.56	4.56	-0.36	0.09	-0.09
0.25	1.2589	0.9942	4.33	4.39	4.37	4.34	-1.41	-0.98	-0.18
0.3	1.2607	0.9836	4.12	4.22	4.20	4.12	-2.44	-2.04	-0.18

0.35	1.2626	0.9726	3.91	4.05	4.03	3.91	-3.46	-3.10	-0.09
0.4	1.2648	0.9612	3.71	3.88	3.87	3.71	-4.57	-4.24	-0.02
0.45	1.2671	0.9494	3.52	3.73	3.72	3.52	-5.85	-5.55	0.00
0.5	1.2696	0.9370	3.34	3.58	3.57	3.34	-7.44	-7.15	-0.14
0.55	1.2724	0.9242	3.17	3.45	3.44	3.17	-8.75	-8.45	0.06
0.6	1.2755	0.9108	3.01	3.32	3.31	3.00	-10.27	-9.93	0.20
0.65	1.2789	0.8969	2.86	3.20	3.19	2.85	-12.02	-11.63	0.22
0.7	1.2826	0.8824	2.72	3.09	3.08	2.71	-13.95	-13.51	0.19
0.75	1.2867	0.8673	2.58	3.00	2.98	2.58	-16.13	-15.63	0.04
0.8	1.2913	0.8515	2.46	2.91	2.90	2.46	-18.24	-17.71	0.05
0.85	1.2963	0.8351	2.35	2.83	2.82	2.35	-20.70	-20.18	-0.12
0.9	1.3017	0.8180	2.25	2.77	2.75	2.25	-22.84	-22.39	0.06
0.95	1.3079	0.8000	2.15	2.71	2.70	2.17	-26.15	-25.85	-1.17
				T=308.15K					
0.05	1.2615	1.0250	3.91	3.83	3.84	3.91	2.16	1.87	0.08
0.1	1.2627	1.0159	3.73	3.69	3.71	3.73	1.05	0.59	0.03
0.15	1.2641	1.0064	3.56	3.56	3.58	3.56	0.04	-0.51	0.03
0.2	1.2657	0.9965	3.39	3.42	3.44	3.39	-1.03	-1.61	-0.03
0.25	1.2674	0.9863	3.23	3.29	3.31	3.23	-1.98	-2.54	0.04
0.3	1.2693	0.9756	3.08	3.17	3.18	3.07	-2.98	-3.49	0.14
0.35	1.2713	0.9646	2.92	3.05	3.06	2.92	-4.26	-4.72	0.04
0.4	1.2736	0.9531	2.77	2.93	2.94	2.77	-5.63	-6.04	-0.09
0.45	1.2760	0.9412	2.63	2.82	2.83	2.63	-7.02	-7.41	-0.13
0.5	1.2787	0.9288	2.49	2.71	2.72	2.50	-8.81	-9.19	-0.43
0.55	1.2817	0.9159	2.37	2.61	2.62	2.37	-10.08	-10.48	-0.17
0.6	1.2850	0.9025	2.26	2.51	2.52	2.25	-11.46	-11.90	0.09
0.65	1.2885	0.8885	2.15	2.43	2.44	2.14	-13.14	-13.64	0.18
0.7	1.2924	0.8740	2.04	2.35	2.36	2.04	-14.87	-15.44	0.33
0.75	1.2967	0.8589	1.95	2.27	2.28	1.94	-16.72	-17.36	0.46
0.8	1.3014	0.8431	1.85	2.20	2.22	1.85	-19.07	-19.75	0.28
0.85	1.3067	0.8266	1.76	2.14	2.16	1.76	-21.73	-22.40	-0.02
0.9	1.3124	0.8094	1.68	2.09	2.10	1.68	-24.81	-25.39	-0.55
0.95	1.3187	0.7915	1.60	2.04	2.05	1.62	-27.63	-28.01	-1.22
				T=318.15K					
0.05	1.2700	1.0171	2.99	2.95	2.96	2.99	1.15	1.00	-0.04
0.1	1.2711	1.0080	2.86	2.86	2.87	2.87	-0.02	-0.26	-0.06
0.15	1.2726	0.9984	2.75	2.77	2.78	2.75	-1.00	-1.29	-0.01
0.2	1.2742	0.9884	2.63	2.68	2.69	2.63	-1.97	-2.26	0.05

0.25	1.2760	0.9781	2.51	2.59	2.59	2.51	-2.94	-3.22	0.11
0.3	1.2780	0.9674	2.40	2.49	2.50	2.40	-3.95	-4.21	0.19
0.35	1.2801	0.9563	2.29	2.40	2.41	2.28	-5.21	-5.45	0.10
0.4	1.2825	0.9448	2.17	2.32	2.32	2.17	-6.61	-6.82	-0.04
0.45	1.2850	0.9328	2.06	2.23	2.23	2.07	-8.28	-8.48	-0.32
0.5	1.2879	0.9203	1.95	2.15	2.15	1.96	-9.91	-10.11	-0.44
0.55	1.2910	0.9074	1.86	2.07	2.07	1.86	-11.25	-11.46	-0.20
0.6	1.2944	0.8939	1.77	1.99	2.00	1.77	-12.57	-12.80	0.18
0.65	1.2981	0.8798	1.68	1.92	1.92	1.67	-14.27	-14.52	0.31
0.7	1.3020	0.8653	1.59	1.85	1.86	1.59	-16.20	-16.50	0.34
0.75	1.3064	0.8502	1.51	1.79	1.79	1.51	-18.42	-18.75	0.22
0.8	1.3114	0.8343	1.43	1.73	1.74	1.43	-20.71	-21.06	0.15
0.85	1.3168	0.8178	1.36	1.68	1.68	1.36	-23.44	-23.78	-0.16
0.9	1.3228	0.8005	1.30	1.63	1.63	1.30	-25.78	-26.08	-0.07
0.95	1.3294	0.7825	1.23	1.59	1.59	1.24	-29.09	-29.29	-1.06

Table 2: Standard deviations of different correlation models

	Standard deviation		
T/K	**Mc_3**	**Mc_4**	**JA**
298.15	0.024	0.011	0.008
308.15	0.019	0.033	0.007
318.15	0.018	0.024	0.005

Conclusion:

From the above discussion it may be concluded that all the correlation models deals fair agreement with experimental findings from 298.15-318.15K but among the three Jouyban Acree model show very less standard deviation in comparison to other two models described by McAllister. Increasing order of overall performance of theses model is McAllister-4 body < McAllister-3-body <Jouyban Acree.

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ROLE OF QUORUM SENSING IN BACTERIA

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Abstract

Quorum sensing is a key behaviour co-ordination mechanism employed by many bacteria to regulate gene expression in accordance with population density through the use of signal molecules, known as auto-inducers, that increase in concentration as a function of cell density. Generally, the quorum sensing pathways in bacteria are composed of several main parts, including bacteria populations, signal molecules, protein activators and target genes. In this system, the bacteria secrete the signal molecules into the environment and the concentration increases gradually as the bacteria population grows. In a certain concentration threshold, the molecules become detectable to the bacteria populations and then activate target genes that regulate various behaviour, such as virulence factors. The detection of a minimal threshold stimulatory concentration of an auto-inducer leads to an alteration in gene expression. Gram-positive and Gram-negative bacteria use quorum sensing communication circuits to regulate a diverse array of physiological activities. These processes include symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation and biofilm formation. In general, Gram-negative bacteria use acylated homoserine lactones as auto-inducers and Gram-positive bacteria use processed oligo-peptides to communicate. Recent advances in the field indicate that cell-cell communication *via* auto-inducers occurs both within and between bacterial species. Furthermore, there is mounting data suggesting that bacterial auto-inducers elicit specific responses from host organisms. Although the nature of the chemical signals, the signal relay mechanisms, and the target genes controlled by bacterial quorum sensing systems differ, in every case the ability to communicate with one another allows bacteria to coordinate the gene expression and therefore the behavior, of the entire community. Presumably, this process provide upon bacteria some of the qualities of higher organisms. The evolution of quorum sensing systems in bacteria could and therefore, have been one of the early steps in the development of multicellularity.

Keywords: Quorum sensing, auto-inducer, homoserine lactone, cell to cell communication, virulence, biofilm development, bioluminescence and Quorum quenching-quorum sensing inhibition.

Introduction:

'Quorum' is a latin word. It means the number of members of the group required to be present to transact business or carry out an activity legally. "Quorum sensing" is a process of bacterial cell to cell communication involving the production and detection of extracellular singling molecules (auto-inducers)."Auto-inducers" contribute to the regulation of the expression

of particular genes. Quorum sensing was discovered and described over 25 years ago in two luminescent bacterial species, *Vibrio fischeri* and *Vibrio harveyi* and these bacteria exist as free living cell or as symbiotic in light producing organ of an animal host, such as the Hawaiian bobtail squid (Nealson *et al.,* 1979). In both species, the enzymes responsible for light production are encoded by the luciferase structural operon *luxCDABE* (Engebrecht *et al.,* 1984) and light emission was determined to occur only at high cell population density in response to the accumulation of secreted auto-inducer signaling molecules (Nealson *et al.,* 1979). Until recently, only a few other cases of bacterial regulation of gene expression in response to cell to cell signaling were known. For example, antibiotic production by *Streptomyces* spp. (Dawson and Sia, 1931), conjugation in *Enterococcus faecalis* (Dunny *et al.,* 1978) and fruiting body development in *Myxococcus xanthus* (Dworkin *et al.,* 1985) were also recognized to be controlled by inter cellular signaling. Generally, the effective factors involved in the development of infection by this bacterium can be divided into two categories: Extracellular virulence factors and structural virulence factors. Extracellular factors include pigments that are active in Fe absorption, prevent the growth of other bacterial species and degrade factors including protease, hemolysins and toxins (Figure 1, Veesenmeyer, 2009 and Bjarnsholt, 2010). These bacterial communication systems were considered anomalous and in general, bacteria as a whole were not believed to use cell to cell communication. Rather, the exchange of chemical signals between cells/organisms was assumed to be a trait highly characteristic of eukaryotes. The recent explosion of advances in the field of cell to cell communication in bacteria has now shown that many or most bacteria probably communicate using secreted chemical molecules to coordinate the behavior of the group. Furthermore, we now know that a vast illumination of different classes of chemical signals are employed, that individual species of bacteria use more than one chemical signal and/or more than one type of signal to communicate, that complex hierarchical regulatory circuits have evolved to integrate and process the sensory information and that the signals can be used to differentiate between species in consortia. It seems clear now that the ability to communicate both within and between species is critical for bacterial survival and interaction in natural habitats.

History

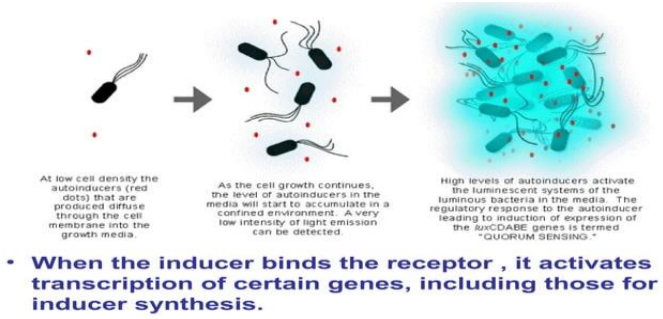
- Nealson *et al.,* 1970) - luminescent in the marine Gram negative bacterium *Vibrio fisheri* controlled by self - produced chemical signal.
- Nealson and Harting (1970) first quorum sensing was described in *Vibrio fisheri* as a symbiotic association with marine eukaryotic hosts that is Squid with light.
- Eberhard *et al.,* 1981) identified the *Vibrio fisheri* auto-inducer signal to be N-3-oxo-hexanoyl-homoserine lactose.
- Eberhard *et al.,* 1983) cloned the genes for the signal generating enzyme the signal receptor and the lux genes.
- Fuqua *et al.,* 1994) introduced the term quorum sensing to describe cell to cell sharing in bacteria.

Cell-to-cell signaling and immune mechanisms

In general, the bacteria control their environmental systems and cell populations through intracellular communications to have the best performance and response according to the demographic and the environmental conditions (Decho *et al.,* 2010). Pathogenic bacteria take the best advantages of communication capability, as an example, they can overcome the host immune system barrier using the community, bacteria will release the virulence factors, thus, the host immune system will be prevented to deliver rapid responses. The process is controlled by a system in bacteria called "Quorum Sensing" (Li and Tian, 2012).

Mechanism of quorum sensing in bacteria

Bacteria showing quorum sensing constitutionally, they must possess three abilities: secretion of a signaling molecules, secretion of an auto-inducers (to detect the change in concentration of signaling molecules) and regulation of gene transcription as a response (Pan and Ren, 2009). This process highly dependent on the diffusion mechanism of the signaling molecules. Quorum sensing signaling molecules are usually secreted at a low level by individual bacteria. At low cell density, the molecules may just diffuse away. At high cell density, the local concentration of signaling molecules may exceed its threshold level and trigger change in gene expression (Bassler, 1999).



Quorum sensing in Gram - positive and Gram - negative bacteria

(A) In Gram - positive bacteria

Gram-positive bacteria use auto-inducing peptides (AIP) as their auto-inducers. When gram positive bacteria detect high concentration of AIPs in their environment, that happen by way of AIPs binding to a receptor to activate a kinase. The kinase phosphorylates a transcription factor which regulates gene transcription. This is called a two-component system (Figer: 2) (Rutherford and Bassler, 2012).

