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Journal of the Technological Association

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Institute of Chemical Technology (Autonomous),

Matunga, Mumbai – 400019.

Editorial

With great pride we present to you Volume 62-63 of the Bombay Technologist. This volume after a long time and a lot of delays finally puts the journal of the Technological Association back on schedule. It involved great efforts from the erstwhile secretaries, editor and of course our team spearheaded by our editor, Prof. V.B. Patravale.

In this volume, we present three invited lectures and ten articles that have been chosen by our esteemed faculty from the numerous articles submitted. The response from students was encouraging, but we do hope for greater participation from our Masters and PhD colleagues. It was great to receive articles from even second year students, and the enthusiasm from first years to learn was commendable.

As pointed out to us by the editorial board, we would like to pass on to one and all, that review articles submitted to a journal must incorporate an in depth study of the subject and most of all referencing must never be neglected. Not to forget, many of the articles submitted were appreciated for the immense hard work by the students.

We would like to take this chance to thank Prof. A. B. Pandit for taking out valuable time to conduct the seminar on 'Scientific Writing' for Bombay Technologist. We would also like to thank each of our evaluators who gave their time and efforts towards Bombay Technologist, and the entire supportive faculty of ICT.

We hope you enjoy reading this volume as much as we enjoyed compiling it.

Regards,

Team Bombay Technologist
Volume 62-63.

The Editorial Board

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September 23, 2013

Message

The Technological Association (TA) of ICT has been actively involved in several students programmes and one such activity is its annual publication of **Bombay Technologist**, which has been in existence for decades. The articles for Bombay Technologist Volume 62-63 have been peer reviewed by the faculty. The 10 articles in this issue are review papers with a variety of topics. Chitosan for skin regeneration in burn patients adequately covers the state of the art. A popular topic is biodiesel which is covered in 2 papers, one based on waste cooking oil and another on palm fatty acid distillate. Gene-replacement therapy for RPE65 associated Leber's congenital amaurosis and organ-on-a-chip as replacement to animal testing, resveratrol as a nutritional supplement, self-assembly of block copolymers and its applications in drug delivery and solar energy storage by polymers have been included. Computation of dynamics of pipe flow in two dimensions is a research article based on in-house code. Strengthening of security paper has been researched using inductive reasoning to establish various hypotheses for increasing the durability of paper. I would have liked to see more papers on emerging areas since several students make excellent seminar presentations.

The TA was always headed by the Director (now Vice Chancellor) as the President with a senior faculty member as the Vice President who is currently Professor P.M. Bhate, Dean (SAHRD). One of his major achievements is to combine academic and extra-curricular activities. The launch of **Vortex ICT** has unified the numerous successful festivals in ICT such **as Exergy, Young Innovator's choice Competition (YICC),** and **Young Researchers' Conference (YRC)** into India's biggest technical festival in the chemical sciences and engineering. YICC-YRC provided a formidable challenge to the most seasoned students, from all over India; they were asked to find pragmatic solution with 72 hours to industry defined problems in the disciplines of chemical engineering, technology, sciences, pharmacy, biotechnology, and bio-processing. Several industries participate to encourage them. PG and Ph D Students also present their research papers and posters. It would be a great idea to include some of the best papers/posters and also list of participants in technical festivals in Bombay Technologist.

I compliment the Editorial Board for keeping the tradition on with a good number of papers.

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INDEX

1. Chitosan: A Biopolymer for Skin Regeneration

Review Article

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Abstract

Skin regeneration is a growing field of interest following the limited treatment modalities available for burn patients. An ideal treatment for burns is required to be fast, be able to restore complete functionality and be within the reach of patients. Such requirements may be achieved only by re-growth of the skin, but this is often limited in case of serious burns. The use of polymeric scaffolds presents a template for the regeneration of skin, allowing adherence of cells and providing support. Scaffolds of multiple materials have been tried; Chitosan scaffolds have yielded good results in various experimental aspects including biodegradability, low immunogenicity, compatibility, etc. Additionally properties such as angiogenesis, wound healing and induction of fibroblasts make it an ideal candidate for skin regeneration.

Keywords: Tissue engineering, skin regeneration, polymeric, scaffold, chitosan.

1. Role of Tissue Engineering

Tissue engineering is a promising therapeutic approach that involves combining living healthy cells of patients into three dimensional temporary scaffolds, made of natural and synthetic materials, in order to produce functional organs to be replaced back into the body. Despite many advances, tissue engineers still face significant challenges in repairing or replacing tissues that serve predominantly biomechanical functions. A major obstacle identified is, that the scaffold plays an important role as the extracellular matrix but is unable to create the correct microenvironment during the engineered tissue development to promote the accurate in vitro tissue development. [1, 2] Presently

in case of skin injury, the conventional solution is to use a skin substitute constructed using the concept of tissue engineering.

Serious injury to the skin, such as burns, trauma or chronic ulcers, requires immediate coverage to facilitate repair and restore skin function which can be done with skin grafts or tissue engineered skin.

2. Skin Substitutes & their Drawbacks [2]

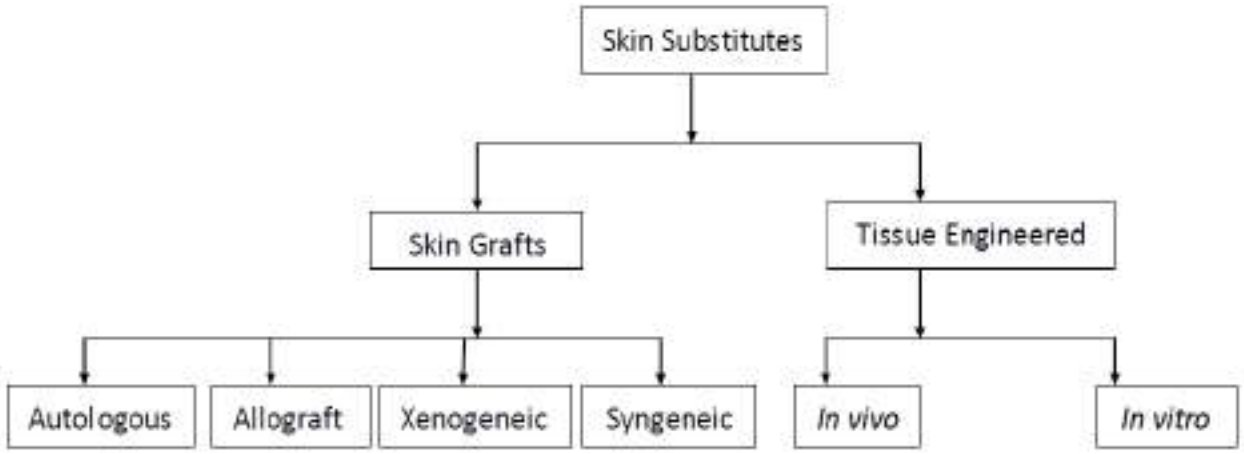

Figure 1: Classification of Skin Substitutes

Conventionally, tissue engineered skin exists as cells grown in vitro and subsequently seeded onto a scaffold or some porous material which is then placed in vivo at the site of injury. The gold standard for skin replacement still remains the autologous skin graft in which an area of suitable skin is separated from the tissue bed and transplanted to the recipient area on the same individual from which it must receive a new blood supply.

3. Problems with existing commercially available Skin Substitutes

- Reduced vascularization: Some existing skin substitutes do not allow angiogenesis to occur. This inability for substitutes to 'take' leads to cells in the replacement dying and ultimately the construct sloughs away from the host.
- Scarring: Scarring at the graft margins is problematic - functionally, mechanically and aesthetically. Scar tissue is not identical to

the tissue which it replaces and is usually of inferior functional quality. It is less resistant to ultraviolet radiation, and sweat glands, hair follicles do not grow back within scar tissue.

- Absence of differentiated structures: Bioengineered skin substitutes are often relatively simple single layered or bilayered structures. The absence of complexity with regard to differentiated structures means that presently available treatments offer none of the many other characteristics of functioning skin.
- Delay involved with cell culturing: Cells for the epidermal and dermal components can take between two and three weeks to expand to sufficient numbers for grafting purposes.
- Persistence of cells in heterologous grafts: Cell persistence may be desirable when covering a large area of missing skin but brings with it long-term safety challenges. E.g. Cell age may affect the duration that allogeneic cells survive in vivo.
- Biocompatibility, mechanical and handling properties: Currently available skin substitutes do not mimic normal skin composition or its mechanical properties. This is in part explained by the fact that the manufacturing processes employed are not sophisticated enough to recapitulate the developmental morphogenesis used to create skin naturally.
- Development, safety and product costs: The early stages of bringing a new tissue-engineered product to the market place can be costly.

4. Tissue Engineering Using Polymeric Scaffolds

The emerging and promising next generation of engineered tissues is relying on producing scaffolds with an informational function i.e. material containing growth factor sequences which facilitates cell attachment, proliferation and differentiation in vivo. A three-dimensional scaffold provides an extracellular matrix (ECM) analog which functions as a necessary template for host infiltration and a physical support to guide the proliferation and differentiation of cells into the functional tissues or organs[1,2], making the process faster and more suited to a particular individual.

4.1. Ideal Properties of Polymers to be used as a Scaffold for wound healing.

- The polymer should restore the epidermal barrier function and become incorporated into the healing wound.

- It should create an improved environment for epidermal regeneration and provide a barrier against infection and water loss.
- The material should be sufficiently permeable to water vapor and allow exudates to leave the wound.
- It would also be advantageous for the material to be haemostatic, transparent, and biodegradable as it restores normal function to the skin.

Some of the polymers currently used in tissue engineering are as follows:

Natural	Synthetic
Collagen	Polyesters
Gelatin	Poly(glycolic acid), poly(lactic acid) and their copolymers
Silk Fibroin	Polylactones
Fibrin	Poly(propylene fumarates)
Chitosan	Polyanhydrides
Starch	Tyrosine-derived polycarbonates
Alginate	Polyurethanes
Hyaluronan	Polyorthoesters

Table 1: Different types of Natural and Synthetic polymers used in tissue engineering

4.2. Natural Origin Polymers [2]

To date, no substitute or replacement for the patient's own skin has been prepared that shows qualities close to those of autologous grafts. Natural polymers have been found to be more suitable for tissue regeneration due to their similarity with normal body components. They have the ability to provide a microenvironment similar to the natural microenvironment and integrate well with growth factors. They perform a diverse set of functions in their native setting. For example, polysaccharides function in membranes and intracellular communication and also as storage, and proteins function as structural materials and catalysts. However, these natural origin polymers also have a few drawbacks.

Due to their similarity to biological substances, they often invoke an immunogenic response. There may be high degree of variability in natural materials derived from animal sources and they are structurally more complex than traditional materials; manipulation thus becomes more elaborate and complicated. They also tend to

degrade faster than the synthetic ones and they also have limited processing routes in many cases. Many different naturally derived polymers like collagen, hyaluronan, gelatin, etc. have been considered for use in tissue engineering with variable success, but the most striking and versatile polymer to date has been chitosan.

5. Chitosan

Chitosan is a cationic polymer obtained from chitin (A natural polysaccharide) comprising copolymers of β (1→4) glucosamine and N-acetyl-D-glucosamine. It is a derivative of chitin (poly-N-acetylglucosamine), which is the second most abundant biopolymer after Cellulose. [1]

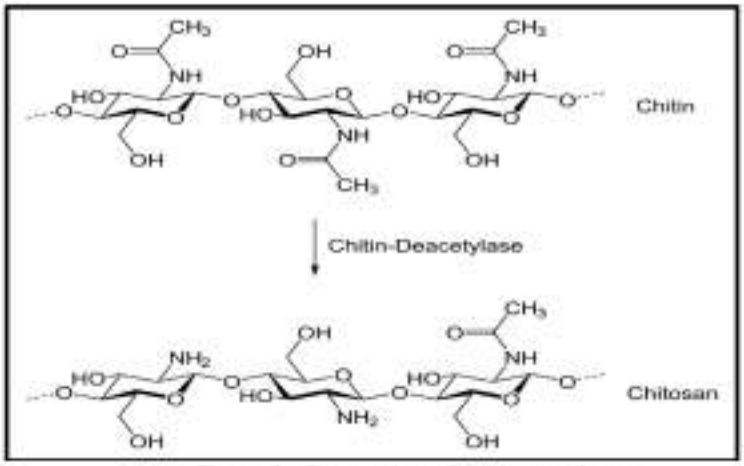

Figure 2: Conversion of chitin to chitosan

5.1 Properties of Chitosan crucial for wound healing

- Chitosan's property of binding with red blood cells allows it to rapidly clot blood. It has recently gained regulatory approval in the USA for use in bandages and other haemostatic agents.
- Chitosan modulates the functions of inflammatory cells and subsequently promotes granulation and organization.
- As a semi-permeable biological dressing, it maintains sterile wound exudates beneath a dry scab, preventing dehydration and contamination of the wound, to optimize conditions for healing.[4]
- It has been proved to be biologically renewable, biocompatible, non-antigenic, non-toxic and bio-functional.
- It is metabolized by certain human enzymes, especially lysozyme and thus can also be considered biodegradable.[1]
- It can act as an ideal wound dressing as it exhibits a positive charge, film-forming capacity, mild gelation characteristics and a strong tissue adhesive property.

- Chitosan induces fibroblasts to release interleukins, which are involved in migration and proliferation of fibroblasts.
- Chitosan based systems at micro and nano scales in combination with other polymers have been developed for skin tissue engineering, using electrospinning method and lyophilization.[1]

Chitosan has two major properties required for skin regeneration:

Antimicrobial property: Chitosan has been widely investigated as an antimicrobial agent due to its destabilizing effect on the outer membrane of gram-negative bacteria and permeabilization of the microbial plasma membrane by binding with sialic acid in phospholipids. [4]

Wound-healing effects of Chitosan: Chitosan and its derivatives can accelerate wound healing by enhancing the functions of inflammatory cells, such as polymorphonuclear leukocytes, macrophages, and fibroblasts or osteoblasts. [4]

6. Evaluation of Chitosan based scaffolds

6.1. Comparing the Biocompatibility of a Bilayer Chitosan Skin Regenerating Template, Human Skin Allograft, and Integra Implants in Rats [3]

A comparison by Shah Jumaat Mohd Yussof and his colleagues with regarding the biocompatibility of chitosan skin regenerating template with other skin substitutes i.e. Integra and Human skin allograft (HAS) for temporary coverage of clean burn wounds proved that, chitosan can physiologically stimulate the tissue repair process and favors angiogenesis. This evaluation provides the in vivo results of the angiogenic activity, inflammatory reactions and level of invagination by chitosan.

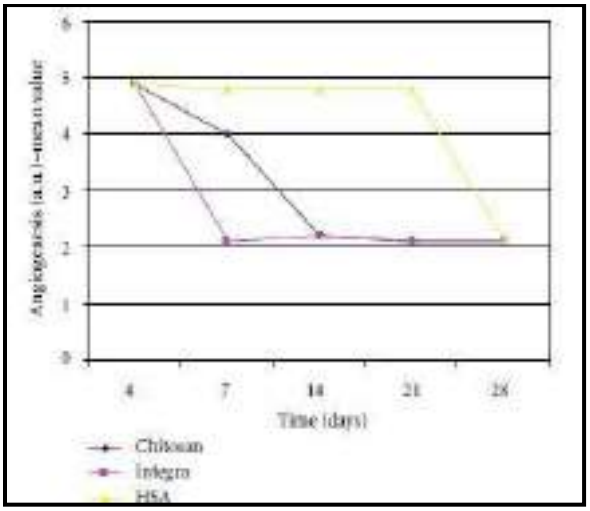

Figure 3: Level of angiogenesis v/s time. [3]

6.2 In vitro evaluation of a Biomedical Grade Bilayer Chitosan Porous Skin [5]

In this study by Chin Keong Lim et al chitosan porous skin regenerating template (CPSRT) was observed to support cell attachment. It was claimed that this growth

was likely due to the initial adaptation of cells to the 3D environment by adhering to the chitosan motifs in the construct. This study strengthens the possible application of chitosan as a template for skin regeneration providing an adequate microenvironment.

Figure 4: Live/dead cell staining of pHDF cultures seeded on CPSRTs. (a) Three-dimensional (3D) surfaces were scanned using a CLSM a day 5. (b) Viable pHDFs (green) were confluent at day 14. (c) Cross-sectional view of a CPSRT seeded with pHDF at day 14. [5]

6.3. Chitosan Nanofibrillar Scaffold for Skin Repair and Regeneration [6]

A Chitosan scaffold produced by accumulation of electrospun nanofibers was observed to be a better substrate for adhesion, growth, and differentiation of the three main skin cell types (keratinocytes, fibroblasts, and endothelial cells) than other types of chitosan device (films, sponges, gel, etc.). Moreover, they are fully biocompatible along with its progressive integration and colonization in vivo, in contrast with lamellar 3-D chitosan sponge that elicits the formation of a foreign body granuloma.

7. Modifications in the Chitosan Moiety

In order to realize the full potential of chitosan and bring a breakthrough in its utilization, there have been attempts made to modify chitosan to obtain various derivatives. [4] The probable methods are as follows:

- Chemical modification of chitosan molecules has been done at three reactive positions: the amino group at C-2 and the primary and secondary hydroxyl groups at C-3 and C-6, respectively [4]. This is of great significance because the fundamental skeleton of chitosan and its original outstanding physicochemical and biochemical properties as a biomaterial are retained after modification, while achieving desirable properties for tissue repair and regeneration applications.
- Cell Specific Ligands or Signaling Molecules have been introduced into chitosan in order to enable desirable interaction with cells and mediate specific cell responses and behavior. From the viewpoint of biomimetics, combination with extra-cellular matrix (ECM) proteins like collagen, fibronectin, and laminin is an attractive strategy for chitosan modification. However, direct modification by proteins

has been found to have several limitations due to possible immunogenicity, low cell-binding efficiency, and easy denaturation. These drawbacks can be overcome while maintaining the biofunctions by using small peptide sequences derived from the ECM.

- Cross linking is another effective approach for modulating characteristics of chitosan-based biomaterials for tissue repair. Typically, in the case of chitosan porous scaffolds, the mechanical strength and biostability can be enhanced by the cross linking treatment. Two types of cross linking are used for the chitosan porous scaffolds: Ionic cross linking and covalent cross linking.

7.1. Examples of Modified Chitosan

i. The hydrophobic character of the acetyl group on chitosan renders it water insoluble [7]. This water-insolubility of chitosan is disadvantageous for its wide application as an antibacterial agent. Hence to improve its water solubility, it is necessary to prepare anionic side chain- grafted, water-soluble chitosan (WSC) derivatives having zwitterionic properties [8]. To prepare these derivatives, mono (2-methacryloyl oxyethyl) acid phosphate and vinylsulfonic acid sodium salt have been grafted onto chitosan.

ii. Biopolymer blends between collagen and chitosan have been found to have potential to produce cell scaffolds with biocompatible properties since collagen is the major protein component of the extracellular matrix, providing support to connective tissues. [1] The chitosan – collagen blend showed promising properties including mechanical strength, biodegradability, and cell proliferation stimulating ability, which are crucial for tissue engineering. This biopolymer blend can further be cross linked with Glutaraldehyde in order to improve its biostability. [9]

iii. Due to the pH-sensitive character of chitosan, combination of chitosan with a thermo-responsive polymer, poly(N-isopropylacrylamide), has produced dual stimuli- responsive polymeric systems that can be used as delivery vehicles that respond to localized conditions of pH and temperature in the human body. [10]

iv. The poor solubility of chitosan at neutral and basic pH limits its biomedical applications, especially in physiological environments. Attempts have been made to boost the positive charge density, resulting in an enhanced solubility of chitosan over a broader range of pH [11]. Development of trimethyl chitosan (TMC) was an effort in

this regard. Chemical modification of chitosan to trimethyl chitosan provided derivatives that are soluble at neutral and basic pH.

8. Conclusion & Future Prospects

Chitosan has been proved to be biologically renewable, biodegradable, biocompatible, non-antigenic, non-toxic, hemocompatible and biofunctional. Due to these desirable properties, chitosan has been widely proposed as a scaffold material in tissue engineering applications and as a carrier for various drug delivery systems. Apart from the applications of the chitosan polymer itself, applications of chitosan in combination with other materials have also been studied. Such advances in the applications of chitosan are hinting towards the future of chitosan based scaffolds for skin regenerations. The low cost of chitosan in comparison with other biopolymers such as collagen is an incentive for further research and applications. Chitosan allows us to overcome the disadvantages of currently available skin substitutes and thus could soon replace them as a suitable, effective alternative providing a solution to thousands of burnt patients. It can be hypothesized from these findings that if the required in-vivo studies of chitosan are carried out, a fast-track solution to burns and scarring could be found, simultaneously enhancing the healing process of the body.

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2. Production of Biodiesel from waste cooking oil

Review Article

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Abstract

High variation in global price of petroleum crude oil has an inauspicious impact on national economy of crude oil dependant countries like India. As per the survey, petroleum crude oil consumption in India was 3.182 million barrels/day in 2010 and oils consumption is growing fast, in 2002 consumption rate increases by 3.28% while in 2010, it increases by 6.77%. Therefore, looking for the eco friendly alternative path to produce replacement for the petroleum based fuel like diesel is the recent task for green technologists. Transesterification reaction of frying oil generally known as waste cooking oil with alcohol (methanol or ethanol or both) in presence of various different catalysts to synthesis transesterified product i.e. biodiesel is the future need as the alternative energy source because of depletion of fossil fuels.

The comparisons of the biodiesel production from waste cooking oil using various methods were also reviewed in this paper. This paper reviews the work that has been already studied in biodiesel production technologies from waste cooking oils.

Keywords: Biodiesel, waste cooking oil, Trans-esterification, frying oil etc.

1. Introduction

Currently, intensive efforts to reduce green house gases (GHG) emission from petroleum-based diesel usage have initiated vast production of biodiesel worldwide. It is biodegradable in which the fuel is derived from renewable sources; non-toxic, less emission of hazardous gases during combustion and has been categorized as the most promising substitute to petroleum-based diesel [1]. However, the bottleneck to produce

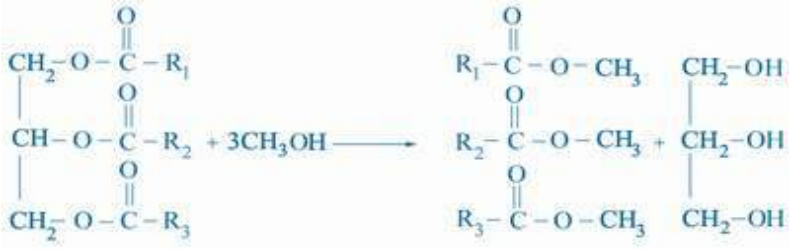

Figure 1: Transesterification reaction [5]

biodiesel in commercial scale is the high cost of edible virgin oil, in which account for more than 70% of the overall biodiesel production cost. In addition, using edible virgin oil such as rapeseed, sunflower, soybean and palm oil in biodiesel production has raised the concern of food versus fuel debate. Thus, recent biodiesel development has shifted to use non-edible and waste cooking oil (WCO) as a new and sustainable feedstock for long term production [2]. It is strongly believed that using these oils will help in improving economical feasibility of biodiesel and minimize the hurdle of food versus fuel phenomena. See Table 1 for physical and chemical properties.

Table 1. Physical and chemical properties of waste cooking oil. [3]

Properties	WCO
Physical	
Density at 27^0C, g cm^{-3}	0.9168
Acid Value, mg KOH g^{-1}	5.0
Free fatty acid, %	2.54
Moisture content, %	0.162
Saponification value	183.5
Chemical	
Fatty acid composition, wt.%	
C16:0	39.3
C16:1	0.18
C18:0	2.3
C18:1	46.3
C18:2	11.9

2. Transesterification

The most common way to produce biodiesel is by transesterification, which refers to a catalyzed chemical reaction involving oil and an alcohol to yield fatty acid alkyl esters (i.e. biodiesel) and glycerol. Triglycerides, as the main component of vegetable oil, consist of three long chain fatty acids esterified to a glycerol backbone [4]. When triacylglycerols react with an alcohol (e.g., methanol or ethanol or both), the three fatty acid chains are released from the glycerol skeleton and combine with the alcohol to yield fatty acid alkyl esters. Glycerol is produced as a by-product.

Methanol is the most commonly used alcohol because of its low cost and is the alcohol of choice in the processes developed. In general, a large Excess of methanol is used to shift equilibrium towards the product side.

A simple molecular representation of the reaction is shown in Fig. 1.

3. Various methods to produce Biodiesel from WCO

3.1 Alkali catalyzed transesterification reaction

In alkaline catalyzed transesterification process, Strong base catalysts e.g. sodium methoxide, sodium hydroxide, potassium hydroxide and potassium methoxide are used. This process is most effective in converting triglycerides into esters when free fatty acid level is less than 1%. It is the most widely used process because this reaction happens at moderate temperatures and lower pressures and also there is high conversion efficiency (98%). This process requires only a small time and there is a direct conversion of biodiesel without any intermediate steps. However, it becomes less effective when the free fatty acid level exceeds 1% because the FFA reacts with the most common alkaline catalysts (NaOH, KOH, and CH3ONa) and forms soap which adversely affects on the separation of ester from glycerin i.e. ultimately reduces the conversion rate. Certain amount of alkaline catalyst is consumed in producing soap and hence, catalyst efficiency decreases [6].

3.2 Acid catalyzed transesterification reaction

In acid transesterification process, acidic catalysts like, sulfuric acid, phosphoric acid, hydrochloric acid and organic sulfonic acid are used. In this process, a strong acid is used as a catalyst for esterification of the FFAs and the transesterification of triglycerides [8]. This process does not yield soap due to the absence of alkali material. The esterification rate of the FFAs to alcohol esters is relatively fast however; the transesterification of the triglycerides is very slow, taking several days to complete.

3.3 Two step method

The combined process with acid catalyzed pretreatment is developed to improve the yield of biodiesel by the WCO. The pretreated step of this process is to esterify the FFA with methanol by acid catalysis. When the FFA content is lower than 0.5%, the sulfuric acid drained and the solid alkali is introduced into the system to complete the transesterification [10].

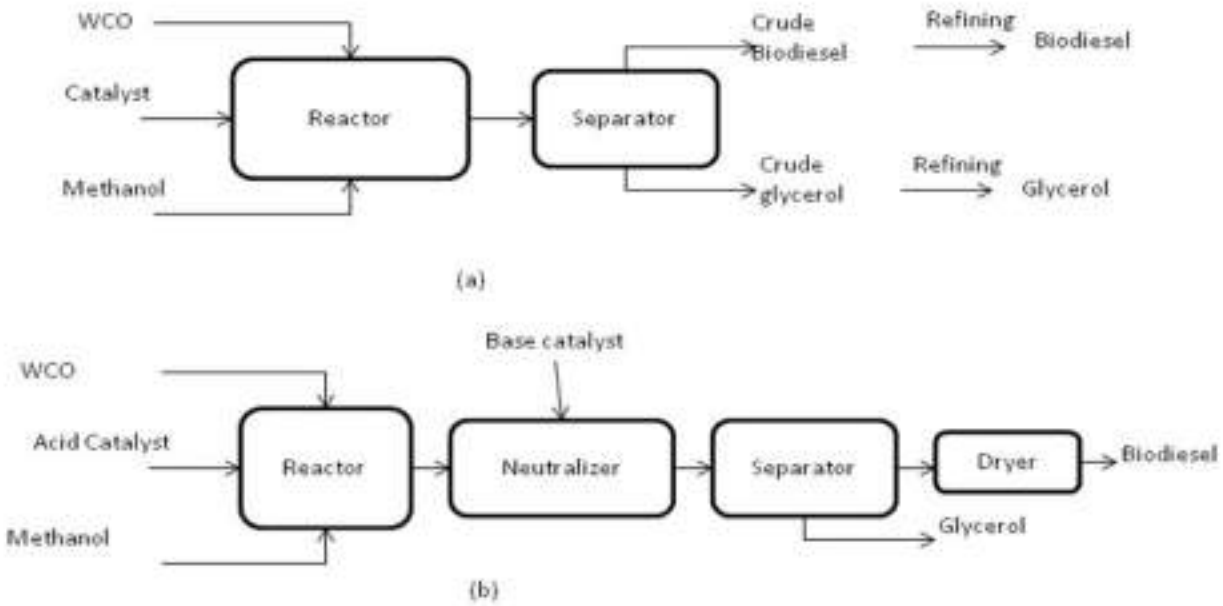

Figure 2: a) Alkali catalyzed method [7], b) Acid catalyzed method [9]

However, long reaction time, no recovery of catalyst and high cost of reaction equipment are disadvantages of this process. To overcome the disadvantages of acid catalyzed process or pretreatment, the homogeneous Lewis acid catalyst (carboxylic salts) is used [11]. However, the reaction temperature is too high (≤200 ◦C), and the conversion ratio is relatively low (≤90%). That is why heterogeneous catalyst is used in this method and also this catalyst can be recycled and reused. At the first step, ferric sulfate introduced to catalyze the esterification reaction in which FFA in the WCO reacted with methanol. The ferric sulfate that has very low solubility in the oil is separated from the liquid after the methanol recovery, and could be recovered by ashing process [12]. At the second step, potassium hydroxide is added to catalyze the transesterification reaction in which triglyceride (TG) reacted with methanol. Without wastewater, reusable catalyst and low cost of reaction tank, this two-step catalyzed process exhibits potential application in the biodiesel industry. At the first step, ferric sulfate introduced to catalyze the esterification reaction in which the Fig. 3. Mechanism of synthesis biodiesel by two-step catalyzed process. FFA in the WCO reacted with methanol. The ferric sulfate that has very low solubility in the oil is separated from the liquid after the methanol recovery, and could be recovered by ashing process [13]. At the second step, potassium hydroxide is added to catalyze the

transesterification reaction in which triglyceride (TG) reacted with methanol. Without wastewater, reusable catalyst and low cost of reaction tank, this two-step catalyzed process exhibits potential application in the biodiesel industry.