(B) In Gram-negative bacteria

Gram-negative bacteria produced N-acyl homoserine lactose (AHL) as their signaling molecules (Rutherford and Bassler, 2012). Usually AHL do not need additional processing and bind directly to transcription factors to regulate gene expression (Bassler, 1999). Some gram-negative bacteria may be use the two component system as well as gram-positive bacteria (Figer:2) (Rutherford and Bassler, 2012).

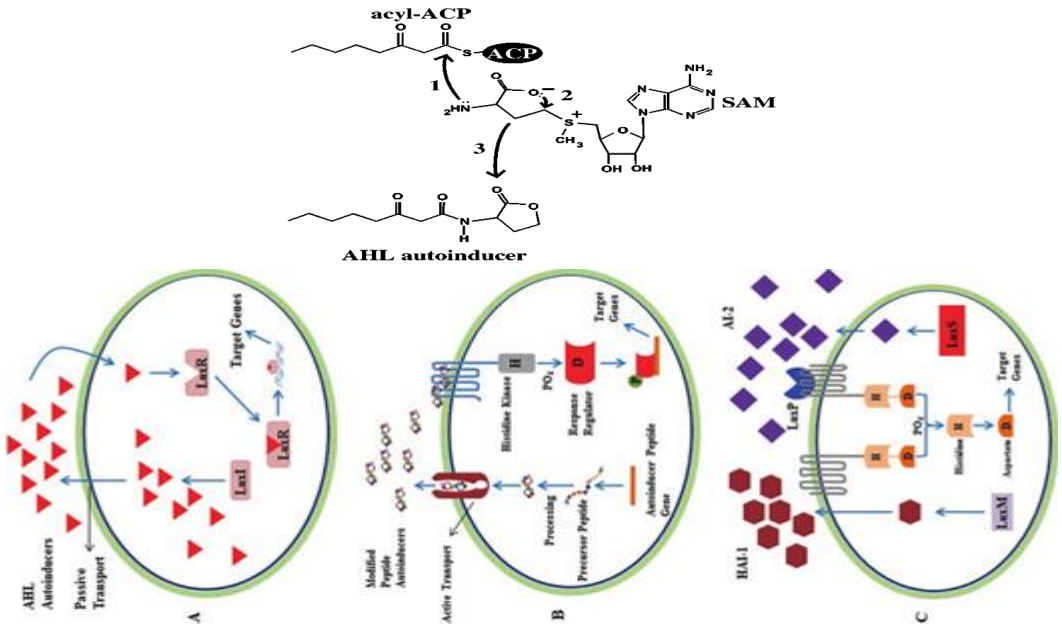


Fig. 2: Mechanism of quorum sensing (A) Gram-negative bacteria (B) Gram-positive bacteria (C) *Vibrio harveyi* bacteria

Example of the quorum sensing bacteria:

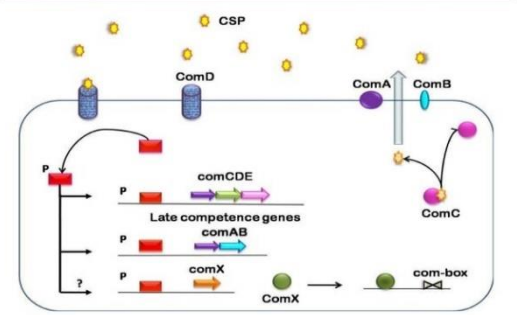
Vibrio fisheri	Gram - negative
Curvibacter sp.	Gram - negative
Escherichia coli	Gram - negative
Salmonella enterica	Gram - positive
Pseudomonas aeruginosa	Gram - positive
Acinetobacter sp.	Gram - positive
Aeromonas sp.	Gram - positive
Yersinia	Gram - positive
Archaea (Methanosaeta harundinacea 6Ac)	Mathanogenic bacteria
Social insects	

Microorganism	Major signal molecules	Regulatory system	Group-derived benefit
Bacillus subtlis	ComX CSF (PhrC) PhrA, -E, -F, -K,-H	ComP/ComA Rap proteins	Competence, sporulation, biofilm formation, antibiotics production
Pseudomonas aeruginosa	3O-C12-HSL and C4-HSL	LasI/LasR-RhlI/RhlR	Structured biofilm formation and virulrnce facters
Staphylococcus aureus	AIP-I and AIP-II	AgrC/AgrA	biofilm formation and virulrnce facters
Streptococcus mutants	CSP (ComC) and XIP (ComS)	ComD/ComE/ComR	Bacteriocins, biofilm formation, Competence

Streptococcus pneumoniae	CSPs	ComD/ComE	Competence biofilm formation, fratricide and virulrnce facters
Vibrio fisheri	HSL auto-inducer *N*-(3-oxohexanoyl)-homoserine lactone (hexagons	LuxI/LuxR	Bioluminescence emission, symbiosis

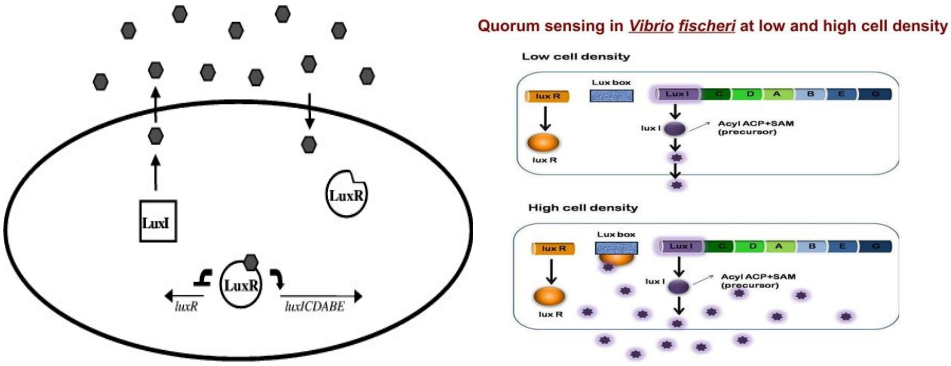
Gram-positive bacteria- peptide mediated Quorum sensing (*Streptococcus pneumoniae* ComD/ComE Competence System

Genetic transformation in bacteria was originally described in *S. pneumoniae* (Dawson and Sia 1931). This process requires that the recipient bacterium become "competent" in order to acquire exogenous DNA molecules. The progression of events that results in *S. pneumoniae* and *B. subtilis* achieving the "competent state" is complex and partial control of this phenomenon is *via* a well-studied quorum sensing system (Haverstein and Morrison, 1999). The peptide signal required for development of the competent state in *S. pneumoniae* is called CSP (competence stimulating peptide). CSP is a 17 amino acid peptide that is produced from a 41 amino acid precursor peptide called ComC (Pozzi *et al.,* 1996). The ComAB and ABC transporter apparatus secretes processed CSP. Detection of accumulated CSP at high cell density occurs *via* the ComD sensor kinase protein. High levels of CSP induce auto phosphorylation of ComD and subsequent transfer of the phosphoryl group to the response regulator ComE. Phosphate-ComE activates transcription of the *comX* gene. ComX is an alternative σ factor that is required for transcription of structural genes that are involved in the development of competence (Lee and Morrison, 1999). The *S. pneumoniae* competent state is transient and occurs only during exponential growth. In later stages of growth *S. pneumoniae* loses the ability to take up exogenous DNA (Dawson and Sia, 1931). Therefore, other, as yet unidentified, regulators must exist that are responsible for transitioning *S. pneumoniae* out of the compatent state. What is remarkable about competence in *S. pneumoniae* is that this bacterium is able to take up DNA irrespective of its sequence and therefore its species of origin. This quorum sensing–controlled process could allow *S. pneumoniae* to assimilate DNA only under conditions that would favor the likelihood of the presence of heterogeneous DNA that contain a collection of genes specifying novel functions that have not evolved in *S. pneumoniae*.



Gram-negative bacteria-LuxI/LuxR bioluminescence System type quorum sensing in *Vibrio fischeri*

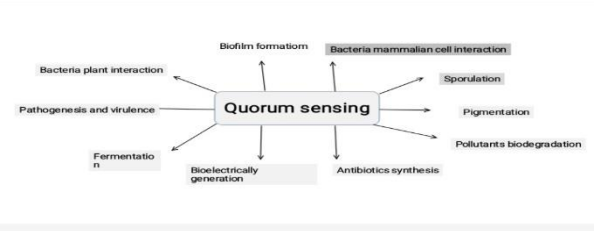
The most obdurately studied of quorum sensing system is that of the bio-luminescent marine bacterium *Vibrio fischeri*. *Vibrio fischeri* bacterium lives with the eukaryotic hosts *i.e*. Squid with light as a symbiotic association. In each case the host has developed a specialized light organ that is inhabited by a pure culture of a specific strain of *Vibrio fischeri* at very high cell density. In these symbiotic associations the eukaryotic host supplies *Vibrio fischeri* with a nutrient rich environment in which to live. The role of *Vibrio fischeri* is to provide the host with light (Visick *et al.,* 2000). The *Vibrio fischeri* have LuxI/LuxR quorum sensing circuit. There are five luciferase structural genes (*luxCDABE*) and two regulatory genes (*luxR* and *luxI*) required for quorum sensing controlled light emission in *Vibrio fischeri*. The genes are arranged in two proximate but diver-gently transcribed units. The regulatory genes *luxR* is transcribed to the left and the *luxICDABE* operon is transcribed to the right. The regulatory genes *LuxI* protein (square) is responsible for synthesis of the HSL auto-inducer *N*-(3-oxohexanoyl)-homoserine lactone (hexagons). As the cell population density increases, the concentration of the auto-inducer increases both intra and extra cellularly. At a critical auto-inducer concentration, the LuxR protein (circle) binds to the auto-inducer. The Lux R-auto-inducer complex binds at the *luxICDABE* promoter and activates transcription of this operon. This action results in an exponential increase in auto-inducer synthesis *via* the increase in transcription of *luxI* and an exponential increase in light production *via* the increase in transcription of *luxCDABE*. The LuxR auto-inducer complex also binds at the *luxR* promoter, but in this case the complex represses the transcription of *luxR*. This negative action compensates for the positive action at the *luxICDABE* promoter. The oval represents a bacterial cell (Melissa *et al.,* 2001) .



Why do bacteria talk to each other? or what is the need of quorum sensing?

As environmental condition often change rapidly bacteria need to respond quickly in order to:

I. Quorum sensing enables bacteria to coordinate their behaviour.
II. It is very important for pathogenic bacteria during infection of a host to coordinate their virulence in order to be establish a successful infection.



Molecules involved in quorum sensing

Three dimensional structures of proteins in quorum sensing were first published in 2001 when the crystal structures of three LuxS orthologs were determined by X-ray crystallography (Lewis *et al.,* 2001). In 2002 the crystal structure of the receptor LuxP of *Vibrio harveyi* with its inducers AI-2 (which is one of the few bio-molecules containing boron) bound to it was also determined (Chen *et al.,* 2002). The genomics analysis indicates that the LuxS enzyme required for AI-2 synthesis is widespread in bacteria, while the periplasmic binding protein LuxP is only present in *Vibrio* strains. Thus, other organisms may either use components different from the AI-2 signal transduction system of *Vibrio* strains to sense the signal of AI-2 or they do not have such a quorum sensing system at all (Sun *et al.,* 2004).

Evolution (Sequence analysis)

Quorum sensing is a widespread form of bacterial communication in which individual cells produce and respond to specific N-acyl homoserine lactone signal metabolites. The different auto-inducer syntheses that generate these signals and the receptor/activator proteins that mediate the cell's response to them constitute evolutionary conserved families of regulatory proteins known as the LuxI and LuxR families, respectively. We have performed a phylogenetic analysis of 76 individual LuxI and LuxR homologous present in diverse members of the Gram-negative *Proteobacteria*. The results were consistent with an early origin for these regulators during the evolution of the *Proteobacteria*, with functional pairs of luxI and luxR genes possibly co-evolving as regulatory cassettes. In many cases, specific LuxI and LuxR family members appeared to have been inherited horizontally. In particular, those species containing multiple LuxI and/or LuxR homologous usually appeared to have obtained each individual homologue or functional pair of homologue from an independent source. Because multiple homologues interact to form regulatory cascades, this finding suggests that hierarchical signaling pathways can potentially evolve by the sequential integration of preexisting regulatory circuits acquired from diverse sources (*Gray et al., 2001)*. Phylogenetic analyses of the relevant gene families (LuxI/R and LuxS) show that the genes annotated as LuxI/R inducer and receptor elements comprise two families with virtually no homology between them and with one family restricted to the *gamma-Proteobacteria* and the other more widely distributed. Within bacterial phyla, three for the LuxS and the two LuxI/R families show broad agreement with the ribosomal RNA tree, suggesting that these systems have been continually present during the evolution of groups such as the *Proteobacteria* and the *Firmicutes* (*Lerat and Moran*, *2004*).

Role of quorum sensing in biofilm development

This phenomenon is worrisome and represents an area of interest for both clinical practice and fundamental research. One important mechanism whereby bacteria acquire

resistance to antibiotics and evade the immune system is by forming biofilms. It is estimated that 80% of the bacteria producing chronic infections can form biofilms. During the process of biofilm formation microorganisms have the ability to communicate with each other through quorum sensing. Quorum sensing regulates the metabolic activity of plank-tonic cells and it can induce microbial biofilm formation and increased virulence. In this review we describe the biofilm formation process, quorum sensing, quorum quenching, several key infectious bacteria producing biofilm, methods of prevention and their challenges and limitations. Although progress has been made in the prevention and treatment of biofilm-driven infections (Preda and Sandulescu, 2019).