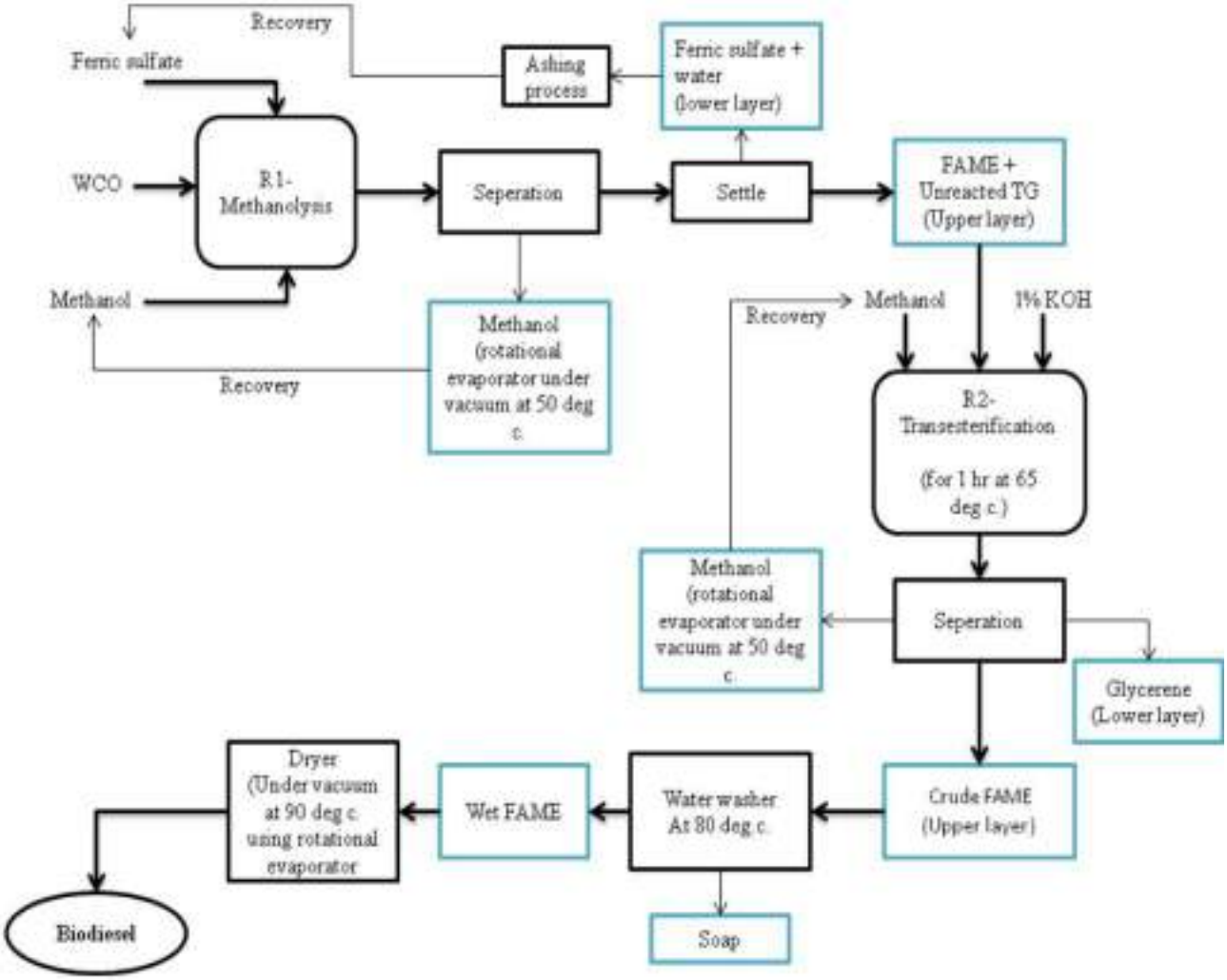

Figure 3: Two step method

3.4 Supercritical Method

A combination of acid- and alkali-catalyzed processes has been developed to overcome disadvantages of Alkali and acid catalyzed method caused by the presence of FFA and water; these can be essentially overcome if a non-catalytic biodiesel production is realized [14]. In such a situation, supercritical fluid has received a special attention as a new reaction field due to its unique properties.

Following figure 4(a) shows a schematic diagram of the supercritical methanol method. In supercritical methanol, TG in oils/fats is found to be converted to FAME

without any catalyst due to its methanolysis ability. Compared to the alkali-catalyzed method, there are some advantages, the production process becomes much simpler the reaction is so fast, FFA in oils/fats can be converted to FAME through methyl esterification and Yield of FAME is higher. This method, therefore, offers potentially a simple process for producing biodiesel fuel [15]. Although this process has many disadvantages, it requires restrictive reaction conditions. In such conditions, special alloys (e.g.Inconel and Hastelloy) are required for the reaction tube to avoid its corrosion. In addition, FAME particularly from poly-unsaturated fatty acids, such as methyl linolenate, are partly denatured under this severe condition [10].

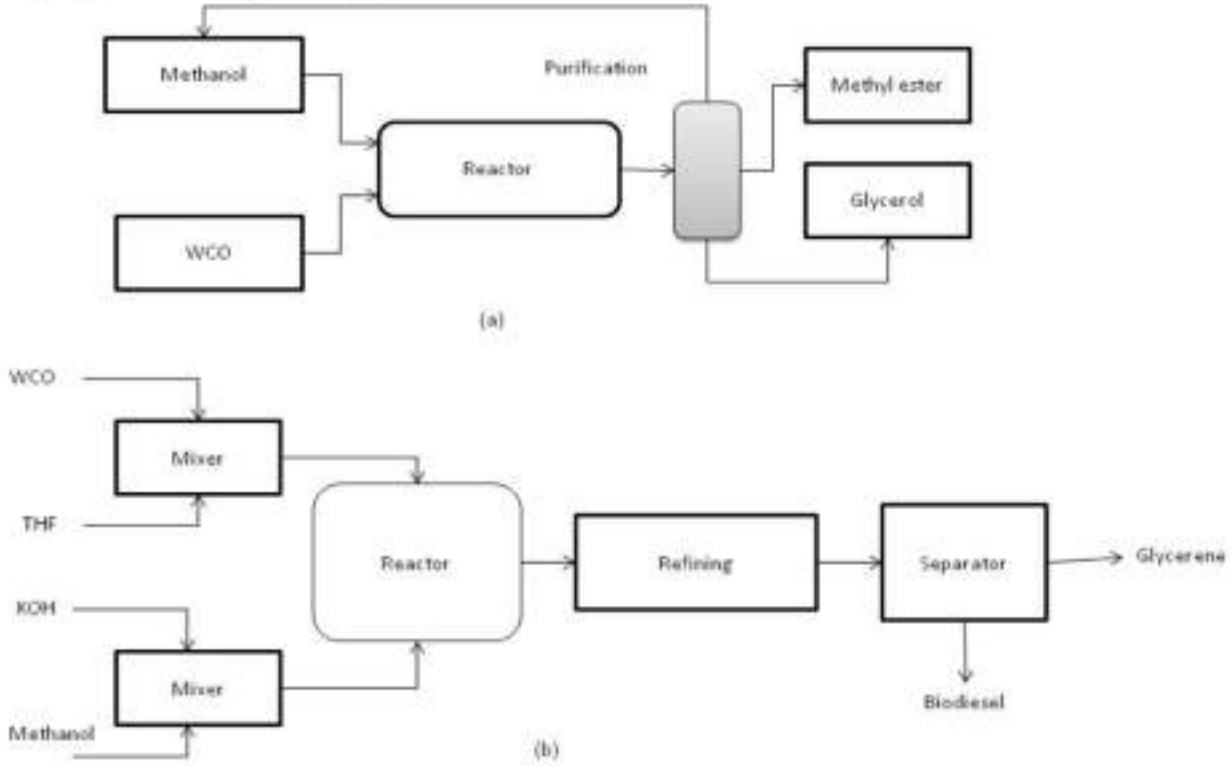

Figure 4: (a) Supercritical method [36], (b) Biox method [19]

3.5 Biox Method

The main problem for the lower rate of transesterification is that the reaction mixture is not homogeneous because the oils and alcohols are not miscible each other because of their chemical structures. Oil disperses in the methanol medium, so the probability and the rate of collision of the glyceride and the methoxide (the mixture of methanol and the alkaline catalyst KOH or NaOH) molecules becomes lower. This lowers the rate of collisions of molecules and so the rate of reaction causes longer

reaction times, higher operating expenses and labor [17].

To overcome this difficulty of the heterogeneous mixing of the reactants, a single phase reaction has discovered. The proposed model includes a cyclic solvent introduced into the reaction mixture which makes both the oil and methanol miscible. This solvent can be a numerous of different solvents with the boiling point up to 100°C. THF (tetrahydrofuran) is preferred because of close boiling point to that of methanol so that after reaction both methanol and THF can be recycled in a single step to use again [18]. This process uses a co-solvent, tetrahydrofuran to stabilize the methanol. It requires only 5–10 min to complete the reaction. This system requires low operating temperature 30 °C [18].

Tetrahydrofuran is the most extensively used co-solvent because; its boiling point is close to that Tetrahydrofuran is the most extensively used co-solvent because; its boiling point is close to that of methanol. Firstly the process converts the free fatty acids (up to 10% free fatty acids contents) followed by the triglycerides through the addition of a cosolvent in two steps, single phase continuous process at atmospheric pressure and temperature within 90 min of reaction time. The co solvent is then recycled and reused continuously in the process. A lot of research work has been carried out to produce biodiesel that is cost competitive with conventional diesel by using this method. See table 2 for overview of parameters involved in all the above processes.

4. Conclusion

The objective of this paper is to review the work that has been already done in the technologies of conversation of triglycerides into biodiesel. The following specific conclusions are based on the review from this paper.

4.1 Selection of a transesterification process depends on the amount of free fatty acid and water content of the feedstock.

4.2 Alkaline catalyzed transesterification process is most effective in converting triglycerides into esters when free fatty acid level is less than 1%. KOH is the most commonly used alkaline catalyst for producing biodiesel from waste cooking oil.

4.3 When the FFA content of feedstock is >1 wt. %, then an acid catalyzed transesterification process is most effective. However, this process requires high catalyst concentration and high molar ratio leading to corrosion problems.

4.4 A new technology (Biox process) is developed in order to overcome the problem low homogeneity of reactants, methanol and oils. This process uses a co-solvent to stabilize the methanol. THF is most commonly used co solvent and this method is commercialized.

Table 2: Overview of parameters involved in all the above processes.

Reaction Variable	Acid catalyzed	Simple base catalyzed	Two step reaction	Biox method	Supercritical method
Feedstock	WCO	WCO	WCO	WCO	WCO
FFA content	High	Low (<1%)	High	High	High
Reaction time (min)	240	60	90	10-15	30
Temperature (d. C.)	55-80	60-70	60-70	30	350
Catalyst used	H_2SO_4	NaOH	1- Ferric sulfate 2-NaOH	Catalyst- NaOH Co solvent- THF	No catalyst
Catalyst loading	1.3:1	1%	1% ferric sulfate	-	-
Conversion (%)	91.7	98.4	99.3	>99	90
Glycerine recovery	Difficult	Difficult	Easy	Easy	Easy
Purification	Difficult	Difficult	Easy	Difficult	Difficult
Cost of catalyst	Cheap	Cheap	Costly	Costly	-

4.5 Problems which are occurred during alkali and acid catalyst process, two step process is used to overcome. In this case heterogeneous catalyst is used in first step which can be recycled and reused while in second step solid alkali catalyst is used.

4.6 Non-catalyzed supercritical methanol transesterification process has been developed in order to overcome the limitations of catalyzed transesterification process. It requires very short time under supercritical conditions (temperature 350–400 °C and pressure more than 80 bar. However, it requires a high alcohol to oil (42:1) ratio and higher capital and operating cost. It also consumes more power

4.7 Currently the cost of biodiesel is high as compared to conventional diesel oil because most of the biodiesel is produced from pure vegetable oils. However, it can be reduced by using low cost feedstock such as animal fat and used cooking oil.

4.8 The fuel properties of biodiesel derived from used cooking oil are in accordance with biodiesel standards. Thus, biodiesel produced from used cooking oil can be used in diesel engines without any engine modifications.

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3. A hope for vision : Gene-replacement therapy for RPE65 associated Leber's Congenital Amaurosis

Review Article

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Abstract

Leber's Congenital Amaurosis (LCA) is a group of inherited blinding diseases which leads to congenital retinal dystrophies and is difficult to treat. However, gene replacement therapy holds great potential in treating LCA type 2 caused to Rpe65 gene mutations. This restores the isomerohydrolase activity of the expressed RPE65 protein by correcting the defect at the molecular level. Adeno-Associated Virus mediated subretinal delivery of RPE65 cDNA regulated by constitutive or tissue specific promoter shows dramatic improvement in vision in terms of subjective and objective aspects which indicates efficacy of the therapy. Absence of immune responses, adverse effects and vector dissemination underlines the safety of the therapy. Successful findings observed in pre-clinical and clinical trials pave a way to its practical application. Response to the treatment however varies with the extent of retinal degeneration and age of the patient. Moreover, currently developing non-viral gene delivery and nanoparticle mediated approaches gives an insight to its future prospects too.

Keywords: Leber's congenital amaurosis (LCA) type 2, Rpe65 gene, gene replacement therapy, Adeno-associated virus, subretinal delivery, efficacy, safety.

1. Introduction

1.1. Leber's Congenital Amaurosis

Leber's Congenital Amaurosis (LCA) is a group of inherited retinal dystrophies characterized by severe loss of retinal and visual functions from birth due to progressive degeneration of the cellular structure of the retina.[1, 3, 5] LCA which was first reported by Theodor Leber, affects almost 1/81,000 individuals and accounts for

at least 5% of all retinal dystrophies.[2,3]This degenerativedisorder is identified by nystagmus,highly attenuated electroretinographicresponses and pupillary light reflexes, oculodigital sign (habitual rubbing or poking the eyes), keratoconus, cataracts and fundus abnormalities. Since childhood, vision starts getting deteriorated and ultimately total blindness is observed by the third or fourth decade of life.[4, 5]No effective treatment for LCA has been reported yet. [1]

1.2. RPE65 gene, its function and pathogenesis of LCA-Type 2

LCA is reported to be caused due to mutations in 15 different genes and is inherited in an autosomal recessive manner although autosomal dominant forms are also reported. LCA2 resulting due to mutations in the Rpe65 gene accountfor 10% of LCA. [5] Rpe65 gene is expressed predominantly in the retinal pigment epithelium (RPE) cells producinga 65kD protein which isessential for the functioning of a visual retinoid cycle. The RPE65 protein is a carotenoid oxygenase whose isomerohydrolase activityconverts all-trans-retinylesters to 11-cis-retinal.This facilitates constant supply of a light-sensitive 11-cis-retinal to the photoreceptor outer segments for re-association with opsin to form a visual pigment, Rhodopsin. Moreover, RPE also contributes to the survival of the photoreceptors through the phagocytosis of rod and cone outersegment discs. Hence, an optimum functioning of the photoreceptors ultimately depends on the RPE.[2, 4, 6,7]

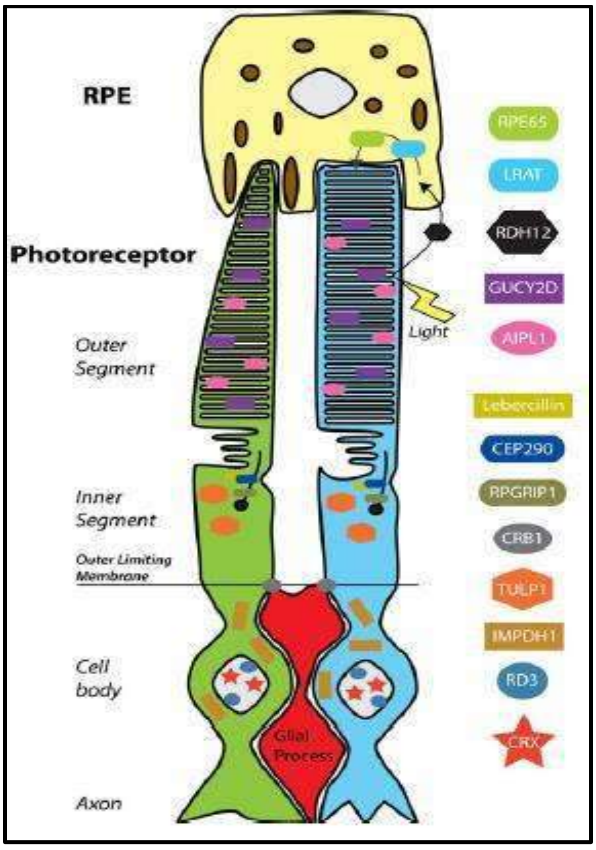

Figure 1: Location of the LCA-associated proteins within the photoreceptor-RPE complex.[2]

Mutations in Rpe65 gene resulting in RPE65 deficiency cause biochemical blockade of the visual cycle. This leads to the accumulation of all-trans-retinyl esters in RPE cells and the diminished levels of 11-cis-retinal is observed in LCA2 patients. As

a result,rod photoreceptor cells are unable to respond to light. Cone photoreceptor cellscan sustain their function through an alternative pathway that does not depend on RPE65, thus, allowing cone-mediated vision initially in children with LCA. However, cone photoreceptor cells progressively degenerateleading to loss of cone-mediated vision. Overall,a profound impairment in visual function along with delayed histological degeneration of retinal cells is the ultimate outcome. [1,5]

1.3. Potential of Gene Therapy

Gene-replacement therapy may serve as a potential therapeutic strategy to correct the defects in Rpe65 gene owing to the features of the disorder. Vector mediated delivery of a wild typeRPE65cDNA shows improvement in the visual function. [1,5, 8]

2. Gene therapy-Process

2.1 RPE as a target for gene therapy

The eye seems to be a promising target for gene therapy as it's immune privileged, has a small compartment size, easily visualized and examined, and readily accessible with minimal risk to patients undergoing surgery. The prime targets for gene therapy include the photoreceptors and the RPE (the phagocytotic nature of the RPE further facilitates gene delivery). Retina is exploited for the accurate delivery of therapeutic agent to the photoreceptors and RPE by injecting a fluid suspension containing the therapeutic particles into the subretinal space. Temporary detachment of the retina is seen following to the injection; however serious damage to the other parts is not reported. [6]

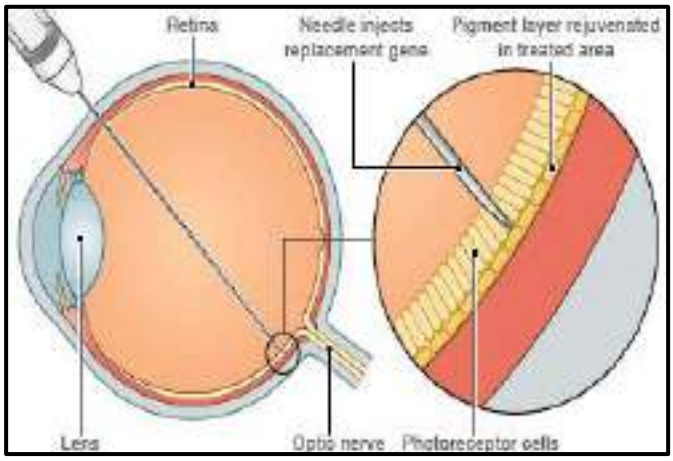

Figure 2: Gene-replacement therapy through Rpe65 genesubretinal delivery. [17]

2.2. Adeno-associated virus mediated gene therapy

The efficiency with which the transgene is delivered to the appropriate cell type determines the success of gene therapy technique. Of the two major approaches for the gene delivery - viral and non-viral types, varied tropisms are exhibited by different viral vectors for the specific eye tissues. Of all the viral vectors, adeno-associated virus

proves to be a major vector in the gene therapy for LCA2. [6]

2.2.1. Adeno-associated Virus (AAV) - Vector construction and gene packaging

AAV is a non-pathogenic, non-enveloped virusapproximately 25 nm in diameter containing a single stranded DNA genome with a maximum length of 5.1 kb. [6, 9]For constructing the recombinant AAV (rAAV) vector, the coding sequence of AAV (rep and cap genes) is replaced with the transgene cassette comprising of a promoter (either constitutive or tissue-specific), gene of interest (here Rpe65 gene) in the form of cDNA (human RPE65 cDNA is 1.6 kb long)[11] and a poly-Adenylation tail. [9] The Inverted terminal Repeats (ITRs) sequences of the AAV are retained as they are required for cis-packaging of a recombinant vector genome. Due to the necessary inclusion of ITRs, the total coding capacity of AAV vectors is reduced to approximately 4.7 kb, which limits the size of the therapeutic gene that can be incorporated. [6]rAAV-mediated Rpe65 gene delivery resulted in a stable transgene expression in RPE cells, by maintaining the transgene mostlyin an episomal fashion.[12]

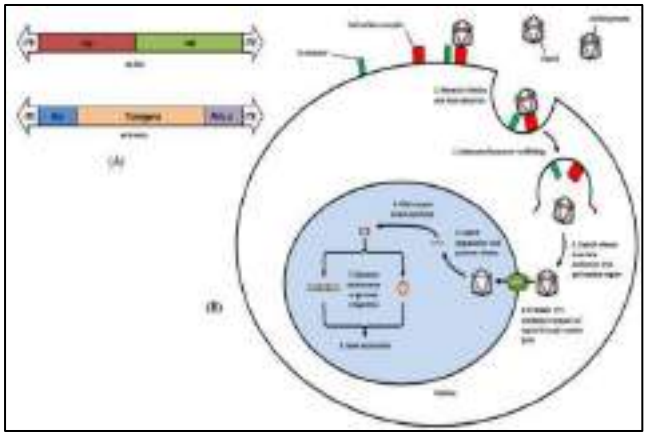

Figure 3: (A) rAAV vector genome, (B) Ways of transgene expression on viral transduction. [6]

2.2.2. Pseudotyping of rAAV Serotypes

By the 'pseudotyping' process, rAAV vectors are produced. For example, an AAV2 (AAV serotype 2) based transgene cassette is packaged into a capsid from a virus originating from a different serotype. By using this technique, gene delivery to RPE occurs effectively with several pseudotypes, particularly rAAV2/1 (AAV2-based genome in AAV1 capsid), rAAV2/2 and rAAV2/4. [6]

2.2.3. Regulation of Transgene Expression

Expression is primarily regulated by inclusion of appropriate promoters such as constitutive ones like Chicken β actin (CBA) promoter or RPE-tissue specific promoter. [1, 4, 6, 10] Temporal regulation of

transgene expression is achieved by using tetracycline, doxycycline, etc. [6]

2.3. Methodology

Following two processes are carried out each based upon the different promoter types used. Methods A and B utilize CBA and tissue specific promoters respectively.

Method A: The transgene cassette in the AAV2.hRPE65v2 vector carrying a CBA promoter drives the expression of the human RPE65 (hRPE65) cDNA. Co-administration with a surfactant prevents the loss of the vector to surfaces which are in contact with the product. During surgery, an injection of vector genome of AAV2.hRPE65v2 in a phosphate-buffered saline supplemented with Pluronic F-68 NF Prill Poloxamer 188is administered into the subretinal space which creates a localized retinal detachment which ultimately resolves later. [4, 11]

Method B: The rAAV2 (specifically tgAAG76) vector containing the human Rpe65 coding sequence driven by the hRPE65promoter and terminated by the bovine growth hormone polyA site is filled in a buffered saline solution and allowed to freeze in 1-ml aliquots at −70°C. rAAV2 efficiently transduce RPE cells following subretinal delivery. The possibility of the occurrence of intraocular inflammation was minimized by using immunosuppressive agents. [1]

2.4. Efficacy and safety of the therapy

Administered AAV2-hRPE65v2 is well tolerated and improvement in subjective and objective measurements of vision (i. e, dark adaptometry, pupillometry, electroretinography, nystagmus, and ambulatory behaviour) is noticed in pre-clinical and clinical trials as described elsewhere. The response to subretinal Rpe65 gene therapy depends onthe extent of retinal degeneration andthe ageof the patient. The greatest improvement is seen in children as seen in a study. [8] Absence of the clinically significant adverse effect of subretinal vector delivery underlines the safety of the therapy at all administered doses whereas absence of the systemic dissemination of the vector is indicative of the minimal extraocular leakage of vector from the subretinal space. [1, 8, 9] However, a macular hole is reported during a trial conducted in a patient but it occurred due to contraction of a preexisting membrane stimulated by the surgical procedure,and does not affect the vision. [4] No significant humoral immune responses and neutralizing antibodies areobserved against viral capsid or expressed RPE65. [11] There's a

possibility that rAAV particles can spread to adjacent cells and transduce ganglion cells. This can result in expression in the visual pathways present in the brain. However, evidence which can support the occurrence of CNS toxicity in preclinical or clinical studies is not reported. [12]The safety, extent, and stability of improvement in vision as noted in trials described elsewhere support the use of AAV-mediated gene therapy for the treatment of LCA2.[8]

3. From Research to Practice

3.1. Preclinical Trials

3.1.1. Description

In this trial, a naturally occurring murine model of LCA with a recessive nonsense Rpe65 mutation, the rd12 mouse, was employed to test the potential of recombinant adeno-associated virus (rAAV) -mediated gene therapy in rescuing this defect and the associated loss of retinal structure and function. rd12 mouse is a natural model of RPE65. Due to mutation in Rpe65 gene, small lipid-like droplets in RPE cells were first detected at 3 weeks of age, followed by progressive retinal degeneration. Fundoscopic examination also revealed evenly dispersed yellowish- white spots throughout the retina from 5 months onward.

The recombinant adeno-associated virus constructs with AAV2 inverted terminal repeats and pseudotyped AAV5 capsids were based on the pTR-UF2 vector. Serotype 5 rAAV vectors carry a normal hRPE65 cDNA (AAV5-CBA-hRPE65). The viral preparations were made, with average physical particle titers of 10^{13} viral particles/ml and biological titers of approximately $2x10^{11}$ infectious particles/ml. In each mouse, 1 microlitre of vector suspension was administered subretinally. The other eye remained uninjected. Animals with indications of ocular damage, and other complications were removed from further study. For this study, C57BL/6 mice were used as controls for various later tests. The control mice received no injections whatsoever.

3.1.2. Results

a) rAAV-mediated Rpe65 gene therapy was employed into rd12 mice with the aims of rescuing the RPE-origin functionaldefect and ultimately restoring vision in these animals. 3 weeks after single injection, stable RPE65 expression was observed in the injected eye. No RPE65 expression was noted in untreated rd12 retinas of the same animals.

b) Changes in fundus were also noted. Retinas treated with AAV5-CBA-hRPE65 appeared nearly normal, in contrast to yellow-white spots devoid of pigment observed in untreated eyes.

c) Rhodopsin levels in retinas injected with AAV5-CBA-hRPE65 were restored to 70% of normal levels and had a molecular weight identical to those noted in the control mice.

d) Using light and electron microscopy, the retinal morphology after vector treatment was noted. Retinas of normal control C57BL/6 mice and the age-matched rd12 eyes treated with AAV5-CBA-hRPE65 were comparable, with 10-12 layers of outer nuclear layer (OLN) cells. In contrast, untreated rd12 eyes displayed striking differences. The OLN contained only 6–8 layers of cells.

3.1.3. Discussion

Through this trial, it was concluded that gene therapy could reverse the congenital defect, improve retinal structure and vision. Retinas treated with AAV5-CBA-hRPE65 showed results comparable to those in normal eyes. Several other preclinical studies have concluded that gene replacement therapy using a recombinant adeno-associated virus (rAAV) vector carrying a wild-type canine Rpe65 cDNA restored partial retinal function. Success has also been noted in similar rescue using gene therapy in a strain of Swedish briard dogs carrying a functionally null allele of canine $Rpe65^{-/-}$ that also exhibit a retinal dystrophy. Hence, there was a further need to validate existing data through human clinical trials. [7]

3.2 Clinical trials

3.2.1 Description

Three consecutive patients who had LCA2 and were between the ages of 19 and 26 years were selected. The eye with worse function was selected for delivery of AAV2.hRPE65v2. Method utilized for the therapy was the same as described in the Method A under the section 2.3 (Methodology) of this article.

3.2.2. Results

a) Pupillometry: Baseline testing showed that the pupillary light reflexes of the three patients prior to injection were much less sensitive to light than those of control subjects, even when the strength of the stimulus was increased. After injection, the responsiveness of the patients' injected eyes was reliably greater than that of their uninjected eyes. In summary, after injection of AAV2.hRPE65v2, each of the three eyes that received injection became more effective in driving the pupillary response,

more sensitive to light and surpassed the sensitivity of the other eye.

b) In the weeks following injection, patients reported having improved vision in dimly lit environments. In tests of ability to navigate an obstacle course before the injection of AAV2.hRPE65v2, patients collided with most of the obstacles and remained off-course throughout. However after, administration of injection, patients were able to navigate through the obstacle course.

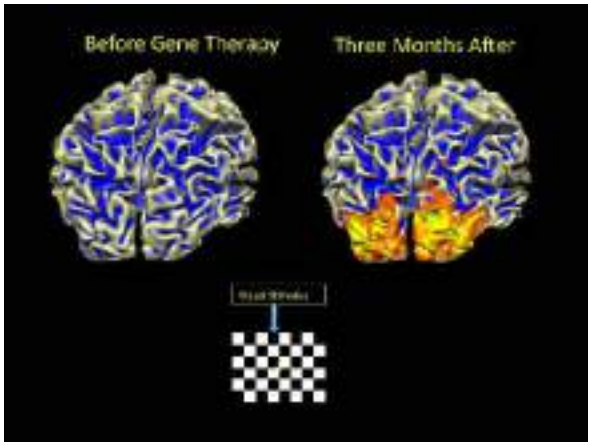

Figure 4: Comparative analysis of the responses to visual stimuli before and after the gene therapy.[18]

3.2.3 Discussion

All three patients with LCA2 who received AAV2.hRPE65v2 by subretinal injection showed evidence of improvement in retinal function. There was improvement in the pupillary light reflexes and increase in vision. There were no apparent local or systemic adverse events observed throughout. Thus, this trial provides the foundation for gene-therapy approaches in humans for treatment of LCA and other forms of retinal degeneration.[4]

4. Advantages and Limitations of the Therapy

AAV mediated subretinal Rpe65 gene delivery show promising results in the trials. Vision improvement both in objective and subjective aspects along with the safety of the therapy underlines the clinical significance. Even the re-administration in the previously treated eye shows dramatic improvements in the vision. However, the delivery of larger genes using AAV is still a problem. Efforts to expand AAV packaging capacity artificially are in progress. [2]

5. Conclusion

Practical application of gene therapy for Leber's Congenital Amaurosis has not yet started. . However, various preclinical and clinical trials have yielded optimistic results. The many benefits reaped by the patients after gene therapy, suggests that practical use of gene therapy for Leber's Congenital Amaurosis should be carried out at the earliest. These findings along with earlier

successes are very promising in terms of the potential of gene therapy for patients with certain forms of congenital and degenerative eye disorders and open the possibility of rescue of a wider variety of recessive mutations originating in RPE and rod photoreceptors.

6. Future Prospects

Alternative functions of RPE65 in photoreceptors, if discovered, may play a role in the disease process and treatment effects. Apart from AAV, other gene delivery vectors encompassing various viral (example- lentivirus) or non-viral vectors may modify the efficacy of the therapy. Non-viral gene delivery techniques like protein-based nanoparticle carriers, electroporation, lipofection, etc., if exploited, may prove as more effective approaches. Nanoparticle-mediated therapy prove to be an effective technique since nanoparticles are easily manufactured, may have less immunological responses, and can readily pass through membranes. Nanoparticles (NPs) can be modified to produce sustained-release compounds.[2, 13]CK30PEG10k NPs have shown high levels of expression by distributing throughout the RPE cell layer. A major clinical phenomenon is observed by transduction of CK30PEG10k NPs in RPE cells away from the site of injection. Moreover, targeted expression in RPE cells is also reported in the case of VMD2-eGFP NPs. [13, 14] Targeted gene delivery using Zinc Finger Nucleases also have potential to prove as a promising strategy in the coming years. In addition to this, oligochitosan polyplexes and low-molecular weight oligochitosan (non-viral technique) mediated gene delivery may serve as an active research area, too. [15, 16]

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4. Synthesis of Biodiesel from Palm Fatty Acid Distillate

Research Article

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Abstract

Palm fatty acid distillate is a by-product of palm oil refining industries and it consists of 83% fatty acids and 12% glycerides. Biodiesel was synthesized using palm fatty acid distillate (PFAD) as raw material in two steps. The first step involved hydrolysis of PFAD to convert glycerides into free fatty acids (FFA). The second step was esterification of these free fatty acids using methanol and homogeneous acid catalyst (sulfuric acid) to give methyl esters (biodiesel). Hydrolysis was studied at two different catalyst loadings. Esterification reaction was executed by recycling methanol and sulfuric acid in the aqueous phase. Recovery of methanol and sulfuric acid from aqueous phase of esterified PFAD was carried out by distillation. Biodiesel was purified firstly by water washing followed by neutralization of unreacted FFA. Neutralization was studied with different bases like aqueous sodium hydroxide, aqueous sodium carbonate, sodium hydroxide in 80:20 and 90:10 glycerol:water mixture.