Examples of bacterial species involved in biofilm formation and their biological effects

Bacterial strain	**Gram stain**	**Types of infections**
Staphylococcus aureus	Gram-positive	Chronic biofilm infections, right valve endocarditis, chronic wound infection, lung infections in patients with cystic fibrosis
Staphylococcus epidermidis	Gram-positive	Endocarditis, catheter-related infection, joint prosthesis infection
Streptococcus pneumoniae	Gram-positive	Lung infections, bacterial meningitis, acute or chronic otitis media
Listeria monocytogenes	Gram-positive	Co-culture interactions with *Pseudomonas*, *Vibrio* strains, listeriosis, contamination of food products
Burkholderia cepacia	Gram- negative	Opportunistic infections in patients with blood cancer
Escherichia coli	Gram-negative	Hemolytic uremic syndrome, acute diarrhea syndrome, urinary tract infections
Klebsiella pneumoniae	Gram-negative	Bacteria, liver abscess, urinary tract infections
Pseudomonas putida	Gram-negative	Urinary tract infection
Pseudomonas aeruginosa	Gram-negative	Osteomyelitis, ventilator-associated pneumonia, lung infections in patients with cystic fibrosis, opportunistic infections in neutropenic patients, nosocomial infections
Pseudomonas fluorescens	Gram-negative	Bio-remediation, bio-control *Pythium, Fusarium*, antimicrobial properties – production of mupirocin
Rhizobium leguminosarum	Gram-negative	Biocontrol properties – *Pythium*
Lactobacillus plantarum	Gram-positive	Prevention of Salmonella infection
Lactococcus lactis	Gram-positive	Antimicrobial properties in the human gastro-intestinal tract

Particularities of biofilm formation phases

Phase	Characteristics
Adhesion of plank-tonic cells	Biofilms generally start by the adhesion of microbial cells to a biotic or abiotic surface. Biotic surfaces may include endothelial lesions, necrotic tissues, mucous, etc., while abiotic surfaces may include indwelling devices: vascular catheters, urinary catheters; prostheses, surfaces from the clinical environment. This surface adhesion or primary attachment, can be active or passive depending on microbial factors such as motility, or expression of adhesive. Cellular physiology changes, affecting surface membrane proteins. Removal of irreversibly attached cells is difficult as it requires use of specific enzymes, surfactant, sanitizers. Bacteria behave as hydrophobic particles presenting negative charge, but this varies with growth phase.
Formation of an extracellular polymeric substance (EPS) matrix	Genes responsible for adhesion and matrix assembly are activated when stimulated by factors including population density and nutrient limitation. The EPS matrix is composed of a mixture of bio-polymers. The matrix produced in broth culture is not similar to the one produced when strains are attached to a surface, and biofilms also differ between in vivo and in vitro conditions. EPS can also be produced by plank-tonic cells resulting in enhanced attachment.
Accumulation of multi-layered clusters of microbial cells	Micro colony development is the result of simultaneous bacterial aggregation and growth. The tulip biofilm arrangement was established as a discrete model, using con focal laser microscopy.
Biofilm maturation	During biofilm maturation, canals are created in the biofilm structure. These will allow gradient-based passage of nutrients and signaling molecules, favoring organized agglomeration and differentiation of cells based on their metabolic state.
Detachment and dispersal of plank tonic bacteria	Following maturation, biofilms become thicker, developing an anaerobic environment on the interior, while external layers may begin separating. Detachment and dispersal can also occur when there is a nutritional imbalance. For instance, low carbon availability increases EPS synthesis. Detached cells or clusters of cells can travel as septic embolism, and may colonize new sites, then generating infection with potentially new biofilm formation.

Quorum Sensing Inhibition

Quorum sensing helps bacteria to communicate with each other and in coordinating their behavior. Many diseases of human beings, plants and animals are mediated by quorum sensing. Various approaches are being tried to inhibit this communication to control the diseases caused

by bacteria. Periodontal pathogens also communicate through quorum sensing and new approaches to treat periodontal disease using quorum sensing inhibition need to explored.

Quorum quenching-quorum sensing inhibition

The bio-film formation can be disrupted by disturbing the quorum sensing mechanism utilized by the various species of bacteria that together form the plaque bio-film. The inhibition of quorum sensing is commonly referred to as "quorum quenching." It initially meant stopping quorum sensing by enzymatic hydrolysis of AHL auto-inducers, there have been changes and presently the phrase quorum quenching is now commonly used in a more general sense to refer to any inhibition of quorum sensing due to the use of enzymatic or non-enzymatic molecules (Galloway *et al.,* 2014).

Quorum sensing can be blocked by stopping the signal molecule production, destroying the signal molecule and by preventing the signal molecule from binding to its receptor that are following:-

a) Blockage of AI synthesis - AHL production can be blocked by developing structural analogs of S-adenosyi methionine and acyl carrier protein. E.g. Molecules like-L/D-S-adenosyl homocysteine, *S*-adenosylcysteine and sinefungin suppress production of AHL. Some macrolide antibiotics like erythromycin are capable of repressing AHL synthesis when applied at lower concentrations. It is not known clearly how these antibiotics interfere with bacterial quorum sensing.
b) Inactivation of AI in Gram-negative bacteria - Enzymes such as-acylase, lactonase, oxireductases can selectively inactivate AHL in Gram-negative bacteria and due to this AHL accumulation in the extracellular environment does not occur and QS regulated genes are not expressed (Chen et al., 2013). Dong *et al.* found a *Bacillus* species that produced an enzyme termed AiiA that catalyzed the hydrolysis of AHL molecules. Many AHL lactonases similar to AiiA have been recognized. E.g. AttM in *Agrobacterium tumefaciens*, AiiB in *A. tumefaciens* C58, AiiS in *A. tumefaciens* K84, AhlK in *Klebseilla pneumoniae*.
c) Inhibition of AHL signal reception - Quorum sensing can be inhibited by preventing the AHL molecule from binding to its receptor. It can be competitive inhibition by molecules that bind to the receptor in preference to the AHL molecule. Slight changes in AHL acyl side chain or in the lactone ring or changes in both acyl side chain and lactone ring produce molecules that can bind with LuxR type receptor protein, but will not cause the signal generation. Quorum sensing blockage by molecules produced by various plants, algae and other organisms. Various plants, algae, fungi, etc. produce molecules which might play a role in inhibiting quorum sensing in bacteria. Some of them are:

1. Horseradish-Iberin Garlic-ajoene.
2. Turmeric-curcumin.
3. Citrus flavinoids-flavonine naringenin.
4. Sponge *Agelas oroides*-alkaloid oroidin.
5. Red marine alga known as *Dalea pulchra*-halogenated furanones.
6. Grape fruit extract-furocoumarins, carotenoids, limonoids, pectin, and coumarin.
7. Nutmeg (*Myristica-cinnamomea*)-Malabaricone. Nutmeg (*M. cinnamomea*) - Alabaricone.
8. Sweet basil-osmarinic acid. Garlic-disulfides and trisulphides.
9. Clove extract-eugenol. Clove extract-hexane and methanol.
10. *Piper nigrum*, *Piper betle* and *Gnetum gnemon*-hexane, chloroform, and methanol.
11. Coffe extract-caffeine.

Conclusions and future perspecctives:

Bacteria occupying diverse niches have broadly adapted quorum sensing systems to optimize them for regulating a variety of activities. In every case quorum sensing confers on bacteria the ability to communicate and to alter behavior in response to the presence of other bacteria. Quorum sensing allows a population of individuals to coordinate global behavior and thus act as a multi-cellular unit. Although the study of quorum sensing is only at its beginning, we are now in a position to gain fundamental insight into how bacteria build community networks. Quorum sensing inhibition in periodontal treatment is still in the research stage, more research needs to be done on natural products that can inhibit quorum sensing in periodontal pathogens. How quorum sensing has evolved to facilitate species-specific and inter species cell-cell communication. We will learn how quorum sensing allows populations to act synergistic and how quorum sensing can be used to conquer competitors. We will learn about the assortment of signals that are employed by bacteria and about the biosynthesis of these signals as well as how the information encoded in these chemical signals is processed and transduction to control gene expression. Furthermore, novel antimicrobial strategies could be designed based on information garnered from studies of quorum sensing, which suggests that research on quorum sensing could have enormous practical applications. Many bacteria use quorum sensing as a multicellular system to coordinate gene expression according to the density of their local population. By this mechanism bacteria can regulate metabolic, host interactions and environmental processes. So this system can be used as a useful target in medicine and other applications such as the production of biochemicals, microbial biosensors and mixed-species fermentation.

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PATHOPHYSIOLOGICAL ROLES OF ANGIOTENSIN- II

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Introduction:

- **Historical Background:** At the end of the 19th century, the discovery of the renin-angiotensin system (RAS) had a profound impact on our understanding of diseases and their treatment. Over a century of research has revealed the widespread involvement of angiotensin II (Ang II) in the pathophysiology of various diseases.
- **Angiotensin II Overview:** Angiotensin II is a vasoconstricting peptide hormone generated through the proteolytic cleavage of angiotensin I by the angiotensin-converting enzyme (ACE) in endothelial cells. The RAS, in which Ang II plays a key role, is implicated in the heart, liver, lungs, and kidneys [1].
- **Mechanism of Angiotensin II:** Angiotensin II is a potent vasoactive peptide that regulates blood pressure and fluid homeostasis. It is generated through a complex pathway involving the renin-angiotensin-aldosterone system (RAAS), which is activated in response to changes in blood pressure and fluid volume. Angiotensin II acts on different receptors, primarily the AT1 and AT2 receptors, which mediate its vasoconstrictive and vasodilatory effects, respectively [2].The effects of angiotensin II on blood pressure and fluid balance involve various mechanisms, including direct vasoconstriction of arterioles, stimulation of aldosterone secretion in the kidneys, and release of antidiuretic hormone from the pituitary gland.

Clinical Manifestations

- **Dysregulation of the RAAS Pathways:** Dysregulation of the RAAS pathways and excessive activation of angiotensin II can lead to various clinical manifestations and contribute to the pathogenesis of hypertension, heart failure, and kidney disease. Long-term exposure to angiotensin II induces oxidative and endoplasmic reticulum stress and modulates sodium ion transport, which are associated with diseases such as hypertension and chronic kidney disease [3].

Pathophysiological Roles of Angiotensin II

1. **Roles of Angiotensin II Receptors in Blood Pressure Regulation:** Angiotensin II, through its receptors (AT1 and AT2), plays a critical role in regulating blood pressure. The AT1 receptor primarily mediates the vasoconstrictive and hypertensive effects of Ang II, while the AT2 receptor is thought to have vasodilatory and anti-inflammatory effects [4]. The AT2 receptor is also involved in the BK-NO-cGMP vasodilator cascade, which protects against further increases in blood pressure. The imbalances in these receptor-mediated effects can lead to hypertension [5].

2. **Pathophysiological Roles of AT1 & AT2 Angiotensin II Receptors in Metabolic Disorders:-**Pathophysiological Roles of AT1 & AT2 Angiotensin II Receptors in Metabolic Disorders (Hypercholesterolemia and Diabetes) [6]

   Hypercholesterolemia and diabetes together increase the risk of complications. One mechanism linking them is the increased production of reactive oxygen species (ROS) by angiotensin II (Ang II). This leads to atherosclerosis and diabetic disorders. Ang II also causes oxidative stress and endothelial dysfunction, impairing nitric oxide synthesis [7]. Studies show that blocking Ang II receptors can slow down the progression of atherosclerosis and glomerulosclerosis. Ang II contributes to atherogenesis by upregulating adhesion molecules. The balance between AT1 and AT2 receptors may influence the response to Ang II in diabetic nephropathy. Hypercholesterolemia and hyperglycemia can trigger pro-inflammatory responses in coronary arteries and kidney glomeruli, causing injury. **In diabetes,** macrophages are recruited to the glomerulus through Ang II-stimulated MCP-1 expression, implicating them in glomerular injury. Hyperglycemia activates NF-kappaB, which affects intracellular signaling. Insulin can increase NF-kappaB transactivation stimulated by Ang II and hyperglycemia. The renin-angiotensin system activation plays a role in the inflammatory cell infiltration seen in both hypercholesterolemia and hyperglycemia. Although more research is needed, studies suggest that ARBs can protect the kidneys and blood vessels in high-risk patients. Blocking AT1 receptors with ARBs may reduce oxidative effects and stimulate AT2 receptors, providing vascular and renal protection.