Keywords: Biodiesel, esterification, neutralization, free fatty acid, PFAD.

1. Introduction

Biodiesel, an alternative fuel similar to conventional diesel, is produced from vegetable, and/ or animal oils/fats such as rapeseed, soybean, palm, lard, pork etc. No engine modifications are needed. Although diesel is part of its name, there is no

petroleum or other fossil fuels in biodiesel. This environment-friendly fuel reduces emissions, smoke and odors. Biodiesel can also be used in blends with conventional diesel still achieving substantial reductions in emissions. Technically, biodiesel is Fatty Acid Methyl Ester (FAME). It is formed by replacing the glycerol from each triglyceride molecule of vegetable oil with methyl from methanol (that is by the reaction of free fatty acid and methanol). But there is some notable difference. The biodiesel molecules are simple hydrocarbon chains, containing no sulfur, ring molecules, or aromatics that are associated with fossil fuels. Biodiesel is made up of almost 10% oxygen, making it a naturally oxygenated fuel [12].

The Benefits of Biodiesel

Biodiesel has many environmentally beneficial properties. The main benefit of biodiesel is that it is carbon neutral, i.e., no net output of carbon in the form of carbon dioxide (CO_2). This effect occurs because when the oil crops grow they absorb the same amount of carbon dioxide as is released when the fuel is combusted. Biodiesel is rapidly biodegradable and completely non-toxic. Biodiesel has a higher flash point than fossil diesel and so is safer in the event of a crash. Biodiesel production process is straightforward and methodical and can produce consistent results. That is to say, in technical terms, the method of producing biodiesel is feasible.

2. Materials and Methods

2.1. Materials

The palm fatty acid distillate (PFAD) was procured from ROYAL ENERGY, Mumbai, as a yellow color solid having melting point of 40°C. Methanol (AR grade), potassium hydroxide, oxalic acid and phenolphthalein indicator were procured from S.D.Fine Chemicals, Mumbai. HPLC grade acetonitrile and acetone were procured from Thermo Fischer Scientific for analysis.

2.2. Experimental Methods

2.2.1. Hydrolysis of Palm Fatty Acid Distillate

PFAD was firstly washed with water in order to remove water soluble impurities. For this 100 g PFAD was taken in 250 ml glass reactor placed in oil bath maintained at 90^{o}C and washed with water at an agitation speed of 1500 rpm for about an hour with an overhead stirrer. Water to PFAD ratio was 2:1 (v:v) so as to keep water as a continuous phase with PFAD as dispersed phase. The organic (PFAD) and aqueous (H_2O) phases were separated out using separating funnel.

The washed PFAD was then taken for hydrolysis.

Hydrolysis reaction was conducted in a stirred batch reactor of volume 250ml equipped with a six blade turbine impeller running at 1500 rpm. In a typical reaction, 100g of PFAD was first melted in the reactor at 90°C for 10 minutes. Water (30% wt of PFAD) and sulfuric acid (1 and 2% wt of PFAD) as catalyst were added to the melt. The reaction temperature was maintained using an oil bath and the reaction mixture was vigorously agitated at 1500 rpm for an hour. Samples were collected at specific intervals of time (10 minutes). After the completion of reaction, reaction mixture was allowed to stand in an oil bath for 5 minutes to separate out the two phases. The two phases were separated from each other using separating funnel. Pretreated organic phase was subsequently used for the esterification reaction.

2.2.2. Esterification of Palm Fatty Acid Distillate

100g of hydrolyzed PFAD was taken in 250ml reactor kept in oil bath at 60^{o}C. Methanol (PFAD:Methanol = 1:3 molar basis) and 98% sulfuric acid (5% by wt. of PFAD) were added to the reactor to initiate the esterification reaction. The reaction was carried out at agitation speed of 1500 rpm using an overhead stirrer for an hour. Samples were collected at specific intervals of time (10 minutes). When the reaction subsides, the organic phase was separated from the aqueous phase using a separating funnel. The esterification reaction was carried out as shown in Figure 1 in order to study the effect of recycling of aqueous phase on FFA conversion. The organic phase was further treated for neutralization of acid and aqueous phase was used for recovery of sulfuric acid and methanol.

2.2.3. Recovery of Methanol and Sulfuric Acid from Aqueous Phase obtained from Esterification

The aqueous phase was taken in a 250ml three necked round bottom flask. Dean-Stark apparatus was attached to one of the necks and the rest two were stoppered. The fractionating column of the apparatus was stoppered and its burette was attached to the condenser. The mouth of the condenser was connected to gas flow meter to check any formation of gas in the process. The gas flow meter was further connected to gas sampling tube to collect the gas coming out in the process. The round bottom flask was kept in an oil bath and heated stepwise from 65^{o}C to 150^{o}C. The methanol-water mixture

was collected from the burette of the Dean Stark apparatus. The mixtures collected at different temperatures were analyzed in density meter and the composition of methanol and water in the mixture was calculated. Sulfuric acid thus recovered after removal of methanol and water was analyzed for its concentration.

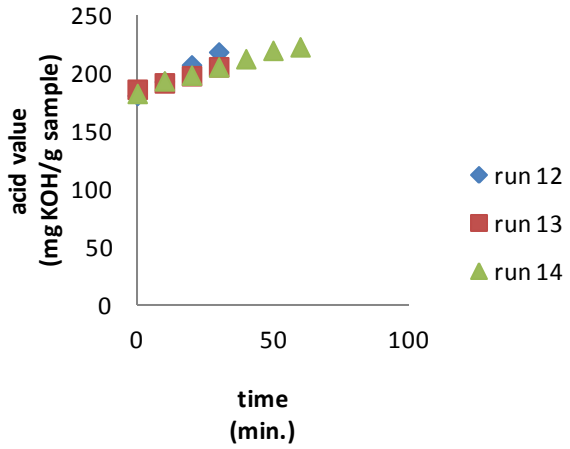
2.2.4. Neutralization of the Unreacted Fatty Acids present in the Organic Phase obtained from Esterification Stage

The entire organic phase was given water wash with excess water in order to remove methanol and sulfuric acid present in the organic phase. The sulfuric acid concentration of the organic phase before and after water washing was determined. The organic phase was then separated from the aqueous phase and its acid value was found out. Sodium hydroxide and sodium carbonate were used to neutralize (saponify) the unreacted FFA. Aqueous sodium hydroxide (50ml), aqueous sodium carbonate (50ml), sodium hydroxide in 80:20 and 90:10 (v:v) mixtures of glycerol: water (50ml each) were prepared. The unreacted fatty acids were removed by saponification reaction on the addition of above mentioned different bases to fixed quantity of organic phase taken in 250ml reactor at room temperature. The two phases were then separated using separating funnel and in case the phases did not separate, the reactor was kept in oil bath at about 60^{o}C till the separation is achieved.

3. Results and discussions

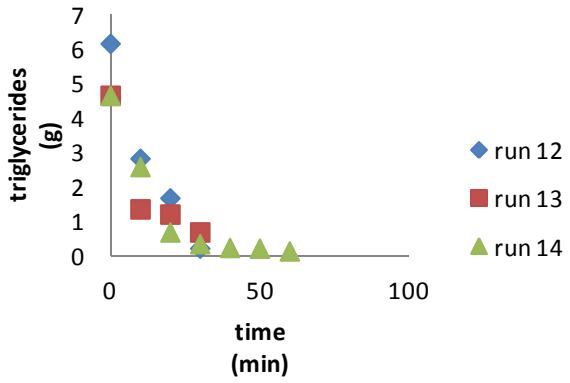
3.1. Hydrolysis of Palm Fatty Acid Distillate

About 100g of PFAD was taken in 250ml reactor kept in an oil bath at 90^{o}C. About 30g of water and 1g of 98% sulfuric acid was added to the reactor for the conversion of triglycerides in PFAD to fatty acids through hydrolysis reaction. PFAD was initially given water wash and water soluble impurities present in the PFAD were removed. The samples collected at intervals of 10 minutes were separated from aqueous phase, given water washing and analyzed for acid value. Also, HPLC analysis was done to find conversion of triglycerides, diglycerides and monoglycerides. Aqueous phase of the hydrolysis was analyzed for glycerol content. Following are the graphs representing the data. Graph 1 represents acid value increment of the PFAD during hydrolysis with time.

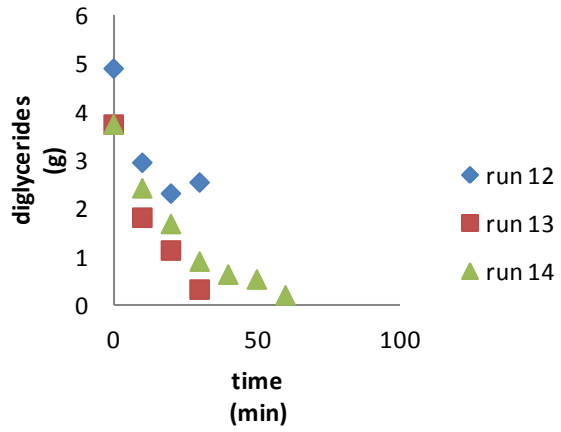


Graph 1: Acid value increment of the PFAD during hydrolysis with time

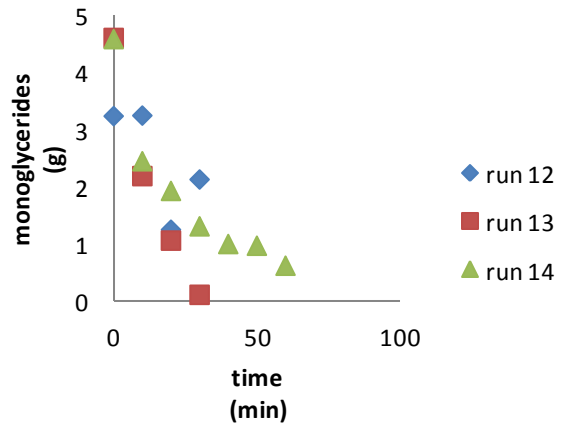
In run 12, 2% sulfuric acid was used to obtain acid value of 218 for reaction time of 30 min and in run 13, 1% sulfuric acid was used to obtain acid value of 205 for reaction time of 30 min. In order to check the conversion of acid value, run 14 was performed using 1% sulfuric acid for an hour and the acid value attained is 222 (nearly same as acid value achieved after half an hour using 2% sulfuric acid).



Graph 2: Conversion of triglycerides into fatty acids



Graph 3: Conversion of fatty diglycerides into fatty acids



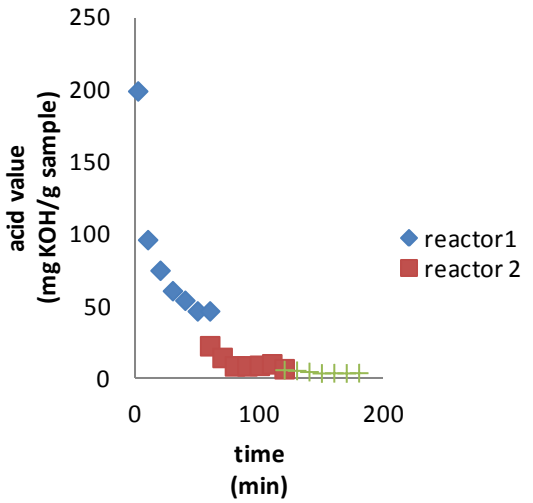
Graph 4: Conversion of monoglycerides to fatty acids

Graphs 2, 3 and 4 represent the conversion of triglycerides, diglycerides and monoglycerides reaction into fatty acids during hydrolysis reaction.

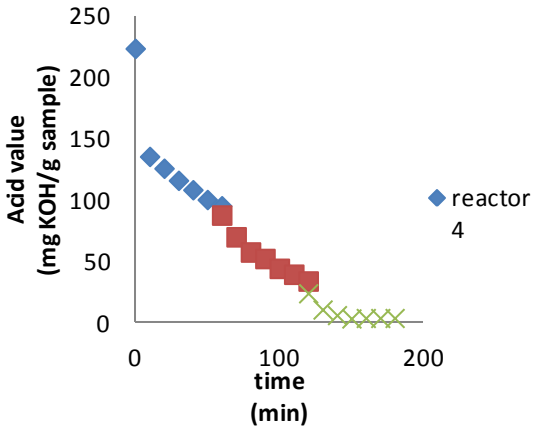
Glycerol content in the aqueous phase =2.004 g/lit (0.0307 mol/lit)

3.2. Esterification of fatty acids:

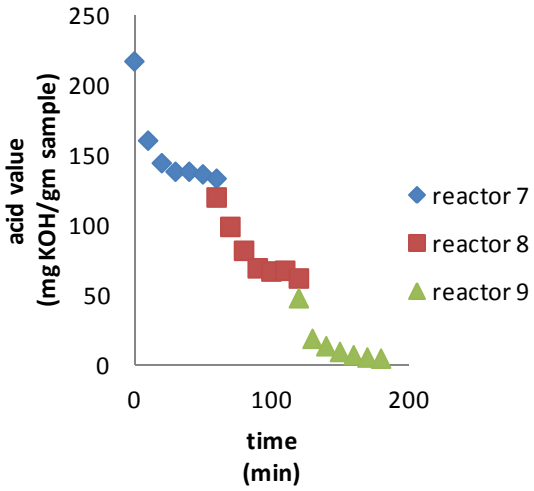
In the following explanation, each reaction was expressed in terms of a run. The block diagram is as shown in figure 1.



Graph 5: Decrease in acid values of the organic phase with progress in time



Graph 6: Decrease in acid values of the organic phase with progress in time



Graph 7: Decrease in acid values of the organic phase with progress in time

Graphs 5, 6 and 7 represent the decrease in acid values of the organic phase with the progress in time as the fatty acids were converted to methyl esters. Three batches in a row are shown in the same graph. The graphs indicate that, as the aqueous phase was recycled the rate of conversion of fatty acids to methyl esters decreased. The rate of conversion of FFA to methyl esters in reactors 6 and 9 increased.

3.3. Recovery of methanol and sulfuric acid from aqueous phase of esterification.

The aqueous phase obtained from esterification stage was taken in 250 ml three necked round bottom flask.