Physiological Roles of Angiotensin II in Inflammation and Tissue Injury

- Recent studies have found that AngII can cause allergic reactions in the body, leading to inflammation. This inflammation occurs when the endothelium of blood vessels becomes activated, and various endothelial cell selectins are expressed, which guide specific types of white blood cells to the site of injury. During the inflammatory process, AngII increases vascular permeability by promoting the expression and secretion of VEGF (vascular endothelial growth factor) and induces the expression of endothelial adhesion molecules such as selectins, VCAM-1, ICAM-1, and integrins.
- AngII also contributes to endothelial dysfunction by activating COX-2, which produces vasoactive prostaglandins and ROS. Additionally, AngII promotes the recruitment of inflammatory cells into tissues by stimulating the production of specific cytokines and chemokines. For example, AngII induces the production of MCP-1, a potent monocyte chemoattractant, in cultured monocytes. In spontaneously hypertensive rats with elevated AngII levels, the increased expression of MCP-1 and its receptor CCR2 is accompanied by significant macrophage infiltration in the aorta. Blocking the AT1 receptor and modulating MCP-1/CCR2 reduces vessel inflammation in these rats. Similarly, CCR2-deficient mice show minimal macrophage infiltration in the arterial wall in response to AngII [8].
- In models of progressive nephropathies, increased renal expression of MCP-1 is observed along with the accumulation of macrophages in the interstitial space. Renoprotection

provided by the ACE inhibitor lisinopril reduces interstitial inflammation and MCP-1 expression. AngII also activates dendritic cells (DC), specialized antigen-presenting cells involved in immune responses. AngII enhances DC migration, maturation, and antigen presentation. In rats with partial kidney removal, inhibiting AngII synthesis reduces local DC accumulation and mitigates tubulointerstitial damage.

- Furthermore, AngII activates toll-like receptors (TLR) in cultured mesangial and vascular smooth muscle cells. AngII, through AT1 receptor signaling, stimulates TLR-4 expression, leading to cellular oxidative injury, apoptosis, and inflammation.
- Angiotensin II, through AT1 receptor signaling, plays a role in the localized activation of the immune system in immune cells, as well as mesangial cells and vascular smooth muscle cells.

Pathophysiological Roles of AT2R in Cardiac Diseases

1. **Remodeling of the Heart:** The effects of circulating Angiotensin II (Ang II) play a critical role in regulating the cardiovascular and renal systems. Ang II is produced in various peripheral tissues, including the heart. It binds to specific receptors in an autocrine or paracrine manner, promoting tissue remodeling [9].

   In cardiac fibroblasts, the dominant expression of AT2R has pharmacological implications. AT2R antagonists may have actions that counteract the effects of Ang II in failing hearts, particularly in cardiac fibroblasts. Studies have shown that AT2R inhibits the synthesis of DNA and extracellular collagenous proteins, such as fibronectin and collagen type 1, thereby inhibiting the progression of fibrosis in the heart. AT2R also modulates arterial hypertrophy and fibrosis in hypertensive conditions [10]. It may induce apoptosis, affecting changes in myocardial structure. The cardioprotective action of AT2R antagonists is partly mediated by the kinin/NO system, which is involved in cardiac fibrosis. AT2R activation of the kinin/NO system also contributes to kidney function, including pressure natriuresis and diuresis.

   In cardiac myocytes, AT2R has distinct actions. Overexpression of AT2R in mice showed that it exerts a positive chronotropic action in response to Ang II. In hypertrophic myocytes, AT2R inhibits the positive chronotropic or hypertrophic actions mediated by AT1R, suggesting a regulatory role.
2. **Heart Failure:** Acute decompensated heart failure occurs when the heart cannot pump enough blood to meet the metabolic demands of essential organs. Neural hormones are released in response, including renin. Renin activates the renin-angiotensin-aldosterone system (RAAS), leading to the generation of Angiotensin II. Angiotensin II causes vasoconstriction in blood vessel walls, including the lungs, and stimulates the release of endothelin, contributing to coronary and systemic artery and vein constriction. Vasoconstriction increases the workload of the weakened heart and limits oxygen flow to the myocardium. Aldosterone, stimulated by Ang II, promotes salt reabsorption, leading to fluid retention. Fluid overload and venous constriction result in pulmonary edema and symptoms such as shortness of breath and fatigue [11].

Chronic volume overload and the activity of Ang II, aldosterone, and other hormones contribute to pathological cardiac remodeling, including interstitial fibrosis and cellular apoptosis. These changes further impair the heart's ability to relax and pump effectively. The body's response to heart failure, including vasoconstriction, fluid retention, and increased heart rate and contractility, initiates a detrimental cycle of myocardial damage and worsening heart failure.

Role of Angiotensin II in Atherosclerosis:

Angiotensin II (Ang II) plays a significant role in promoting atherosclerosis, a condition characterized by the buildup of plaque in the arteries. Ang II is formed in various tissues, including the brain, kidney, heart, and blood vessels. When Ang II stimulates the AT1 receptor, it activates several intracellular signaling pathways that contribute to the development of atherosclerosis [12].

These pathways involve inflammation, oxidative stress, endothelial dysfunction, tissue remodeling, thrombosis, proliferation, fibrosis, and autostimulation. Ang II activates nuclear factor kappa B, a pro-inflammatory transcription factor, leading to the production of adhesion molecules, pro-inflammatory cytokines, and chemokines. These markers attract and bind monocytes to dysfunctional endothelium, causing them to migrate through the endothelium.

Under normal circumstances, nitric oxide, produced by healthy endothelial cells, inhibits platelet and leukocyte adhesion, as well as the growth of vascular smooth muscle cells. However, Ang II impairs nitric oxide activity by creating oxidative stress through the stimulation of oxidase enzymes in vascular smooth muscle cells and endothelial cells. This increased production of reactive oxygen species promotes LDL oxidation and contributes to endothelial dysfunction [13].

Endothelial dysfunction amplifies the vasoconstrictive action of Ang II and stimulates platelet aggregation. Ang II also mediates tissue remodeling by activating enzymes called matrix metalloproteinases and promoting the deposition of extracellular matrix proteins. Furthermore, it enhances thrombosis by stimulating the endothelium to express tissue factor and inhibit tissue plasminogen activator (TPA). Ang II can lead to myocardial fibrosis by increasing the expression of transforming growth factor beta, a cytokine that stimulates the production of collagen and other proteins in the extracellular matrix. In tissue-specific renin-angiotensin systems, Ang II can be produced locally through a process called autocrine stimulation, further contributing to its detrimental effects [14].

Inhibition of the RAAS pathways:

Inhibiting the RAAS pathways is an important treatment strategy for certain conditions. Two commonly used approaches are through ACE inhibitors or Angiotensin receptor blockers (ARBs). Alongside these, there are other medications that target the RAAS pathways, such as renin inhibitors, aldosterone antagonists, and neprilysin inhibitors. Clinical trials have demonstrated the effectiveness of these agents in lowering blood pressure, enhancing cardiovascular outcomes, and slowing the advancement of kidney disease [15].

How does ACE & ARBs work?

- We know that ACE converts angiotensin1 to angiotensin 2Which will result in the increase of sodium , water and aldosterone in the body. So, if we block the ACE enzyme we will automatically block the production of angiotensin II which will be done by the use of ACE inhibitors.

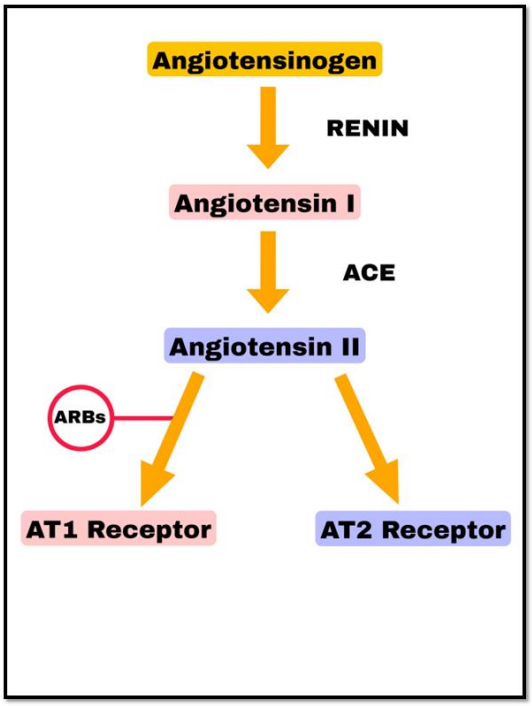

Fig. 1: Angiotesin II receptor blockage system

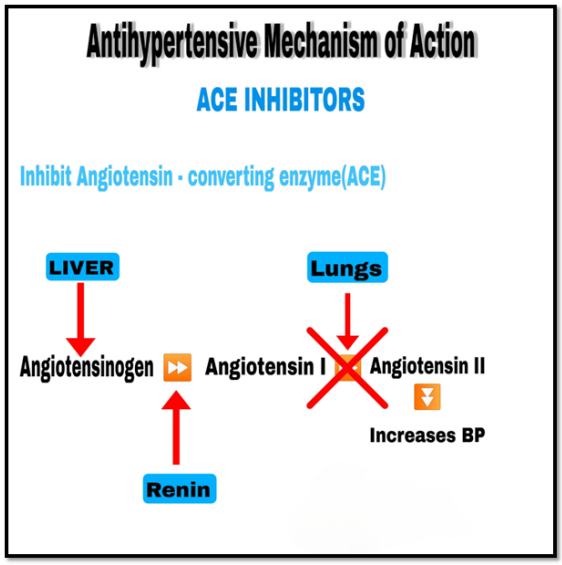

Fig. 2: Angiotesin – convering enzyme

- When angiotensin II is made in our body it goes to kidney & attaches to the AT1R and once it attaches to AT1R we get the increase in sodium, water and aldosterone in the body but if we block the AT1 receptor by using ARBs (angiotensin receptor blockage system) it will result in the decrease of sodium,water and aldosterone retention in the body.

Why do we use ACEs and ARBS?

- Certain condition like when any patient is suffering from hypertension and angioedema meaning swelling due to allergy.
- If patient has congestive heart failure or we can also use it as cardioprotection.
- If patient has chronic kidney disease .
- If patient has type 2 Diabetes mellitus .
- IT also reduces the risk of stroke [16].
- ACE inhibitors and ARBs have similar benefits , and both work equally well in the body . ARBs seems to cause less side effects than ACE inhibitors .

Conclusion:

The above mentioned pathophysiological roles of Angiotensin II is a connecting bridge between anatony, physiology and pharmacology. Therapeutic approaches that targets more complete inhibition of the RAAS may offer additional clinical benefits for patients with all pathophysiological disease caused by Angiotesin I would appreciate the same co-operation and suggestion from readers to assist me in my quest to impart and spread knowledge.

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BIOREMEDIATION: AN EMERGING SCIENCE TO MEET THE CHALLENGES OF ENVIRONMENT POLLUTION

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Introduction:

Bioremediation is a process that uses living organisms, such as bacteria, fungi, or plants, to degrade, detoxify, or remove contaminants from the environment. It is an environmentally friendly approach to remediate polluted sites and restore them to their natural state. Bioremediation can be applied to various types of contaminants, including organic pollutants such as petroleum hydrocarbons, solvents, pesticides, and heavy metals.

Bioremediation is a process that uses living organisms, such as bacteria, fungi, or plants, to degrade, transform, or remove contaminants from the environment. It is a natural and sustainable approach to remediate polluted sites and restore them to a cleaner state.

Contaminants can include various types of pollutants, such as organic compounds (e.g., petroleum hydrocarbons, solvents, pesticides) or inorganic substances (e.g., heavy metals, radionuclides). These contaminants can be present in soil, water, sediments, or even in the air.

Bioremediation works by harnessing the metabolic capabilities of microorganisms or plants to break down or transform the pollutants into less harmful substances. The organisms involved in bioremediation use the contaminants as a source of energy or nutrients for their growth and, in the process, convert them into simpler and less toxic forms. This can be achieved through various mechanisms, including biodegradation, bioaccumulation, or volatilization.

There are different types of bioremediation approaches:

Microbial Bioremediation: This approach involves the use of microorganisms, such as bacteria or fungi, to degrade or transform contaminants. These microorganisms may already be present at the contaminated site (indigenous microorganisms) or can be introduced to enhance the remediation process (bioaugmentation). The microorganisms break down the pollutants into harmless byproducts through enzymatic reactions.

Phytoremediation: Phytoremediation involves the use of plants to remove, degrade, or immobilize contaminants. Plants can absorb and accumulate certain pollutants from the soil or water through their roots, a process known as phytoextraction. They can also break down contaminants through metabolic processes in their tissues (phytodegradation) or transform them into less toxic forms (phytostabilization). Phytoremediation is often used for contaminants like heavy metals and organic compounds.

Mycoremediation: Mycoremediation utilizes fungi to remediate contaminated environments. Fungi have the ability to break down or transform various contaminants, including petroleum

hydrocarbons, pesticides, and even some persistent organic pollutants. They can also help in the breakdown of complex materials like wood or paper.

Role of microorganisms in bioremediation

Microorganisms are the key players in bioremediation. The microbes are present almost everywhere so the question of their availability becomes unimportant. They are best suited for the task of contamination removal as they produce a vast array of enzymes which help in degradation of the pollutants or their transformation into some other non- toxic products. They may even sometimes use these heavy metals or radioactive wastes for themselves. These microbes internalize the metals and use them as a part of their own metabolic or regulatory mechanisms.

The microorganisms get stimulated by some specific substrates such as nutrients; organic or inorganic acids etc. and then undergo a series of changes which ultimately lead to the degradation of the contaminants.

The process of Bioremediation requires that the microorganisms should be active and healthy so that they can perform their function efficiently. It is not necessary that all the microorganisms detoxify the same toxins, different microorganisms may be required. Also, it is possible that one microorganism can decontaminate more than one kind of toxin.

Bioremediation can take place both aerobically as well as anaerobically, i.e., in presence of air (hence oxygen) and in absence of air (hence oxygen) respectively. Under former conditions the resulting products are generally carbon and water; and in the latter conditions the resulting products are one or the other forms of energy such as light or heat.

There are two main types of bioremediation:

***In-situ* bioremediation:** This method involves treating the contaminated material at the site where it is found, without the need for excavation or removal. In situ bioremediation can be achieved through various techniques such as bioaugmentation, where specific microorganisms are introduced to enhance the degradation of contaminants, or biostimulation, which involves providing the necessary nutrients and conditions to promote the growth of indigenous microorganisms already present at the site.

***Ex-situ* bioremediation:** In this approach, the contaminated material is excavated and treated at a different location. It is often used when the contamination is too severe or when the site conditions are not favorable for in situ bioremediation. The excavated material can be treated through techniques such as composting, landfarming, or bioreactors, where the conditions can be carefully controlled to optimize the degradation process.

Bioremediation has several advantages over traditional remediation methods. It is cost-effective, as it can be less expensive than other techniques such as excavation and disposal. It is also less disruptive to the environment since it allows the contaminants to be broken down naturally rather than being transported elsewhere. Bioremediation is often a sustainable solution that can be applied to large areas and can be tailored to specific contaminants and site conditions. However, it's important to note that bioremediation is not suitable for all types of contaminants or environmental conditions. The success of bioremediation depends on factors such as the

availability of suitable microorganisms, nutrients, oxygen levels, pH, temperature, and the presence of inhibitory substances. Extensive site characterization and feasibility studies are necessary to determine the suitability and effectiveness of bioremediation for a specific contaminated site.

Overall, bioremediation is a promising and environmentally friendly approach to address pollution issues, and ongoing research and advancements continue to expand its applications and effectiveness in cleaning up contaminated sites.

Types of bioremediations

There are generally three major kinds of BR.

a) Biostimulation

It is the process in which the microorganisms require an external stimulus to start the bioremediation of any sort of harmful substances (here heavy metals and radioactive wastes) from the surroundings. This stimulus is generally in the form of any environmental modification. However, it has been seen in many instances that in terrestrial environments the indigenous microorganisms become adapted to the environment and its contaminants over a period of time; hence the bioremediation stops (Fredrickson et.al 1988). In such cases it becomes more important to know the biogeochemistry of that site. By making some suitable changes in the biogeochemistry of the desired site the microorganisms also get a stimulus and hence they start responding to the environment and its contaminants.

b) Bioaugmentation

It is the process in which the microorganisms are induced to undergo multiplication and growth at specific sites such as in sewage canals to remove the contaminants. The process requires a periodic addition of the nutrients which are necessary so as to speed up the bioremediation of the contaminants. The process has also proved to be very successful in agricultural land and in forests over many years (Jasper 1994). This process is however a bit risky as there is an uncontrolled growth of microorganisms which may become dangerous and fatal to the environment. So, a continuous monitoring and maintenance of this process becomes mandatory.

c) Intrinsic Bioremediation

This process is also known as Natural Attenuation. As the name suggests, it occurs on its own in the nature without any human help. It occurs primarily in the soil and water as both these sites are usually very rich in vast diversity of microorganisms and also often these sites are most heavily contaminated with heavy metals, radioactive wastes and also other sorts of hazardous substances. Intrinsic Bioremediation generally involves the microorganisms which are extremophiles, i.e., which grow in the extreme environments such as highly acidic or highly alkaline environments, in deep underground pipes, petroleum tanks etc. Not much work has been done so far in understanding the kinetics and durability of intrinsic bioremediation and more studies and research needs to be made (Van Ras et.al 2007).

Types of microbes

Microorganisms are microscopic living creatures. They might be single celled organisms or multicellular and are tiny (Madigan & Martinko 2006). All prokaryotes and some eukaryotes are among the microbes. They are normally not apparent to the human eye, but certain macroscopic ones can be seen by the naked eye ("Max Planck Society Research News Released Accessed 15 Sept'12").

Microbes comprise of protozoa, bacteria, archaea, fungi, algae and some members of the Animalia kingdom like rotifers as well as planarians. Viruses also fall under this category but few scientists even consider them as non-living (Rybicki 1990) (LWOFF 1956).

Microbes can be found in every sphere of the Earth. They live in the harshest settings, where no other living thing can survive. "Extremophiles" are bacteria that live in extreme environments. The following are some examples:

a) **Acidophiles:** include those organisms which can live in extremely acidic conditions or at very low pH which is generally less than 2. Acidophiles belong to all the three classes namely Archaea, Bacteria and Eukarya. Example: *Acetobacter aceti, Dunaliella acidophila, Acidianus infernus* etc.
b) **Basophiles:** this class include organisms which can thrive in extremely alkaline conditions with a pH of more than 10. These are also called as **Alkaliphiles**. Example: *Halorhodospira halochloris, Natromonas pharaonis, Thiohalospira alkaliphira* etc.

Thermophiles: these are those organisms which thrive easily at very high temperatures such as 45 degrees Celsius and up to 122 degrees Celsius. At such high temperatures no other living organisms are found to survive except this certain class of microorganisms. Examples: *Sulfobolus solfataricus, Sulfobolus acidocaldarius* etc.

c) **Psychrophiles:** this class include organisms which can easily survive at very low temperatures of -15 to +10 degree Celsius. Example: *Arthrobacter spp., Psychromonas spp., Pseudomonas spp.* etc.
d) **Xerophiles:** these are the organisms which can grow and live at sites where water availability is very less. Example: *Trichosporonoides nigrescens* etc.
e) **Halophiles:** are creatures that can survive in a saline environment. Water is frequently scarce at these locations. *Haloferax spp., Dunaliella salina*, and other bacteria are examples.
f) **Barophiles:** these are also called as "**Peizophiles**." These are organisms that can survive in extreme pressure environments, such as the deep sea. *Xenophyophores sp., Halomonas salaria,* and others are examples.
g) **Mesophiles:** they are also called as "**Neutrophiles**." These are creatures that can survive under moderate temperatures, acidity, alkalinity, salinity, and other factors. Only optimally balanced conditions are required for their survival. Almost all living organisms, with the exception of those stated above, are an example.

Active volcanoes, Sulphur springs hundreds of feet below sea level, acid springs, ice and glaciers, and other places may have them. They can be found on and inside both living and non-living things. Microbes have the reproductive capacity in both sexual and asexual ways.

On 17th Mar'13, microbes were discovered in the Mariana Trench, the Earth's deepest location (Glud et al. 2013). They've also been discovered as deep as 1900 ft beneath rocks and 8500 ft below sea level (Oskin and Becky 2013). "You can find bacteria everywhere," one of the researchers said. "They are incredibly adaptive to environments and live wherever they are." (Choi and Charles 2013).

Microorganisms are very useful for many important processes such as weathering of rocks, decomposition, nitrogen fixation; organic manure production and recent studies have also shown the microbes present in air play a role in weather control and precipitation (Christner et al. 2008).

Usefulness of microorganisms in bioremediation

The food and water that living beings consume are many a times contaminated with various different kinds of organic and inorganic contaminants such as chemicals, heavy metals like lead, arsenic, chromium etc. which lead to a vast range of diseases in living organisms. The contamination is not a recent phenomenon but has been seen since times immemorial. Some people are of the opinion that it started when the civilizations began, whereas, another school of thought date this phenomenon with the onset of urbanization and increased anthropogenic activities which destroyed the balance of environment.

To overcome this problem is a matter of serious concern and many technologies have been developed so far but most of them have proved to be in effective. This is attributed to the fact that they are too expensive and the range of toxic materials that they can decontaminate is very less.

This has led the researchers to look for alternative solutions. This is how the use of microorganisms for bioremediation first started. This use of microorganisms provides an environment friendly technique of remediation of harmful substances without having any alternative effect which may disturb the homeostasis of environment in any manner.

Many microorganisms live in the body of human beings and other animals also. Most common of these is bacteria *Lactobacillus* which is found in the human mouth, intestine and vagina of females. *Lactobacillus* has the ability to bind to detoxify some of the most harmful radionuclides and toxic metals. Due to this property, Lactobacillus is now also used in packed frozen foods and fermented preparations also.

The presence of radionuclides and HM in our environment has risen to the top of the ecological priority list. These contaminants do not decompose naturally and must be removed or neutralised. In the conversion of harmful metals into non-toxic metals, microbes operate as natural catalysts. As a result, there is a growing interest in understanding microbial activities that aid in the removal of harmful wastes from the environment (Francis 1990).

HM and radioactive wastes are mostly mobilised and immobilised by microbes. Processes such as "methylation of wastes," which turns them into volatile compounds, "chelation

of metals" to particular ligands, which helps to neutralise their toxic effects, and autotrophic and heterotrophic leaching are all examples of mobilisation.

Alternatively, immobilization occurs as an outcome of sorption to cell components, transport into cells, precipitation as insoluble compounds etc. (Gharieb *et al.,* 1998; Sayer *et al.,* 1997).

In nature, the radioactive wastes and heavy metals can be present in a number of forms such as oxides, superoxides, peroxides, sulphates, nitrates, citrates, carbonates etc. Microorganisms change these forms or states of toxic substances into less toxic or nontoxic states. They carry out these transformations due to their sensitivity towards presence or absence of free electrons. Under aerobic conditions, the microorganism's direct oxygen to act as electron acceptor and under anaerobic conditions the sulphate group, nitrate group, phosphate group or carbonate group etc. take up the electrons (Francis 1990).

The process of microbial transformation of radionuclides and heavy metals is considered important because of its significant commercial applications. It is easy to perform, is less expensive and can be applied to a large number of radionuclides and heavy metals present in various forms. These properties make microbial transformation a promising approach in bioremediation.

Mechanisms and pathways of microbial bioremediation (mbr)

When microbes are present in close proximity to poisons or pollutants, they aid in their removal in some way, cleaning up the environment. These unique bacteria are regarded as "Natural Cleansers" of the ecosystem, making them extremely beneficial to the environment.

MBR occurs in a variety of environments, including soil, water, sludge, sewage, inside plants and animals, agricultural areas, etc. In other words, based on availability of suitable bacteria, BR can occur in a wide range of environments. This characteristic has also shown to be extremely advantageous. Though, the mechanism and pathways of remedial processes are different in each case.

Let us first discuss the mechanism of remediation and sequestration of harmful heavy metals. The microorganisms, mainly bacteria, have a tendency of binding the metals to their cell walls.

The three main mechanisms for the binding of these heavy metals to bacterial cell wall are as under:

a) Reactions of exchange of ion with "peptidoglycan and teichoic acid" (Beveridge & others 1980).
b) Precipitation through nucleation reactions (Beveridge *et al.,* 1985).
c) Complexation with Nitrogen and Oxygen ligands.

It has been observed that gram positive bacteria have relatively high contents of peptidoglycan and teichoic acid in their cell wall: whereas, gram negative bacteria have lower content of these molecules. This is why gram +ve bacteria have high metal adsorptive capacity and gram-negative bacteria have poor metal adsorptive capacity (Gavrilescu 2004).

The major examples of gram-positive bacteria are Bacillus spp., Clostridium spp., Lactobacillus spp. etc. These bacteria are also present in considerable amount in gastrointestinal tract of human beings and all other mammals and several other animals also. Thus, they help in uptake of any metal species which may enter the intestinal tract with food by peristaltic movements. The gram +ve bacteria play a vital part in sequestration of these HM from human body.

It should be noted, however, that these microbes do "Detoxication" rather than "Detoxification." The latter is the process of removing harmful medicines, mutagens, and other toxic agents from the body, while the former is the act of preventing dangerous chemicals from entering the body (Jin & others 2009).

Detoxication occurs predominantly in the colon, liver, and kidney, where the bacterium sequesters the hazardous compounds before they reach the target tissues and inflict lethal or non-lethal damage (Berhane & others 1994). As a result, it may be concluded that gram-+ve bacteria, including probiotic bacteria, play a critical role in HM binding and consequently body protection.

Advantages of bioremediation

a. Unlike many other remedial processes which use various chemical as well as/ or physical techniques, bioremediation is a natural phenomenon. It occurs spontaneously wherever the correct microorganisms are found in close vicinity of the hazardous wastes. Also, this process generally requires continuous stimulus and it continues dynamically unless all the hazardous substances get depleted.
b. It is an environment friendly phenomenon in all the respects. It continues to occur without causing any sort of imbalance in the surrounding environment and does not interfere with other ecosystems. The process of bioremediation generally does not produce any harmful or toxic byproducts which can pollute or harm or affect the environment in any possible manner.
c. Bioremediation process can occur in a very vast range of habitats and surroundings. This is primarily due to the fact that microorganisms are ubiquitous in distribution. They are found from normal to the most extreme environments.
d. Bioremediation has proved to be efficient at places which are otherwise inaccessible without excavation. Microbes (mostly bacteria) have been found in deep rocks and 8500 ft below sea level, according to studies (Oskin and Becky 2013). HM and radioactive chemicals found at such depths can only be remediated or changed into less poisonous or non-harmful products by microbes. These depths would otherwise be unattainable without the use of excavation or mining techniques, both of which are damaging to the environment.
e. Bioremediation helps in efficiently reducing pollution and thereby protecting the environment from hazardous and toxic substances. As bioremediation uses living organisms so the chances of further damage to the environment become minimal which is

not the case when chemical or physical methods are used for the removal of hazardous wastes.

f. BR is a very cost-effective option. Because the expense of producing remedial approaches is small in compared to other accessible remedial technologies, it is less expensive than other technologies. This is due to the fact that microbes are naturally abundant in the environment, lowering the cost of production.
g. It can be simply carried out on the contaminated site, eliminating the requirement for trash transportation. This also saves money on transportation. It's also advantageous because the environment might get even more contaminated when waste is transported from one location to another. Bioremediation can easily overcome this problem.