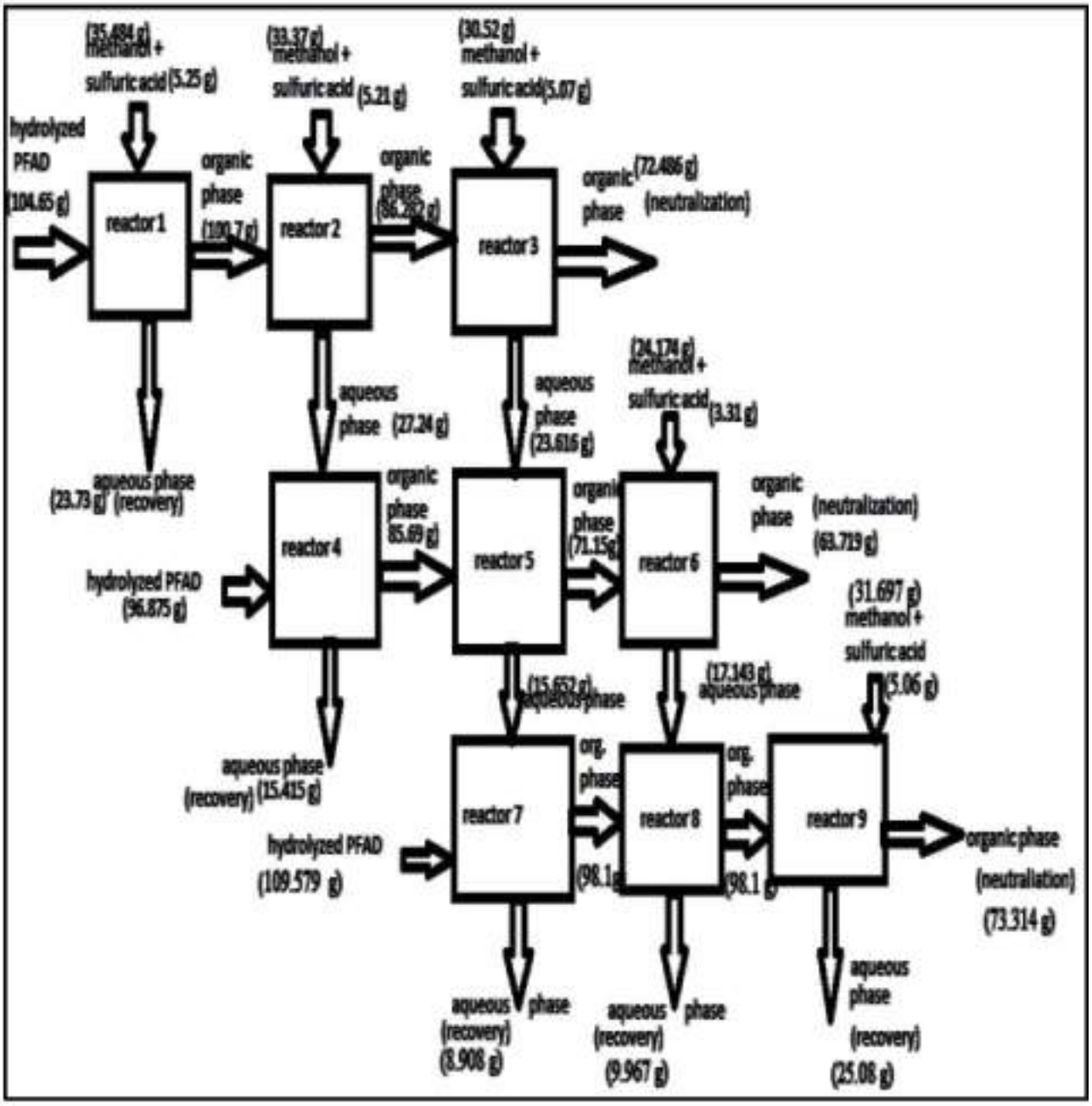


Figure 1: Esterification reaction

The setup has Dean Stark apparatus, condenser, gas flow meter, gas sampling tube. The Dean Stark apparatus along with condenser is shown in figure 2. The glycerol, if present in aqueous phase, will get dehydrated by sulfuric acid and gases will be trapped in gas sampling tube. The gas collected in the gas sampling tube was analyzed by gas chromatography. The analysis showed presence of carbon dioxide. The samples collected at different temperatures were weighed and analyzed in

density-meter. On the basis of density of the mixture achieved, composition of the mixture was found out. The methanol content was given by,

Percentage of methanol

$$= \frac{(1-\text{density of mixture})}{(1-\text{density of methanol})} * 100$$

Density of methanol was found in density meter to be 0.787.

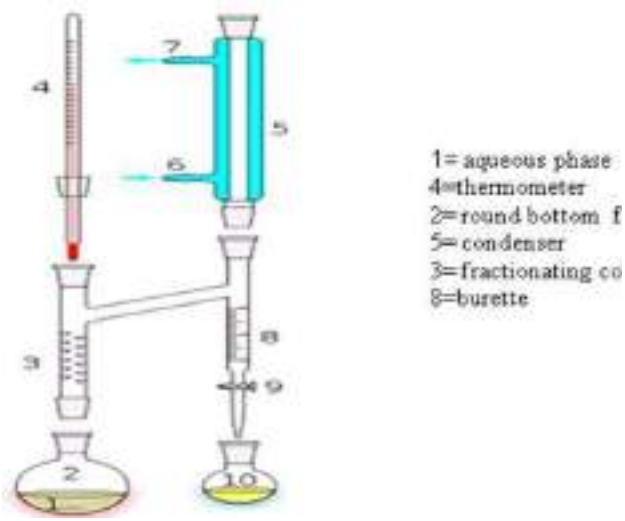


Figure 2: Dean Stark apparatus along with condenser

Following were the observations and calculations:

The concentration of sulfuric acid recovered =16.974N

Recovery of sulfuric acid =83.52%

Total amount of carbon dioxide present in the gas = 0.45 ml (in 150 ml of gas)

3.4. Neutralization of fatty acid in organic phase of esterification

The acid value of the organic phase was calculated to be 2.125.

Exact amounts of sodium hydroxide and sodium carbonate required to neutralize the fatty acids was calculated and 50 ml of these aqueous solutions were prepared. Also, sodium hydroxide solution in 80:20 and 90:10 mixture of glycerol: water was prepared. The bases were added to the organic phase containing methyl esters and fatty acid in a reactor of 250 ml using a six bladed impeller for 30 minutes at room temperature.

50 ml of aqueous solutions of sodium hydroxide (56.0 mg of NaOH pellets) was added to 37.487 g of organic phase in a reactor at room temperature. On the addition of the base, the aqueous phase changed its color from colorless to turbid yellow. When the agitation was started at 1500 rpm, the two phases appeared to be homogenous (pale turbid yellow) and foam formation took place at the top of the phases. The phases were allowed to separate for about 1 hour 15 minutes, yet the organic phase showed presence of soap (a whitish layer present in organic phase). The aqueous and

organic phases were allowed to separate in separating funnel. The organic phase was given water washing in a reactor for the removal of soap in heating condition at 60^{o}C by placing the reactor in an oil bath. A clear phase separation was observed and the organic phase appeared completely reddish. Acid value of the organic phase was calculated to be 0.548. The organic phase was separated by using a separating funnel.

50 ml of aqueous solution of sodium carbonate (76.6 mg of Na2CO3) was added to 37.696 g of organic phase in a reactor at room temperature. The observations were same as in previous case. Acid value of the organic phase was calculated to be 0.812. The organic phase was separated by using a separating funnel.

50 ml of 90:10 glycerol: water mixture was prepared and 58.6 mg sodium hydroxide was added to it. This solution was added to the reactor containing 37.91 g of organic phase. The neutralization was carried out at 60^{o}C with agitation of 1500 rpm provided by six bladed impeller. On the addition of the base, the two phases remained unchanged, i.e., aqueous phase remained clear and organic phase remained reddish. As the stirring was started, the two phases appeared as homogenous phase of red color and no foam and soap formation was observed. When the agitation was increased to 2500 rpm, bubbles of aqueous phase were observed in the system. On further increase in agitation to 3500 rpm, the reactor content turned turbid yellow and foam formation appeared at the top. When the agitation was stopped after half an hour, the reactor content showed phase separation slowly. After 85 minutes, phases separated out and organic phase was separated using a separating funnel. The acid value of the organic phase sample was calculated to be 0.438.

4. Conclusions

This two-step synthesis which is hydrolysis followed by acid catalysed esterification method reduces the overall production cost of the biodiesel, as it uses low cost unrefined feed. The initial water washing of PFAD is an important part of the synthesis because the water soluble impurities can cause problems with the conversion of glycerides to fatty acids.

Table 1

Time taken (min)	Temperature (^oC)	Weight (g)	Density (gm/lit)	Methanol (%)	Water (%)
138	85	9.718	0.803	92.50	7.50
83	90	8.119	0.803	92.50	7.50
69	100	9.634	0.812	88.30	11.70
42	120	9.057	0.830	79.81	20.19
112	150	11.79	0.891	51.20	48.80

The hydrolysis of PFAD with 2% acid catalyst loading shows the same results as 1% acid catalyst loading and takes half of the reaction time, comparatively. The esterification reaction was successfully performed by recycling the aqueous phase. The neutralization was best performed with sodium hydroxide in 90:10:: glycerol: water mixture. The analysis reveals that biodiesel from unrefined PFAD is quite suitable as an alternative to diesel.

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5. Organ-On-A-Chip

A replacement to animal testing

Review Article

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Abstract

Here we describe an Organ-on-a-Chip (OC) which is a biomimetic microsystem. These are engineered microchips that can sum up the microarchitecture and functions of living organs, such as the lung, heart, artery etc. Each individual organ-on-chip is composed of a clear flexible polymer - about the size of a computer memory stick - that contains hollow microfluidic channels lined by living human cells. Because the micro devices are translucent, they provide a window into the inner-workings of human organs without having to invade a living body. There are envisions of creating the entire human body on chips. They might be useful for high-throughput analysis and screening of cellular responses to drugs, chemicals, particulates, toxins, pathogens, or other environment stimuli relevant to pharmaceutical, cosmetic, and environmental applications. Such a creation could spell an end to animal testing which is an expensive, ineffective and lethal process. It has the potential to revolutionize the whole drug testing process.

Key words: flexible polymer, microfluidic channels, animal testing.

1. Organ-On-A-Chip - an Overview

Microscale engineering technologies such as microfabrication and microfluidics, were first used to develop microchips. This enables extraordinary capabilities to control the cellular microenvironment with high spatiotemporal precision and to present cells with mechanical and biochemical signals. This has made it possible to microfabricate models of blood vessels, muscles, bones, airways, liver, brain, gut, and kidney. However, it has not yet been possible to engineer integrated microsystems that replicate the complex physiological functionality of living organs by incorporating multiple tissues,

including active vascular channels, and placing them in relevant organ-specific microenvironment.

Each individual organ-on-chip is composed of a clear flexible polymer - about the size of a computer memory stick that contains hollow microfluidic channels lined by living human cells. Because the microdevices are translucent, they provide a window into the inner-workings of human organs without having to invade a living body.

The researchers, now, seek to build 10 different human organs-on-chips, to link them together to more closely mimic whole body physiology, and to engineer an automated instrument that will control fluid flow and cell viability while permitting real-time analysis of complex biochemical functions. [1, 9, 10, 11]

2. Organs

2.1. Lung-on-a-Chip

Lung-on-a-chips are being designed in an effort to improve the physiological relevance of existing in vitro alveolar-capillary interface model which is the fundamental functional unit of the living lung.

It can be used for testing the effects of environmental toxins, absorption of aerosolized therapeutics, safety and efficacy of new drugs which may help accelerate pharmaceutical development by reducing the reliance on current costly models.

With every human breath, air enters the lungs, fills microscopic air sacs called alveoli, and transfers oxygen through a thin, flexible, permeable membrane of lung cells into the bloodstream. This lung-blood interface recognizes invaders such as inhaled bacteria or toxins and activates an immune response.

The lung-on-a-chip microdevice works by placing two layers of living tissues -- the lining of the lung's air sacs and the blood vessels that surround them -- across a thin (10µm) porous, membrane made of polydimethylsiloxane (PDMS).

The compartmentalization of the channels facilitates not only the flow of air as a fluid which delivers cells and nutrients to the apical surface of the epithelium, but also allows for pressure differences to exist between the middle and side channels. During normal inspiration in a human's respiratory cycle, intrapleural pressure decreases, triggering an expansion of the alveoli. As air is pulled into the lungs, alveolar epithelium and the coupled endothelium in the capillaries are stretched. Since a vacuum is connected to

the side channels, a decrease in pressure will cause the middle channel to expand, thus stretching the porous membrane and subsequently, the entire alveolar-capillary interface. The pressure-driven dynamic motion behind the stretching of the membrane, also described as a cyclic mechanical strain, significantly increases the rate of nanoparticle translocation across the porous membrane. (Fig 1)

In order to fully validate the biological accuracy of a device, the researchers inflicted injuries to the cells:

2.1.1 Pulmonary infection

The researchers tested its response to inhaled living E. coli bacteria. They introduced bacteria into the air channel on the lung side of the device and at the same time flowing white blood cells through the channel on the blood vessel side. The lung cells detected the bacteria and, through the porous membrane, activated the blood vessel cells, which in turn triggered an immune response that ultimately caused the white blood cells to move to the air chamber and destroy the bacteria.

2.1.2. Pulmonary inflammation

The researchers introduced a variety of nano-scaled particles into the air sac channel.

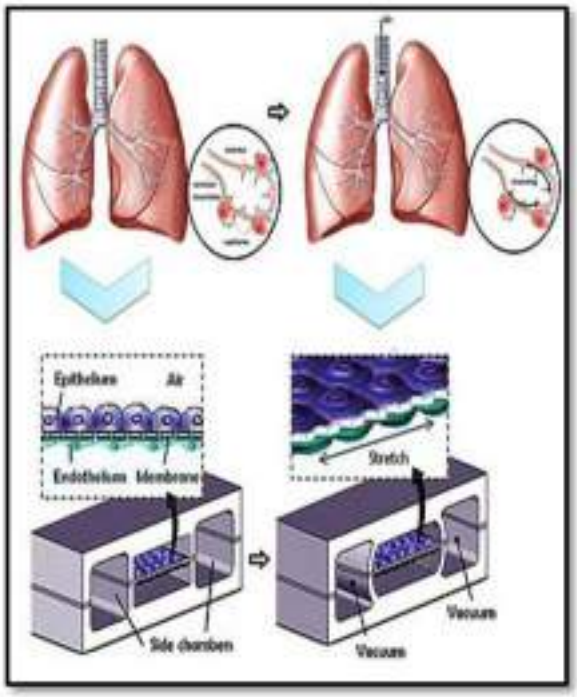

Figure 1: Lung-on-a-Chip [14]

The device consists of three hollow microchannels, and only the middle channel contains a horizontal porous membrane, coated on either side by either an endothelium or an epithelium tissue. The side channels are connected to a vacuum and can therefore simulate the stretching of the membrane. The contraction of the diaphragm triggers the intrapleural pressure to decrease, leading to an expansion of alveoli. This is the phenomenon essentially mimicked by this lung-on-a-chip.

Several types of these nanoparticles entered the lung cells and caused the cells to overproduce free radicals and to induce inflammation which is indicated by an increased production of epithelial cells and an early response release of cytokines.

A microfluidic lung-on-a-chip can more exactly reproduce the mechanical properties of a living human lung, its

physiological responses will be quicker and more accurate. [1, 9, 10, 12, 13, 14, 15]

2.2. Heart-on-chip

Traditionally, muscle physiology experiments require multiple tissue samples to obtain morphometric, electrophysiological, and contractility data. Furthermore, these experiments are commonly completed one at a time on cover slips of single cells, or in isolated muscle strips

Cardiomyocytes, are the cells that constitute heart which generate the electrical impulses that control the heart rate. "Heart on a chip is a device that exploits muscular thin film technology – biohybrid constructs of an engineered, anisotropic ventricular myocardium on an elastomeric thin film – to measure contractility, combined with a quantification of action potential propagation, and cytoskeletal architecture in multiple tissues in the same experiment. The device was created using small thin strips of tissue made from heart muscle cells that are connected to electrodes to stimulate contraction.

The design and fabrication process of this particular microfluidic device entails first covering the edges of a glass surface with tape (or any protective film) such as to contour the substrate's desired shape. (See fig 2). A spin coat layer of PNIPA [poly(N-isopropylacrylamide)] is then applied. After its dissolution, the protective film is peeled away, resulting in a self-standing body of PNIPA. The final steps involve the spin coating of protective surface of PDMS over the cover slip and curing. These Muscular thin films (MTF) enable cardiac muscle monolayers to be engineered on a thin flexible substrate of PDMS. A micro contact printing technique was used to lay out a fibronectin "brick wall pattern on the PDMS surface generated an anisotropic monolayer.