Disadvantages of bioremediation

i. Bioremediation is only applicable to those substances which are biodegradable or which can be acted upon by microbes and enzymes or which can be affected by changing pH of the environment.
ii. It is not always possible to predict the outcome of the bio remedial process. Sometimes the products formed may be toxic or hazardous to the environment. Also, the waste may often get transformed into one or more forms of which a few might be toxic or hazardous.
iii. Bioremediation is no doubt a more or less environment friendly process but it is also a very slow process. It takes longer duration of time as compared to the other physical or chemical degradation technologies. Due to this reason the practical application becomes doubtful as the harm done by the waste has a faster pace than the removal or transformation of those wastes by the microorganisms.
iv. It is very important to keep track of the progress of any process or phenomenon. However, it is a very tedious job to regulate the bioremediation process as the microorganisms may show different behavior in different environments depending upon the environmental conditions and different weather conditions.
v. Sometimes the concentration of toxic substances is very high in comparison to the concentration of microorganisms. In such cases the microorganisms may also fail to grow and survive. So, an adequate level of toxicant-microbe concentration is mandatory for the adequate interaction also.
vi. To prevent these problems there should be proper and continuous monitoring and maintenance of the bio remedial process which is an extremely big task in itself.

Future directions and prospects:

Very significant work has been done in understanding the role of microbes in removal or sequestration of hazardous and toxic substances from the environments. However, attempts still need to be made in better understanding of the various mechanisms of various microorganisms which can be put to use for bioremediation. Such additional advances are expected with the newly emerging technical assistances also.

The major principal of BR is that microbes can be used in removal or degradation of harmful and toxic contaminants. Microorganisms are known to be capable of carrying out a large

range of detoxification as well as detoxication reactions. However, the full potential of these microorganisms is still unknown.

"By providing guidance on how to evaluate bioremediation, the researchers hope to eliminate the mystery that shrouds this highly multidisciplinary technology and pave the way for further technological advances" (Semprini *et al.,* 1990).

New frontiers in bioremediation need to be explored. The process of remediation employs several disciplines simultaneously. "As each of the discipline advances and as new cleanup needs arise, opportunities for new bioremediation techniques will also emerge" (Roberts *et al.,* 1990).

So far, most of the bioremediation work has been carried out in the area of petroleum hydrocarbons only. The major limitations for remediation of other contaminants are as under:

i) Limited understanding of behaviors of different microorganisms in different situations in the field.
ii) Stimulation of microorganisms to initiate the process of bioremediation.
iii) No tools to identify whether interaction between microbes and contaminant has been set up or not.

Innovative engineering technologies are being manufactured for providing stimulus to the microorganisms to initiate remediation. Research is also being conducted for optimizing ways to supply such stimulus. These researches, if result as positive, will pave the path for emerging bioremediation techniques.

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THE ANALYSIS OF NEWER AGENTS ON MULTIPLE MYELOMA: A RETROSPECTIVE OVERVIEW

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Abstract:

A malignancy known as multiple myeloma develops in a type of white blood cell known as a plasma cell. Healthy plasma cells produce antibodies that bind to and destroy bacteria, assisting your body in fighting illnesses. Cancerous plasma cells build up in the bone marrow and crowd out healthy blood cells in multiple myeloma.

Keywords: Myeloma, salvage therapy, cross resistance

Introduction:

Over the past ten years, there have been significant changes in how multiple myeloma is treated. Numerous potential medication combinations that might be employed in first-line and relapsed situations have been created as a result of the rise in the number of active drugs. As a result, there is a great deal of ambiguity regarding the selection of initial therapy regimens, the function of transplantation in the age of new medications, therapeutic endpoints, and the function of maintenance therapy. Whether treatment methods should aim to cure or control disease is a strongly contested topic that has a significant impact on the chosen treatment plan. The treatment of multiple myeloma is updated in this article, with an emphasis on recent developments, newly discovered disease, role of transplantation, and maintenance therapy.

The discovery of entirely new classes of agents exhibiting activity in myeloma that has become refractory to even high doses of melphalan has stimulated their testing in various clinical scenarios. Combinations of thalidomide, bortezomib, and lenalidomide with conventional agents or among each other have resulted in enhanced response rates and efficacy. These studies have provided the framework for identification and validation of novel targeted therapies to overcome drug resistance and improve patient outcome. Immunomodulatory+Protease inhibitors combinations including lenalidomide, bortezomib, dexamethasone and lenalidomide, carfilzomib, dexamethasone has had high response rates and good tolerability. The combination improved overall and extent of response, as well as prolonged time to progression and overall survival. This resulting in FDA approval of lenalidomide with Dexamethasone for therapy Multiple Myeloma relapsing after prior therapy. Lenalidomide showed little degree of cross-resistance with thalidomide, and bortezomib can have some restrictions in patients with preexisting thalidomide-related neuropathy. In those patients a less-aggressive approach with lower doses of dexamethasone and thalidomide or thalidomide in combination with melphalan-prednisone seems to be a more appropriate treatment strategy. Treatment consisted of thalidomide given orally at a dose of 200 mg/d for 2 weeks, then increased by 200 mg/d every 2

weeks, up to a maximal dose of 800 mg/d. Our retrospective analysis demonstrates that the cumulative 3-month Thalidomide dosage is one of the major prognostic factors for overall survival. The addition of thalidomide to dexamethasone and chemotherapy for the management of post-transplant relapses results in higher response rates. Patients who experienced relapse after initial treatment and received salvage therapy had a median survival of nearly 1.5 years.

Table 1: Stages of multiple myeloma

Stage I **5-year survival=82%**	**Stage II** **5-year survival=62%**	**Stage III** **5-year survival=40%**
All of the following: ■ Serum albumin ≥3.5 g/dl ■ Serum beta-2-microglobulin <3.5 mg/litre ■ No high-risk cytogenetics ■ Normal serum lactate dehydrogenase level	Not fitting stage I or stage III	Both of the following: ■ Serum beta-2-microglobulin >5.5 mg/litre ■ High-risk cytogenetics (t(4;14), t(14;16) or del(17p)) or elevated serum lactate dehydrogenase level

Etiology

Multiple myeloma's authentic cause is unknown. The promoter genes, particularly those on chromosome 14, are frequently altered and translocated in multiple myeloma, though, and they probably contribute to the onset of the illness. Additionally, other oncogenes like NRAS, KRAS, and BRAF may take involvement in the growth of plasma cells. Obesity, alcohol usage, environmental triggers such insecticides, organic solvents, agent orange, and radiation exposure are additional risk factors for illness.

Pathophysiology

In terms of the monoclonal gammopathy spectrum, MM is essentially a stage. Monoclonal gammopathy of Undetermined Significance (MGUS), a pre-malignant, asymptomatic stage of clonal plasma cell development, is assumed to be the source of it. Detecting monoclonal immunoglobulins in the blood or urine without signs of end-organ damage is referred to as MGUS. This is quite typical and can be found in more than 3% of people over the age of 50. The cell of origin appears to be a post-germinal centre plasma cell. Although it has been mentioned above, this illness is normally benign with a 1% annual risk of progressing to MM.

Whatever the underlying genetic cause, an excess of monoclonal immunoglobulins can cause renal tubular injury, hyperviscosity, platelet dysfunction, and neurologic derangements, which can all result in bleeding and renal failure. Anaemia, thrombocytopenia, and leukopenia are frequently seen as a result of the increasing plasma cell clone occupying one marrow. Furthermore, the interaction between myeloma cells and the bone microenvironment eventually causes osteoclasts to be activated and osteoblasts to be suppressed, which results in bone loss.

This intricate process involves multiple chemokines, interleukins, and intracellular and intercellular signalling pathways.

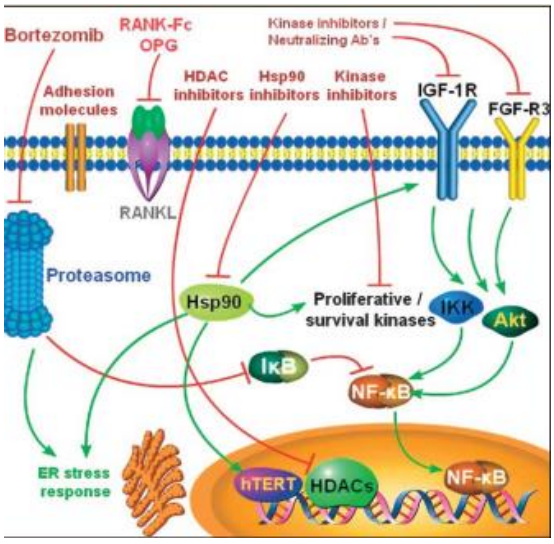
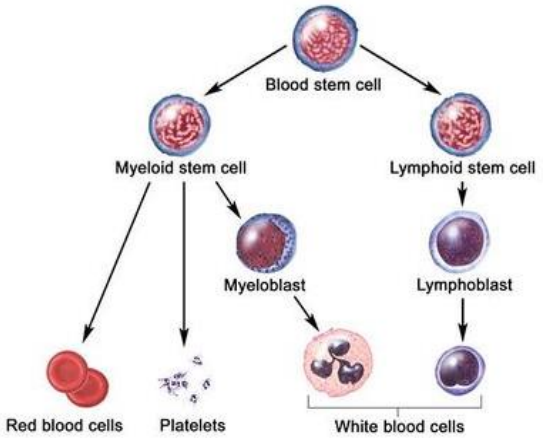


Fig 1. Schematic illustration of the mm treatment target



Evaluation

There are various methods for screening and diagnosing multiple myeloma when there is cause for concern.

The following diagnostic studies are suggested by the NCCN guidelines:

Platelet count and differential complete blood count

Levels of albumin, calcium, electrolytes, creatinine, and BUN

Serum LDH and beta-2 microglobulin; Serum immunoglobulins; Serum protein electrophoresis (SPEP); Serum immunofixation electrophoresis (SIFE); 24-hour proteinuria; Urine protein electrophoresis (UPEP); Serum-free light chain (FLC) assay; Whole Body low dose CT or PET CT; Unilateral bone marrow aspirate and biopsy; Immunohistochemistry and/or flow cytometry; and

In the past, clonal bone marrow plasma cells have been used to diagnose multiple myeloma (MM) if they were greater than or equivalent to 10% during a bone marrow biopsy (or if serum calcium concentration of more than 0.25 mmol/L (or 1 mg/dL) higher than the maximum limit of normal or 2.75 mmol/L (or 11 mg/dL) or higherHaving insufficient kidney

function (creatinine clearance of less than 40 mL per minute or a creatinine level greater than 2 mg/dL [greater than 177 micromol/L]),Anaemia is defined as haemoglobin levels that are less than or greater than 2 g/dL below the lower limit of normal.On skeletal radiography, CT, or PET-CT, one or more osteolytic bone lesions can be seen as punched-out, rounded, radiolucent lesions. While most of the instances that required treatment were found using this set of criteria, many others went unnoticed. The International Myeloma Working Group (IMWG) discovered additional indicators in November 2014 that are 80% more likely to increase the risk of organ damage due to myeloma within two years.

Conclusion:

Survival outcomes were poor despite increased use of multiple agents based chemotherapy regimens. Greater access to available diagnostic and treatments is required to achieve treatment and increased survival rate.

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ELUSIVE X-RAY LASER

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Historical milestones:

Though the topic is termed "Elusive X-ray Laser", it was elusive 50 years ago. It should not be elusive now. For a number of years it has been our field of interest. Laser has been termed by many as pure technology in the same way we make a distinction between applied and pure science. As a matter of fact our progress in science and technology during last 50 years may be measured conveniently by the progress made by us in laser technology.

The effort to produce X-ray laser that is lasing beyond the visible region towards shorter wavelength began as early as 1960 when the 1st laser was made (1). When one considers the possibility of making coherent sources in the X-ray region there are many difficulties. In fact only hope of success lies in the so called Clogston's law, enunciated by A.M. Clogston which states that any device you can think of can be built unless it actually violates the conservation of energy and momentum. It was speculated and estimated in 1970 that a practical X-ray would be developed by the turn of this century. It is now hardly two years left for the year 2000 to appear. So, whenever a real X-ray laser has been made is a question of sufficient interest. The answer is yes in the sense that there are many who occasionally claim to have achieved lasing in the X-ray region and according to others, the claim is not justified. At present a large number of laboratories in Untied States alone and also in other countries are busy in the work related to the development of a practical X-ray laser.

By way of comparison some historical milestone may be compared. A period of about 50 years passed between the discovery of superconductivity and a universally accepted theory. A similar period passed between the formulation of the concept of stimulated emission and Maiman's demonstration of the ruby laser. Whereas BCS theory was followed by continued research and practical applications superconductivity, but no significant increase in critical temperature until the year 1987, the ruby laser prompted a flurry of activities and a series of developments and advantages that is still expanding. In this decade we may have already seen the beginning of a revolution in the area such as the production of quantum well laser, quantum wire and quantum dot structures and the realization X-ray lasing. The current and the beginning of twenty – first century, the new trend is the issue of the short wave lasers, uses of radiation produced by relativistic electron beams in various spatially periodic structures. Such an alternative approach will result in the exponential growth of the frequency of coherent radiation sources. The evolution of sources of radiation beginning from the invention of an electric bulb to ruby laser is almost exponential. It is reasonable to speculate that after 2000 year the curve will assume a saturation stage. Of course, this approach just like that using the population inversion of multiple charged ions and nuclei will require sufficiently high powers. Production of high

power electron beams is however well established in experimental nuclear physics. Therefore, we can expect much progress with the generation of coherent X-rays even in the current century through the good relation of the potentials of lasers and nuclear physics.

Basic difficulties

There are many practical difficulties to achieve laser action in externally short wavelength region. In the far ultraviolet and X-ray region no material is transparent and all solids absorb vigorously. In these regions apart from the valence electrons, outer electrons, the electrons close to the nucleus can also be excited. Thus absorption comes from all atomic electrons, while it is difficult to excite more than one of them at a time. Therefore if there is any hope for coherent radiation in this region it would use a light material with few electrons in the inner shell.

X-ray laser discussions have been confined mainly in the region 1000>λ>10A° and this is the region for which experimental demonstrations of lasing are likely and hence work on X-ray laser are being concentrated in this region. Lasing actions at very short wavelength will be hard to achieve because of three major difficulties not present at longer wavelengths. They are

(i) Mirror: The lasing operating at very short wavelengths must do it without the benefit of mirrors. This is due both to the fact that all materials have low reflectivity for λ< 10 A° and the fact that an XRL (X-ray laser) or γRL (Gamma ray laser) would operate at such a high level that any mirror would be destroyed. Since high reflectance cavities do not appear to be realistic for wavelength shorter than ~ 500 A°, significant gain must be achieved in a single pass. This immediately implies an increase by order of magnitude in the population inversion density required for a given gain at a particular wavelength. Thus at extremely short wavelength amplified emission (ASE) devices are considered for production of highly coherent radiation. This also means that if a laser is to operate without mirrors then the gain down the length of the laser must be so large enough to allow a travelling wave to grow by stimulated emission. In practice, the gain will have to exceed 100 dB i.e. the small gain coefficient α times the length L of the laser cavity must satisfy the relation

$$\alpha L > 25 \qquad \text{----- (1)}$$

This equation translates into the following threshold condition on the net inversion density given by

$$N^* = N_2 - \left(\frac{g_2}{g_1}\right) N_1$$

$$N^* > \Delta\nu(eV)/L \times 10^{18}\, cm^{-3} \qquad \text{----- (2)}$$

Where, $\Delta\nu$ is the width of the transitions measured in electron volts. The exact inversion density required for an X-ray laser will be determined by the line width $\Delta\nu$. In cold matter this will be equal to the Auger width for transitions of interest will be of the order of 1 eV. Therefore, the net inversion density in cold matter must exceed $\approx 10^{18}\, cm^{-3}$. In hot matter the width will be determined by the Stark effect at most densities of interest. In fully stripped plasma (high Z) this will be given by

$$\Delta\nu_{Stark} = 30\ \eta^{2/3} eV \qquad \text{----- (3)}$$

Where, η is the density in the units of $5\times10^{22} cm^{-3}$. The conclusion to be drawn from (2) and (3) is that the inversion densities which are much smaller than $10^{18} cm^{-3}$ are possible only in N^*/N is close to unity.

For the amplifying medium ASE gain may be given in the form of product αL as

$$\alpha L = \left(\frac{\lambda_2 A_{21}}{4\pi^2 \Delta\nu}\right) L\left[N_2 - \left(\frac{g_1}{g_2}\right) N_1\right] \qquad \text{----- (5)}$$

Where, g_2, g_1 and N_2, N_1 refer to the statistical weights and population densities of the upper and lower laser states respectively, $\Delta\nu$ refers to the line width in the frequency units and λ refers to the wavelength of the laser transition. Generally for an amplifying medium, length L the ASE gain is given by the relation

$$I = I_0 \exp(\alpha L)$$

With the transition probability A_{21} for spontaneous emission scaling as $f\lambda^{-2}$ and where population inversion is large (i.e., $N_2 >> N_1$) eqn. (4) can be written as

$$\alpha L \propto \frac{N_2 L}{\Delta\nu} \qquad \text{----- (5)}$$

Here, f is the oscillator strength and is fairly constant along an iso-electronic sequence. This shows that narrow lines and needed so that the product becomes very high (i.e., $\alpha L >> 1$). In plasma media where short wavelength occurs in ionized species, $\Delta\nu$ is often dominated by Doppler or collision broadening. The lack of wavelength dependence factor is somewhat misleading since a large population inversion (N_2 large) is difficult to achieve against depopulation rates scaling as λ^{-2}. A high density plasma of necessarily short wavelength for pumping is appropriate for the shortest wavelength since with electron collisional pumping in a plasma N_2 scales as electron density N_e squared. For increased density, N_2 scales more as N_e due to increased collisions and longer lengths can be more appropriate. At very high densities a longitudinal pump absorption length can be more appropriate. At very high densities a longitudinal pump absorption length can decease rapidly and N_2L may actually decrease with increasing density unless transverse pumping is employed. This indicates that a careful modeling for any particular scheme is needed since many parameters are involved. It is worthwhile to note that the presence of outer electron e.g., in schemes where inner shell transitions are involved, the net gain α_1 is decreased through photoionisation losses (A_{pi}) to the main beam i.e., $\alpha_1 = \alpha - \alpha_{pi}$.

(ii) Pump Power: The second problem in achieving laser action at very short wavelength is the pump power P/a required. This is usually expressed as

$$\frac{P}{a} = N_2 L h\nu\,(\Gamma + A)$$

$$\cong \alpha L \nu^4 \left(1 + \frac{\Gamma}{A}\right)\left(\frac{\Delta\nu}{\nu}\right) \qquad \text{----- (6)}$$

Here ν is the laser frequency and Γ is the Auger rate, which enters for inner shell transitions. For low Z and for metastable transitions Γ can be much greater than A which

increases the pump requirements. Since the presence of excess outer electrons also increases the net gain one has to avoid these problems. Therefore, one must use hydrogenic and helium - like ions. For longer wavelengths alkalis may be considered. Since the product N_2L also appears in eqn. (6) similar scaling examples to that above for αL may be applied.

(iii) Life time of Inversion: The life time of inversion is the third major problem to be considered. The life time of a state where population inversion is to be created plays an important role in any laser devices. Any transition can be inverted for a pre equilibrium interval which is approximately limited by A^{-1}, at which time lasing is self-terminating. For wavelength range of about 1000 A° this time is in nano-seconds but becomes fem to second (10^{-15} sec.) for typical 1-2 A° dipole transitions. Longer level meta stable states with lower f values and transition probabilities require higher N_2L products for equivalent gain and comparable pumping flux. The higher densities can then lead to rapid collisional destruction of inverted meta stable state populations so that the advantage is lost. A sustained inversion is the most desirable mode of operation at short wavelengths and is achieved in principle which the final laser level is depleted at a more rapid rate that it is filled.

Mossbauer line narrowing:

Let us consider a favourable case where the line narrowing and the crystal are so perfect that the only source of line width is the radiative damping from the finite life time of the excited state, that is

$$\Delta\nu = 1(2\pi\tau) \qquad \text{----- (7)}$$

Also we have, $f\tau = 1.5\lambda^2$ ----- (8)

So that, $\frac{f}{\Delta\nu} = 9.24\lambda^2$ ----- (9)

For a Gaussian line and an empty laser state, the gain $|\alpha|$ is given by

$$|\alpha| = 2.5\times10^{-2} N \times 9.42\lambda^2 \ = 23.55N\lambda^2 \qquad \text{----- (10)}$$

For an atomic weight such as Tin, the back ground absorption is about 630 cm^{-1}. Since Tin contains 3.7 x 10^{22} atoms/cm^3, to overcome the background radiation N must be of the order of 2.625 x 10^{19} excited atoms per cm^3. It is supposed that the excited atoms come from radioactivity with the line narrowed by the Mossbauer effect. If corresponding to $\Delta\nu/\nu$ = 5 x 10^{20}, an initially undiluted supply to radioactive atoms would be reduced to this level in about 7 seconds. This not only assumes a crystal so perfect as to give such a narrow line, but also assumes no isotopic dilution. It would be necessary to prepare a crystal in a time comparable with this radioactive life time. This seems to offer some difficulties. Moreover the fractional line-width assumed is about 10 times narrower that of any known Mossbauer emitters. Even if such an emitting crystal is available for a true laser it would be necessary to provide end mirrors. A useful device although not a true maser could also be obtained by taling just a long column of the active material. The properties of a long column of excited atoms without reflectors were first pointed out by Dicke and examined in the X-ray region in 1963 by Chirikov [2]. While it would be very nice to have a crystal of radioactive atoms which need no pumping, one might also consider the energies of a pumped X-ray maser. It is yet not clear whether excitation energy can

be supplied fast enough to operate in X-ray laser but at present a large number of schemes describing production of maser action in the far ultraviolet and X-ray region are available. As a matter of fact the operation of an X-ray is closely analogous to the operation of a well known N_2 laser [3].

The effort to produce lasing beyond the visible region towards shorter wavelengths begun as early as 1960. Among the intially proposed schemes are (i) recombination [4] (ii) inner shell photoionization [5] (iii) photopumping [6] and (iv) collisional excitation [7,8]. Since then numerous schemes and approach have been proposed and many experimental investigations have been carried out. The first conclusive demonstrations of lasing action in the X-ray region in the range 206 A° to 209 A° was announced by two groups at Lawrence Livermore National Laboratory [9] and Princeton University [10,11]. This has brought the field of X-ray lasers from the area of speculation into the realm of practical application. The progress since that time has been encouraging. Laboratory demonstrations of gain have been made in the wavelength range down to around 40 A^0. There are reports of application of soft X-ray lasers to X-ray microscopy and holography [12-17]. Current X-ray laser is focused on extending the wavelength range toward and even beyond the range of the "water window" [Oxygen K absorption edge at 23.3 A^0 and carbon K absorption edge at 43.6 A^0]. It is not my purpose to give a review of the subject in detail. However it is worthwhile to discuss briefly certain lasing mechanisms and pumping methods. In the majority of the X-ray laser desings that have appeared in the literature the basic principle for achieving radiation amplification at shorter wavelength is the same as in the optical range.

Current UV laser

It is worthwhile; at this stage to make a quick and brief survey of the experimental related works performed in various centers around the world with the major work being undertaken in the United States only. It may be noted that all the works are the translations of the ideas originally initiated by Dicke and Chirikov thirty years ago. Many are of the opinion that the matter of whether the device is strictly X-ray laser or simply laser are debatable. The experiments performed during 1980 to 1995 do however indicate a demonstration of gain through stimulated emission in the extreme ultraviolet. The gain per unit length was confirmed by a number of independent measurements. By about 1976 it was realized that the discussion of X-ray laser needs an approach entirely different from usual laser. As a result much effort was terminated. Among those remaining in the field it was clear the XUV laser operating in the region 20-500 A° could be constructed with current technology in the presence of a number of constraints. If an appropriate scheme was chosen the laser would in principle be scalable to the X-ray region once the necessary pumping power was available.

In a conventional laser operating in the near ultraviolet visible or infrared spectral bands, using photon energies not exceeding 10 eV, are closely matched to the electronic or molecular energy levels of weakly ionized or neutral atoms. The photon energies (~ eV) and transition lifetimes (~ nanoseconds) closely match the characteristics of fast electrical circuitry feeding a weakly ionized discharge which may be used directly or indirectly, the laser medium. In an X-

ray laser operating at about 10 A° photon energies are about one kilo electron volt and lifetime about 10^{-13} seconds (10 fs). In consequence the power required to pump the laser is expected to increase very rapidly as the wavelength decreases.

The total power loss per unit area P of the medium must exceed that emitted by spontaneous decay of the laser transition

$$P \approx n_2 I h\nu A$$

$$= \left(\frac{4\times10^{21}}{\lambda^4}\right) \times \alpha L \times \left(\frac{\Delta\lambda}{\lambda}\right) \quad W/cm^2$$

In practical X-ray laser devices we must expect that additional losses will increase the total power needed. Not only this power be supplied by an external source but it must be dissipated by the medium.

Multilayer mirrors have been studied intensely in recent years (18-27). They could give normal incidence reflectance up to 66% at 130 Å. Multilayer reflectors have been used in the experimental demonstration of double and even triple pass gain. These studies may aid the progress toward an actual cavity device, but the present technology is still far from allowing the fabrication of efficient resonators for X-ray laser.

It is reasonable to believe that at present there are only two laboratory sources known which can, in principle, deliver the pump power densities $\geq 10^{15}$ W/cm^3 required by an X-ray laser, namely a subsidiary laser or a particle beam. Many believe that there is a third alternative that is a nuclear explosion. In a less restrictive XUV region (≥ 100 Å) one may add fast high voltage discharge such as superfast pinch.

Absence of proper reflecting media at X-ray wavelengths further complicates the problem. At XUV wavelengths one must use metals at grazing incidence for mirrors in order to obtain high reflectivity. At X-ray wavelengths crystal reflectors may be used at subnormal incidence. Under such condition construction of a cavity is very complex. But some significant developments on multilayer reflectors have been made but the reflectivity is not too high. Multilayered mirrors have been studied intensely in recent years [18-27]. They could give normal incidence reflectance up to 66% at 130 A^0. Multilayered reflectors have been used in the experimental demonstration of double and even triple pass gain. These studies may aid the progress towards and actual cavity device, but the present technology is still far from allowing the fabrication of efficient resonators for X-ray lasers.