After the cutting of the thin films into two rows with rectangular teeth, and subsequent placement of the whole device in a bath, electrodes stimulate the contraction of the myocytes via a field-stimulation – thus curving the strips/teeth in the MTF. The contraction experiments were observed by looking vertically down onto the chip and monitoring the change in length as the strips contracted and bent up.

Observing the contraction response of the tissue allows scientists to study the effect of physiological factors or test drugs for cardiotoxicity. Replicating segments of heart tissue makes it possible to rapidly measure contraction data at the tissue level, rather than just studying individual cells. [2, 16, 17]

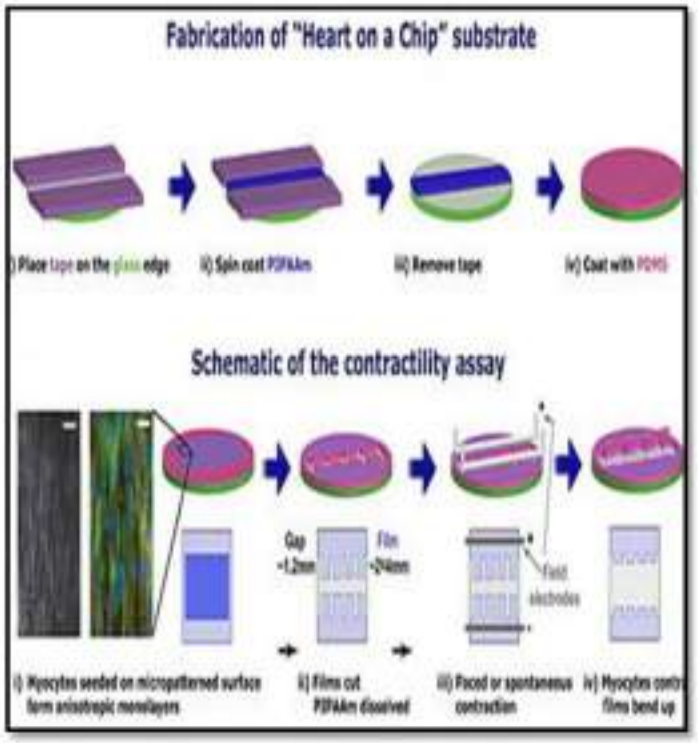

Figure 2: Preparation of the Heart-on-a-Chip Substrate and Contractility Test Samples[34]

After applying a stimulating the contraction of the myocytes via the field electrodes, strips/teeth in the MTF start to curl. Researchers have developed a correlation between tissue stress and the radius of curvature of the MTF strips during the contractile cycle, validating the demonstrated chip as a heart-on-a-chip.

2.3. Artery-on-a-chip

Scientists have developed a microfluidic platform on which fragile blood vessels can be fixed, so as to study the factors that promote and sustain various cardiovascular diseases. Microvascular structure and function are currently studied using two ways,

a. Isometric approach: where small arteries are mounted on two wires, or

b. Isobaric method: where arteries are drained and filled using glass micropipettes. Both of these procedures require manually skilled personnel and good laboratory facilities.

The platform involves loading and immobilising small arteries within a microfluidic channel where they can be maintained and analysed under physiological conditions that are very similar to those experienced in vivo. Forces within ranges that blood vessels experience in their natural environment can be explored without the use of mechanical tools. It acts as the basis of a microfluidic assembly line for complex structures from biological or colloidal building blocks.

Hallmarks of various cardiovascular diseases involve pathologic change of structure and function of small blood vessels, which can be well analyzed, using these chips. This microfluidic device can be used for the assessment of resistance artery structure and function under physiological conditions (37 °C, 45 mmHg transmural pressure). This device could be used to routinely screen drug candidates on viable arteries, potentially speeding up the drug development process and reducing the need for animal experimentation.

Dose effects were checked in mouse artery, of up to ten dose–response sequences of intact mouse mesenteric artery segments (diameter ≈ 250 micrometres and length ≈ 1.5 mm) in a well-defined microenvironment. The application of phenylephrine or acetylcholine (homogeneous condition) yielded dose–response relationships that were identical to conventional myography techniques. Following is the dose response curve seen in mouse mesenteric artery chip on application of phenyleprine.

The microfluidic platform allows us to address new fundamental biological questions, replaces a manually demanding procedure with a scalable approach and may enable organ-based screens to be routinely performed during drug development. [3, 4, 7, 8]

2.4. Kidney on a chip

A kidney-on-a-chip device has the potential to accelerate research encompassing artificial replacement for lost kidney function. Generally, dialysis patients go to a clinic up to three times per week. Artificial kidney research is striving to bring transportability, wearability and perhaps implantation capability to the devices through innovative disciplines: microfluidics, miniaturization and nanotechnology.

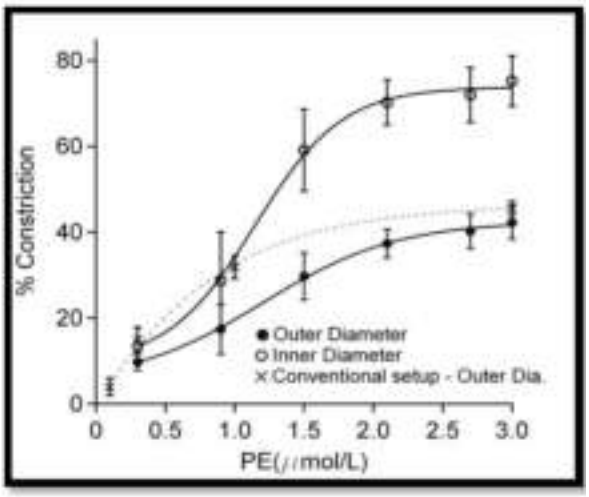

Figure 3: Phenylephrine Dose Response Consistent with Conventional Data

2.4.1. Example: Nephron-on-a-Chip

The nephron is the functional unit of the kidney and is composed of a glomerulus and tubular structures. Nephron on chip is a bioartificial device that replicates the function of the glomerulus, PCT (proximal convoluted tubule), DCT (distal convoluted tubule) and Henle's loop.

Renal tubules are involved in filtering the blood and producing urine. They are important to study as many drugs are secreted into the kidneys via these cells. These cells play roles in many kidney diseases, which are often due to the tubules being damaged or reacting to various molecules.

Each part of the device has its unique design, generally consisting of two microfabricated layers separated by a membrane. The only inlet to the

microfluidic device is designed for the entering blood sample. In the glomerulus' section, the membrane allows certain blood particles through its wall of capillary cells, composed by the endothelium, basement membrane and the epithelial podocytes. The fluid that is filtered from the capillary blood into Bowman's space is called filtrate or primary urine.

In the tubules, substances are added to the filtrate for urine formation, and some substances reabsorbed out of the filtrate and back into the blood. In the PCT, complete absorption of nutritionally important substances takes place. In the device, this section is a straight channel, but blood particles going to the filtrate have to cross the previously mentioned membrane and a layer of renal proximal tubule cells. The second segment of the tubules is the loop of Henle where the reabsorption of water and ions from the urine takes place. The device's looping channels strives to simulate the countercurrent mechanism of the loop of Henle. Likewise, the loop of Henle requires a number of different cell types because each cell type has distinct transport properties and characteristics. These include the descending limb cells, thin ascending limb cells, thick ascending limb cells, cortical collecting duct cells and medullary collecting duct cells.

The micro-model of kidney physiology will also feature two parallel structures – small blood vessels and the surface lining of the renal tubules. This aspect of the device will enable researchers to study the complex interactions between these two structures, which are normally in intimate association inside each of the functional units of the kidney, the nephrons.

One step towards validating the microfluidic device's simulation of the full filtration and reabsorption behavior would include demonstrating that the transport properties between blood and filtrate are identical with regards to where they occur and what is being let in by the membrane. For example, the large majority of passive transport of water occurs in the proximal tubule and the descending thin limb, or the active transport of NaCl largely occurs in the proximal tubule and the thick ascending limb. The device's design requirements would require the filtration fraction in the glomerulus to vary between 15%-20%, or the filtration reabsorption in the proximal convoluted tubule to vary between 65%-70%, and finally the urea concentration in urine (collected at one of the two outlets of the device) to vary between 200-400mM.

However, the flow of blood and urine means that renal tubular cells are exposed to shear stresses. It also brings challenges

to making physiologically relevant models using the cells. To counter this scientists developed a multilayer microfluidic device and optimised the growth conditions for the renal tubular cells. The cells are grown on a permeable support that is placed over a well containing outer tubular fluid (playing the role of blood) whilst a continuous stream of inner tubular fluid (precursor urine mimic) is passed over the cells. This leads to the cells growing and functioning as they would in the body. This device was able to show that hormone stimulation causes a water-transporting protein to move within the cells. The model even mimics how the cells respond in vivo, helping in understanding of cellular mechanisms of disease. [5, 6]

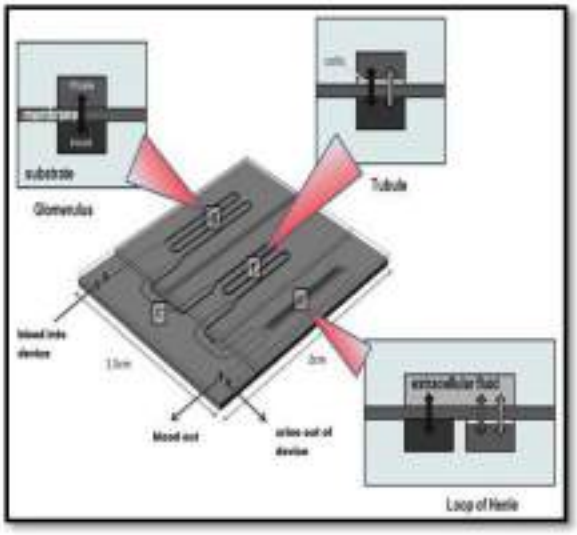

Figure 4: Schematic of a Nephron-on-a-Chip Device with Cross-Sections of 3 functional Units – C – Connector; G – Glomerulus; T – Tubule; L – Loop of Henle / Black arrows: passive transport / White arrows: cell-mediated active transport. [14]

Advantages

With a complete human imitating system, scientists can see the biochemical effects of drugs across the entire human body.

- The concept replicates the function and composition of an organ on a chip that can be easily tested in order to study effects of diseases, toxins, and pharmaceuticals.
- It creates a versatile platform capable of accurately predicting drug and vaccine efficacy, toxicity, and pharmacokinetics in preclinical testing.
- 3D on the chip – allows scientists to assess the effects of a candidate drug on gene expression, on proteins in the cardiovascular system, the neurological system, and more.

Disadvantages

- Are not scalable due to limited the number of laboratories carrying out essential microvascular research.
- Expensive and will take ample amount of time to be put in practice.

3. Conclusion

The goal is to develop human tissue chips that simulate the structure and function of human organs, such the lung, heart, liver, and kidneys. Scientists could then use

these tissue chips to test drug candidates and predict their safety before the next step, human drug studies. This approach is expected be more rapid and cost effective than those currently available. The National Institute of Health, pointed to studies that show that more than 30 percent of promising medications have failed in human clinical trials because the drugs were found to be toxic, despite pre-clinical studies in animal models. Tissue chips may offer more accurate predictions of the side effects of potential therapeutic agents because they contain human cells. These bioengineered devices will produce relevant physiological functions and will reflect the complexity and diversity of living organs, including genetic differences, disease complexity and pharmacological responses.

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6. Comparative study of simulation of incompressible two-dimensional laminar Duct flow in ANSYS FLUENT™ and MATLA□™

Research Article

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Abstract

A finite difference method is described for computation of dynamics of pipe flow in two dimensions. The In-house code solves the pressure –velocity coupled momentum and the continuity equations using Semi Implicit Pressure Linked (SIMPLE) algorithm and post-processes the data. The In-house code has been written in MATLAB™. A run for the same geometry in ANSYS FLUENT™ is also put on for two-dimensional laminar flow and a systematic comparative study is presented between the two codes. The results are tabulated with their percentage accuracy with respect to the analytical solution.

1. Introduction

Traditionally the approach used for design of process equipment involved heuristic knowledge and experience. With the onset Computational Fluid Dynamics the studies of hydrodynamics of process equipment became possible. Knowledge of Fluid dynamics of the equipment enhances the understanding of the physics of the Transfer processes involved, thereby aiding in the design. Pipe flow one of the most commonly flows observed in industry. This paper deals with solutions of the governing continuity and momentum equations for the pipe geometry in two dimensions.

There are many commercially available Solvers which solve the governing partial differential equations of Fluid Dynamics like ANSYS FLUENT™. This paper presents a comparative study of In-house code developed with the commercial

software FLUENT™ and the analytical solutions available for fully developed pipe flow in Laminar Regions.

The transfer of fluid through a pipe is governed by three equations vis-à-vis 'The continuity equation' and 'the momentum equations in X direction' and 'the momentum equation in Y direction'. We will be describing the code to solve these three equations in a uniform rectangular grid. Results of simulations of the In-house code for different number of grid points are also described and compared with the analytical solution. Finally, a comparison between the solution of the exactly same geometry and meshing in ANSYS FLUENT™ are compared with the In-house code.

The flexibility of the In-house code to be modified for Direct Numerical Simulations (DNS) is also discussed in Conclusions as scope for future work.

2. Numerical Analysis

2.1 Governing Equations

The governing equations for an incompressible fluid flow in a rectangular planar duct flow are namely 'the continuity equation' and 'the momentum equations.' The continuity equation arises from mass balance through a control volume. The momentum equations are a result of the Newton's 2nd law applied on a control volume. The equations aforementioned are given below:

$$\frac{\partial \rho}{\partial t} + \nabla.\mathrm{v} = 0 \quad \ldots\ldots\ldots\ldots (1)$$

(The continuity equation)

$$\frac{\partial \mathrm{v}}{\partial t} + (\mathrm{v}.\nabla).\mathrm{v} = \frac{1}{\rho}\{-\nabla.\mathrm{p} + \mu\nabla^2\mathrm{v} + \mathrm{g}\} \ldots (2)$$

(The general momentum transport equation)

Where, 'ρ' is the density of the fluid used, v is the velocity field and 'p' is the pressure.

Now for incompressible fluid flow through a 2D rectangular duct, the above mentioned equations reduce to the following equations:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial x} = 0 \quad \ldots\ldots\ldots\ldots (3)$$

(The continuity equation)

$$\frac{\partial \mathrm{u}}{\partial t} + u\frac{\partial u}{\partial x} + u\frac{\partial v}{\partial x} = \frac{1}{\rho}\left\{-\frac{\partial \mathrm{p}}{\partial x} + \mu\left[\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 v}{\partial y^2}\right]\right\} \quad \ldots\ldots\ldots\ldots (4)$$

(The momentum equation in X-direction)

$$\frac{\partial \mathrm{v}}{\partial t} + v\frac{\partial u}{\partial x} + v\frac{\partial v}{\partial x} = \frac{1}{\rho}\left\{-\frac{\partial \mathrm{p}}{\partial x} + \mu\left[\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 v}{\partial y^2}\right] + g\right\} \quad \ldots\ldots\ldots\ldots (5)$$

(The momentum equation in Y-direction)

where, 'u' is the velocity in X-direction, 'v' is the velocity in Y-direction, all the other symbols suggest the same as above. Solving these governing equations

numerically is the objective of the In-house code.

A finite differencing method is used to solve these partial-differential equations and a central differencing scheme is used for discretisation of convective- diffusive terms and Simple algorithm is followed for solving pressure linked momentum equations. [1]

2.2. Grid Generation and usage

Solving the governing equations involves meshing the geometry (dividing it in smaller areas or volumes) and approximating these partial differentials by the assigning the variables at each grid point and then establishing relationships between these variables by Taylor's theorem or spectral methods. So there a matrix generation, this can be solved at each time step. The algorithm of solution is SIMPLE which requires generation of staggered grid. A staggered grid is used because if pressure and velocities are defined at the same locations a highly non-uniform pressure field can behave like a uniform field in the discretised momentum equations. The In-house code generates a grid of a rectangular shape of 0.01m x 0.6 m. Figure 1 shows a 20 x 20 grid generated by the In- house code.