The absence of cavity implies that the laser must operate in a weakly coherent traveling wave of ASE (amplified spontaneous emission) mode. This form of operation in which the spontaneous fluorescence is amplified by stimulated emission down a rod shaped medium, requires a gain length product [given by eqn. (4)] $\alpha L \approx 20$ i.e. an overall amplification. $e^{\alpha L}$ of about 100 db. Such a device produces a beam whose divergence will be limited by either diffraction or geometry to approximately larger of the two values λ/d or α/L, where d is the diameter of the rod and L its length. Furthermore the beam will be coherent across its wave front. Thus ideally the diameter should have the optimum value $(\lambda L)^{1/2}$. If the diameter is less than this value, diffraction losses will reduce the effective gain length to a value $L^1 = d^2/\lambda$ which is less

than rod length L. Since the total power $P=vd^2$ it is obvious that if the pump power is to be kept within the reasonable limits (~ 100 TW), then $d \geq 10^3 \lambda^2$ cm and therefore $L \leq 10^6 \lambda^3$ cm assuming $\Delta\lambda/\lambda \approx 10^{-4}$ cm^{-3} assuming the oscillator strength of the lasing transition at unity.

From what has been stated above it is clear that the principle limitation on construction of an X-ray laser is the high pump power required. The condition can be satisfied indirectly when the system is pumped to a relatively long-lived reservoir state, which may be slowly filled and transferred into the upper laser state by some appropriate trigger. This approach relaxed the condition on the external pump by using the reservoir energy, but it is limited to short pulse action only. The lasing action must take place either on inner shell transitions of neutral or a weakly ionized atom or on a valence electron (optical) transition of a highly stripped ion. We call them respectively as non plasma and plasma schemes. An alternative approach for the generation of coherent short wavelength radiation is by frequency multiplication of the existing visible and ultraviolet lesser [28, 29]. This method is a convincing way of generation of coherent radiation up to 380 A^0.

In these experiments ultraviolet radiation is frequency multiplied in a gas medium using a near resonance to obtain a strong coupling co-efficient. Although this scheme possesses many interesting features such as feasibility, coherence, high power etc., the application to XUV and X-ray wave lengths are limited.

Non plasma schemes:

Non plasma schemes involve inner shell excitation. Inner shell transitions involve a vacancy within a closed shell of a neutral or weakly ionized atom. The vacancy can be created by particle collision or by photoexcitation. Subsequent decay of the hole can also involve a variety of processes, including radiative. Inner shell transitions are thus inherently complex. In this scheme the pump energy must be restricted to a narrow band to avoid the removal of the outer shell electrons. The basic laser process involves the removal of an inner shell electron to form in inversion with the outer shell vacancy.

For operating at longer wavelengths (~ 400 A^0), one of the outer closed shell electrons may be pumped by suitably filtered X- rays from a neighbouring laser produced plasma. Sodium [30] and neon (2A) have been theoretically studied. For sodium the electronic configuration is $1s^2 2s^2 2p^6 3s$. Here one of the 2p electron is removed to leave an inversion between the 2p-3s level of the Na II with a transition at 372 A°. In practice the self terminating nature of the inversion and heating of the medium by the pump severely limit required operating condition. Thus no gain has been experimentally obtained by these workers. A similar approach [31] has been used for Zinc and Cadmium and gain and laser action have been observed at about 800 A°. One of the methods suggested by Csonka [32] is yet to be realized in practice. This method was interesting in the sense that coherent X-ray could be produced by this method by combined X-ray and low energy photon pumping. In this method it is indicated that under certain condition population can be inverted in a variety of targets by a combination of X-ray (e.g. originating in a storage ring) and low energy photon (e.g., from a laser) pumping. Coherent soft X-rays could then be produced by stimulated emission. The metastable vacancy level Is2s2p4p° is pumped by

X-rays from a laser plasma from a high Z target in the heat pipe oven containing the lithium. A tuned dye laser transfers this population to the level $1s2p^22p$, which forms the upper state of the lasing transition. The lasing then occurs to the level $1s^22p^2p^o$ at 207 A^o.

We should also mention here the nonlinear techniques for generation of coherent radiation in the XUV region [33]. The techniques are interesting in the sense that they demonstrate the tunable pico second radiation across much of the VUV spectral regions. These techniques are, however, not considered as important candidates for generation of coherent radiation in XUV region.

Plasma systems:

Pumping of optical transition of highly ionized species is a promising candidate for consideration in XUV lasers. There are two types of pumping. In the first group the pumping transition is upward through collisional excitation [8, 34] and photo-excitation [8]. In the second group the pumping transition is downward which occurs via recombination and charge exchange. Both excitation and recombination regimes may be described in two steps. The first step is common in both regimes; that is to create a moderate to high density plasma of multiply charged ions. The purpose is always to produce these ions in the in the upper stage of ionization, even though there are several ways of making such a plasma. The difference appears in the second step: the formation of population of population inversion.

Recombination laser: This is one of the most promising candidates for XUV. It is based on the fact that almost any type of collisional recombination prefers the most excited states of an ion. These upper states then cascade down to lower states through collisions and radiation. If one of the excited states decays more rapidly than a higher state, population inversion occurs between these two states.

Let us consider a plasma containing completely stripped ions. Rapid cooling of the plasma introduces a strong non-equilibrium regime such that three body recombination populates predominantly the most excited levels of the recombined H-like ions. Only then does a chain of collisional-radiative transitions follow, acting as a recombination flux to intermediate levels, until finally in reaches the lowest lying levels. On the other hand, owing to the low temperature of the free electrons, electron ion collision can no longer balance the radioactive decay of the low lying levels, while they are still efficient in populating the upper levels of the ions. As a result population inversion may occur between intermediate and lower levels. Fast cooling is considered as very important for an recombination laser to produce high recombination rate, and therefore large population inversion. There have been several observations of gain either measured directly or indirectly from expansions cooled recombination systems. Cooling modes which have been investigated include radiation [35, 36, 37], adiabatic expansion [38 - 40] and conduction [41 - 44]. Recombination X-ray lasers are among the most successful type of X-ray lasers, collisionally pumped X-ray lasers, recombination lasers have the advantage of using of $\Delta\eta = 1$ lasing transition and having higher efficiently [45 47]. Using slab targets irradiated with Nd glass laser pulses of 10 ns duration, Elton and coworkers [48] have indirectly estimated a gain of 2% per cm at 520 A^o on the hydrogen like carbon line CVI ($n = 4$ to $n = 3$). Gain length product

up to 5 on CVI H_α at A^o have been measured by a group of Hull [49, 50] during the expansion of thin carbon fibers of 4 μm diameter following heating by a 1.06 μm laser pulse focused to a line 40 μm wide and 2 mm long. The basic condition necessary for creating inversion during recombination may be understood as follows. The plasma must be first rapidly heated and then cooled over time scales of the order of characteristic ionization time. For CVI ion densities ~ 10^{21} ion/cc the time is of the order of 100 ps. These times are characteristic of the duration of mode locked pulses from a Nd glass laser and of the expansion of plasma of appropriate temperature ~ 10^{-3} in size. In this system the inversion is achieved as sufficiently high density (~ 10^{19} ions/cc) to give an inversion density ~ 10^{15} ions/cc, with a gain co-efficient ~ 10 cm^{-1}. A direct observation of gain at 182 A° in a plasma formed by an expanding laser heated carbon fiber has been made [49, 50].

Excitation pumping: In this process inversion is produced through selective excitation or ionization either by photons through collisional processes. The idea of population inversion by resonant photo absorption is closely analogous to the conventional pumping schemes used in usual optical lasers. It suggests the use of two component plasma. One component consists of the ion of the active medium; the other is composed of the source ions. The source ion radiates a very intense line at a frequency that matches one of the transitions lines in the active medium ions. The upper lasing level of the active medium is selectively populated by resonant photoxitation by the intense line emission from the source ions. Population inversion is created because of the selection. photopumping is a wavelength co-incidence between the pump and pumping transition. Several matched pumping linear pairs have been identified (51). There has been a lot of interest in the field resonantly photopumped X-ray lasers (52-57]. In case of Ne like Ti lasing action has been demonstrated at 326.5 A [85] and this is due to resonantly photopumped schemes.

Electron ion collisional excitation: The idea of producing a steady state population inversion in a plasma by electron collision at excitation pumping is based on the fact that for some particular multicharged ions, there exists a pairs of states for which electron impact excitation from the ground state are comparable but from which the relative decay rates are much different. For example the two levels of Ne- like ions $2p^5$ 3s and $2p^5$ 3p have similar collisional excitation rate from the ground level $2p^6$, whereas the radiative decay is very rapid for the 3s level and strictly forbidden for the 3p one [8, 59]. collisional excitation represents the another successful pumping method. High lasing gains have been demonstrated at many laboratories [60]. The lasing wavelengths have been extending into the "water window" regime [61, 62] a fact which is importing for biological application.

Selective ionization scheme:

In this type of scheme inversion is achieved either as a direct result of inner shell ionization [63] or as a result of Auger transitions which follow the inner shell inversion [64-67]. Selection ionization by photo ionization is based on the fact that the probability of ejecting an inner-shell electron by photo ionization is generally much larger than that for electing an outer shell electron, provided that the photon energy is above the inner shell ionization threshold. As

for an example we may consider the population inversion between $1s2p^1p_1$ and $1s^2\ ^1s_o$ levels of He-like ions by photo ionization of an 1s electron from the $1s^22p^2p$ term. It is assumed that certain ion in the $1s^22p^2p$ term are already present. If they are illuminated by photons with energies higher than there Is shell ionization threshold, one of the two K electrons may be removed and subsequently the ions find themselves in the $Is2p\ ^1p_1$ level which is at a higher ionization state. At the same time the outer shell 2p electros may also be removed and the resulting ion will be in the $1s^2\ ^1S_o$ state. The fact that the photo ionization cross section for removing an inner shell electron is larger than that for an outer shell electron, it is obvious that more ions will be produced in the $1s2p\ ^1P_1$ level than in the $1s^2\ ^1S_o$ state. Thus a population inversion between these two levels is created. Despite the success of recombination and colisional approaches to soft X-ray lasers, photo ionization schemes continue to create sufficient interest in producing X-ray lasing [68-75]. This is because the photo ionization schemes have many attractive features. However for those who are working with hot dense plasma with recombination and collisional pumping, requiring preheating of the material, the immediate problem, is the wastage of energy and increase of line broadening. A typical laser working in a hot intense plasma has relative line width $\Delta\lambda/\lambda$ of the order of 10^{-4} [4]. The use of synchrotron radiation from a high energy storage ring for X-ray laser pumping was originally suggested by Csonka et. al [32]. The exploration of its advantage as an X-ray laser pumping source and its application to photo ionization schemes were also made by a number of workers. We may indicate some of the advantages as an X-ray laser pumping source and its application to photo ionization schemes were also made by a number of workers. We may indicate some of the advantages of using SR as follows. SR leaves a typical electron strage ring with high energy density, high repletion rate and comes in short pulses. This provides us with a very high pumping power necessary for X-ray laser. In this connection the free-electron system (FEL) under study [76] at Stanford Linear Accelerator Center (SLAC) should be considered as a big investment. We should also carefully monitor the recent progress in obtaining ultra short intensity optical laser pulses and extremely bright X-ray pulses, by applying an intense ultra short light pulse to solid targets. New technologies [77-81] have made it possible to generate several to tens of terawatt light pulses of 1-0.1 ps duration. This suggests the possibility of extremely bright X-ray pulses with a peak power greater than 10^{11} W.

Uses of x-ray laser:

In the earlier sections some indications have been given as regards the development of X-ray laser research. We are all waiting anxiously for the results. Once a practical device of this type is made mankind and present civilization will be immensely benefited. As for instance consider the application of X-ray lasers in the field of biological systems. One of the barriers to improving our understanding of molecular basis of life is the difficulty one encounters if one attempts to investigate individual macromolecules in living tissues. To resolve the structure of molecules, one needs radiation of wavelength comparable to the size of the atom. i.e., in the X-ray region. A practical X-ray laser would provide a source with a high enough contrast required for the study of biological macromolecules. It could permit one to make X-ray holograms of bio-

molecules. One of the uses of scientific importance would be to coherent excitation of nuclear states. In that case it should be possible to perform the so called photon echo experiment in unclear region.

Laser research in India: Retrospect and prospect:

I may appropriately conclude my talk with some comments on the field of laser and optical Science in India. During last thirty years the Science of laser and technology has attained certain degree of maturity. Some central institutes like the centre of advanced technology as Indore has presumably the best facilities to carry out research and development work in laser, including X-ray laser. However a major role has to be played by the state universities and Colleges. We have to developed necessary infrastructure in the state Universities and the big man power available should be put to work. Through agencies like University Grant Commission, Department of Science and Technology and many more are involved as funding agency in the development or relevant technology yet something is missing. Maiman built the first laser device in 1960. During this period one renowned physicist of India remarked that we have missed the bus. Laser should have been discovered in India. Atomic and molecular spectroscopy was well developed and precise knowledge in detail about the atomic and molecular energy levels was also known to the physicists of India. Only thing missing was the relevant technology and the necessary infrastructure. In the case of X-ray laser we are concerned with an entirely different technology. It will be unfair to say that we have missed the bus but we should catch bus this time.

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