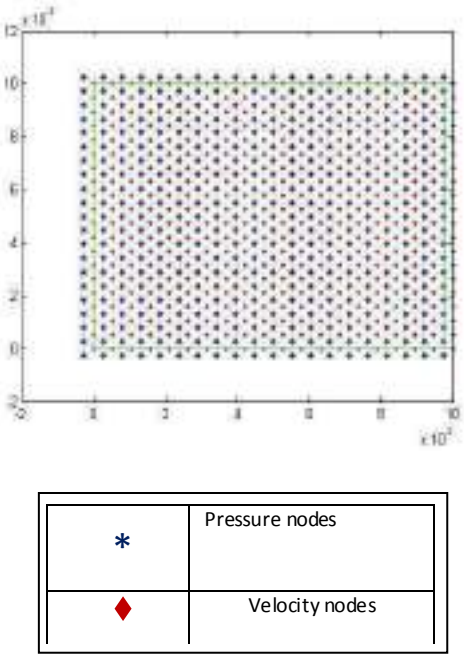

Figure 1: Staggered Grid

2.3. Discretisation

Once the grid is generated then the solvers can be used to find out parameters on respective grid points of pressure and velocity. We now develop all the discretisation involved in the solvers. The discretised parts of the Navier-Stokes equations are (without pressure term, which will be added in the pressure correction equation):

$$\frac{\partial u}{\partial x} = \frac{u_{i+1,j} - u_{i-1,j}}{2hx} \qquad \frac{\partial u}{\partial y} = \frac{u_{i,j+1} - u_{i,j-1}}{2hy}$$

……(6)

$$\frac{\partial v}{\partial x} = \frac{v_{i+1,j} - v_{i-1,j}}{2hx} \qquad \frac{\partial v}{\partial y} = \frac{v_{i,j+1} - v_{i,j-1}}{2hy}$$

……(7)

$$\frac{\partial^2 u}{\partial x^2} = \frac{u_{i+1,j} - 2u_{i,j} + u_{i-1,j}}{hx^2}$$

,

$$\frac{\partial^2 v}{\partial x^2} = \frac{v_{i+1,j} - 2v_{i,j} + v_{i-1,j}}{hx^2}$$

……..(8)

$$\frac{\partial^2 u}{\partial y^2} = \frac{u_{i,j+1} - 2u_{i,j} + u_{i,j-1}}{hy^2}$$

$$\frac{\partial^2 v}{\partial x^2} = \frac{v_{i+1,j} - 2v_{i,j} + v_{i-1,j}}{hy^2}$$

……..(9)

Now we rewrite the transport equations in discretised form:

$$u_{ij,t} + u_{i,j}\frac{u_{i+1,j} - u_{i-1,j}}{2hx} + v_{i,j}\frac{u_{i,j+1} - u_{i,j-1}}{2hy} = \mu\left(\frac{u_{i+1,j} - 2u_{i,j} + u_{i-1,j}}{hx^2} + \frac{u_{i,j+1} - 2u_{i,j} + u_{i,j-1}}{hy^2}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (10)$$

$$v_{ij,t} + v_{i,j}\frac{v_{i+1,j} - v_{i-1,j}}{2hx} + v_{i,j}\frac{v_{i,j+1} - v_{i,j-1}}{2hy} = \mu\left(\frac{v_{i+1,j} - 2v_{i,j} + v_{i-1,j}}{hx^2} + \frac{v_{i,j+1} - 2v_{i,j} + v_{i,j-1}}{hy^2}\right) - g \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

We can now write (10), (11) as

$$u_{ij,t} = f_u(u, v) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (13)$$

$$v_{ij,t} = f_v(u, v) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (14)$$

We will discretise these equations in time with the use of the Adam Bashforth method for time discretisation. We will first calculate a guess for the velocity and use the pressure from the old time level. After that we will update the pressure with the use of equation (6) and calculate the velocities for the new time level.

$$u^* = u^n + \frac{3}{2}f_u(u^n, v^n) - \frac{1}{2}f_u(u^{n-1}, v^{n-1})$$

$$v^* = v^n + \frac{3}{2}f_v(u^n, v^n) - \frac{1}{2}f_v(u^{n-1}, v^{n-1})$$

Or with Euler forward

$$u^* = u^n + f_u(u^n, v^n)$$

$$v^* = v^n + f_v(u^n, v^n)$$

This is the overall discretisation scheme followed, now we use velocities obtained in this level to solve the pressure correction equation to get the pressure. The code also renders a quiver plot of the Velocity Field and plots of velocity in X direction and velocity in Y-direction and the pressure field.

3. Results and Discussions

3.1. Quantitative comparison of velocities the fully developed Flow

We have run the In-house code for different set of grid points and compared the solution with the fluent solution and the analytical solution which is given in table 1. The maximum velocity in the Y-direction is obtained for different grid sizes using the solutions of in-house code. Theses velocities are tabulated with the analytical solution for the fully developed region of flow.

Table 1:- comparative study of error using different grid sizes

Mesh	Obtained Max velocity u (using In-house code)	Analytical Max velocity u	$error = \frac{u_{analytical} - u_{ccalculated}}{u_{analytical}} x100$
20 x 500	0.001136	0.00125	9.066486467
40 x 500	0.001144	0.00125	8.405576532
80 x 500	0.001146	0.00125	8.310331857

We have kept the overall Reynolds's number 500 and calculated the velocity profile using initial velocity of 8.33333×10^{-4} m/s as per standard velocity correlation of fully developed laminar flow. The standard velocity correlation is given below for

$$-\frac{H}{2} \leq y \leq \frac{H}{2}$$

$$v = \frac{3}{2} v_{in} \left(1 - \left(\frac{y}{\frac{H}{2}}\right)^2\right)$$

In Figure 2, we have plotted the solutions of In-house code for different grid sizes vis-à-vis 20 x 500, 40 x 500, 80 x 500 respectively. The solution of the same geometry that is length of 0.6 m and height of 0.01 m, using Gambit meshing and Ansys Fluent™ is also plotted on the same graph. The analytical solution is also plotted on the same plot.

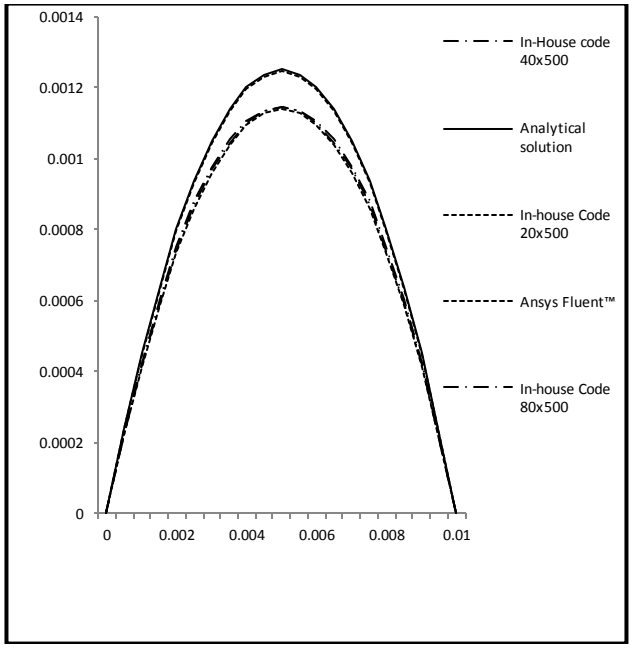

Figure 2: comparative plot for solutions of different grid sizes and analytical solution and Ansys solution

Figure two shows that the In-house code when run for different increasing grid sizes approaches the analytical solution. But after 80 x 500 grid size the solution attains grid independence. Percentage standard deviation of the solution for different grid size is given below:

We see that the error goes down as we increase the grid size from 20 x 500 to 40 x 500, whereas at grid size of 80 x 500 the error decrement is almost negligible that's why the plots in Figure 2 are very close to each other.

3.2. Contour Plots

The In- house code is capable of generating velocity contours and the quiver plots at every 100th iteration. It generates a plot of Velocity in X- direction, Velocity in Y-direction, pressure and mass residual. Figure 3 shows the In-house code generated render of fully developed flow at 65 seconds.

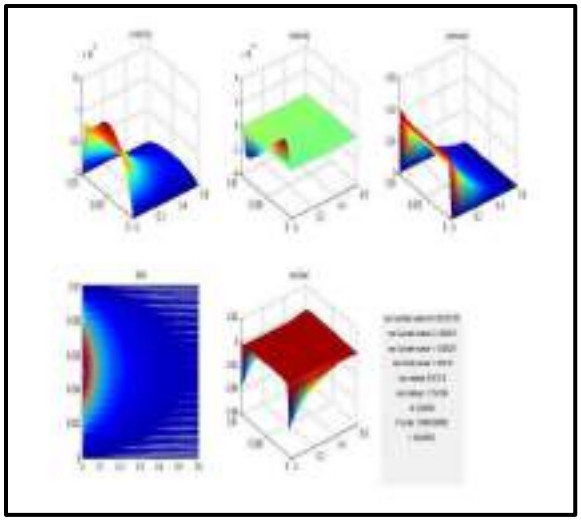

Figure 3: The above figure shows a render of fully developed flow at 65 seconds for 0.01 m x 0.6 m with a grid of 80 x 500 grid size.

4. Conclusion

We see that the solutions obtained from the In-house code in laminar fully developed regions. The velocity profile in Y-direction is indeed parabolic in nature as expected.

The comparison clearly indicates that the code is rendering sufficiently close magnitudes and the trends of flow parameter variation match with the commercial code vis-à-vis ANSYS FLUENT™. The variation in values are due to the change of discretisation scheme as our code implements central differencing scheme and ANSYS FLUENT™ implements 1st order upwind scheme in space. This code can be modified for different geometry and turbulence as well. The velocity profiles generated by the code are matching completely with ANSYS FLUENT™'s solution for incompressible unsteady laminar pipe flow in two dimensions.

This comparison throws into light that this In-house code can be modified with higher order discretisation schemes and extremely fine grid size i.e. upto Kolmogorov length scales and three dimensions to be used as a DNS(direct numerical simulations) code for analysis of Duct flow for high Reynolds numbers.

The pipe flow is a classic example of wall generated turbulence. DNS analysis tracks the turbulence till its lowest possible scales. The near wall turbulence is crucial for transport processes, understanding its structure and associated transport mechanisms is of great importance for scientific and engineering viewpoints. This In-house code can form a basis of this kind of direct numerical simulations.

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7. Strengthening of Security Paper

Review Article

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Abstract

Security papers generally have a very short life span and hence cost a country billions of dollars for making new papers and in disposing of the old ones. It is therefore desirable that novel methods be explored for increasing the life span of such papers. Though numerous additives have been used till date, none of them have been able to increase the life span substantially. We applied inductive reasoning to establish various hypotheses for increasing the durability of paper, and did an exploratory research to determine the results of those hypotheses.

1. Introduction

The history of paper used in currency dates back to as far the 7th century. Generally, banknotes are made from cotton rag paper with a weight of 80 to 90 grams per square meter. Linen, abaca, or other textile fibers are often added to cotton. This type of paper is different from ordinary paper; it is much more resilient, resists wear and tear, and is devoid of whitening agents. Banknote paper is infused with polyvinyl alcohol or gelatin to give it extra strength unlike most printing and writing paper. Polymer banknotes like the ones made from biaxially-oriented polypropylene have also been developed recently to improve durability.

A review of the life of cellulose-based bank notes in various countries providing an interesting cross-section of countries by size, socioeconomic and environmental conditions etc tells us whatever the handling, environmental or quality standards used in these countries, there is nothing unusual about having notes with lives of two years or less. Following generalizations may be made keeping in mind the data conclusively provided by conferences such as the Pacific Rim Banknote Printers Conference.

- Countries have between two and six denominations with life of two years or less;
- Countries have at least two denominations with a life of 18 months or less;
- 75 per cent of all of the countries have at least one denominations with a life of around 12 months or less;
- 63 per cent of all of the countries have at least two denominations with a life of around 12 months or less.

Therefore it can be recognized that there is an immediate need to improve the durability of currency notes. In the recent past a few countries have suggested that we switch to polymeric substrates instead of rag paper. Even though both have their own advantages and disadvantages, we decided to pursue rag paper since it has several properties which are highly desirable in such applications, including dead foldability, tear resistance, printability and embossability. As may be appreciated, these properties combine to give bank notes their familiar feel and functionality. A few of the problems associated with plastic substrates have been listed below.

Problem with the Australian plastic substrate notes:

- They are found to be rather complex in construction and relatively expensive to produce. Moreover, when transmission security devices are laminated between layers in the substrate, an area of weakness and high stress is created which reduced both durability and security.
- First, the oriented polypropylene substrate(OPP) substrate does not dead fold, causing problems in that the film retains either a flat or curved form, jamming cash registers and automatic handling equipment.
- Second, the OPP substrate has poor initiated tear resistance in the processing of currency, which quite frequently creates nicks on the edges of bills, resulting in catastrophic tears.
- The OPP product also does not exhibit the tactility of paper currency, due to the fact that OPP does not emboss well during the intaglio process.

2. Objective

The objective of the paper is to explore different methods to improve the durability of currency notes, and security paper in general. The following properties need an immediate redressal;

- Tear Resistance:
  1. Tear initiation
  2. Tear propagation
- Water Resistance
- Stain Resistance

3. Literature Review

It is necessary that we first understand what gives paper 'strength'. Paper is essentially is composed of cellulose.

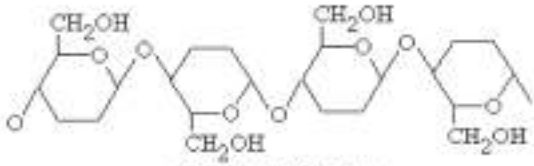


Figure 1: Cellulose

The packing of cellulose can be explained as in figure 2. The important point to note is that these cellulose, and eventually the fibers, are held together by H-bonds. The integrity of a paper sheet is dependent on the hydrogen bonds which form between the fine structures of cellulose fibers during the pressing and drying operations. The bonds between hydroxyls of neighboring fibers are very strong when the paper is dry, but are severely weakened as soon as the paper becomes wet. Bonding between the hydroxyls of cellulose and water is as energetic as bonding between two cellulose hydroxyls. As a consequence, ordinary paper loses most of its strength when it is wet or exposed to very high humidity. The sheet loses its stiffness and bursting, tensile and tearing strength.

If water can be prevented from reaching the sites of the bonding by sizing or coating the sheet, then a measure of wet strength may be attained. High molecular weight species are strongly absorbed on the fibers and are large enough to bridge two fibers.

Low molecular weight species are not retained as well because of fewer charge sites. At this point, it is necessary that we understand some of the relevant properties of paper, and the factors which affect them.

4. Tensile Strength

Some of the factors that affect the tensile strength of paper are listed below,

4.1. Fiber Length

1. A long fiber can have more bonds with other fibers and therefore will be held more strongly than a short fiber.
2. The tensile strength of the wet web increases rapidly with fiber length.
3. Likewise, tensile strength, breaking strain and fracture toughness also increase with fiber length.
4. The probability of two fibers crossing is proportional to the mean value of fiber length squared.

4.2. Fiber-fiber bond strength

- Cellulose molecules are held together by

  1. Covalent bonds;

2. H-bonds;

3. Van der Waal's forces;

4. Any covalent or ionic bond that may be formed between the cellulose and some polymeric mediators.

4.3. Other factors

- Beating: more the beating less will be the TS.
- Bleaching: it may decrease the TS.
- Drying under stress: Drying under an axial tension increases tensile strength but decreases breaking strain.
- Recycled pulp: it swells less and therefore less TS.
- Internal Stresses developed during packing of fiber networks: these are local stresses.

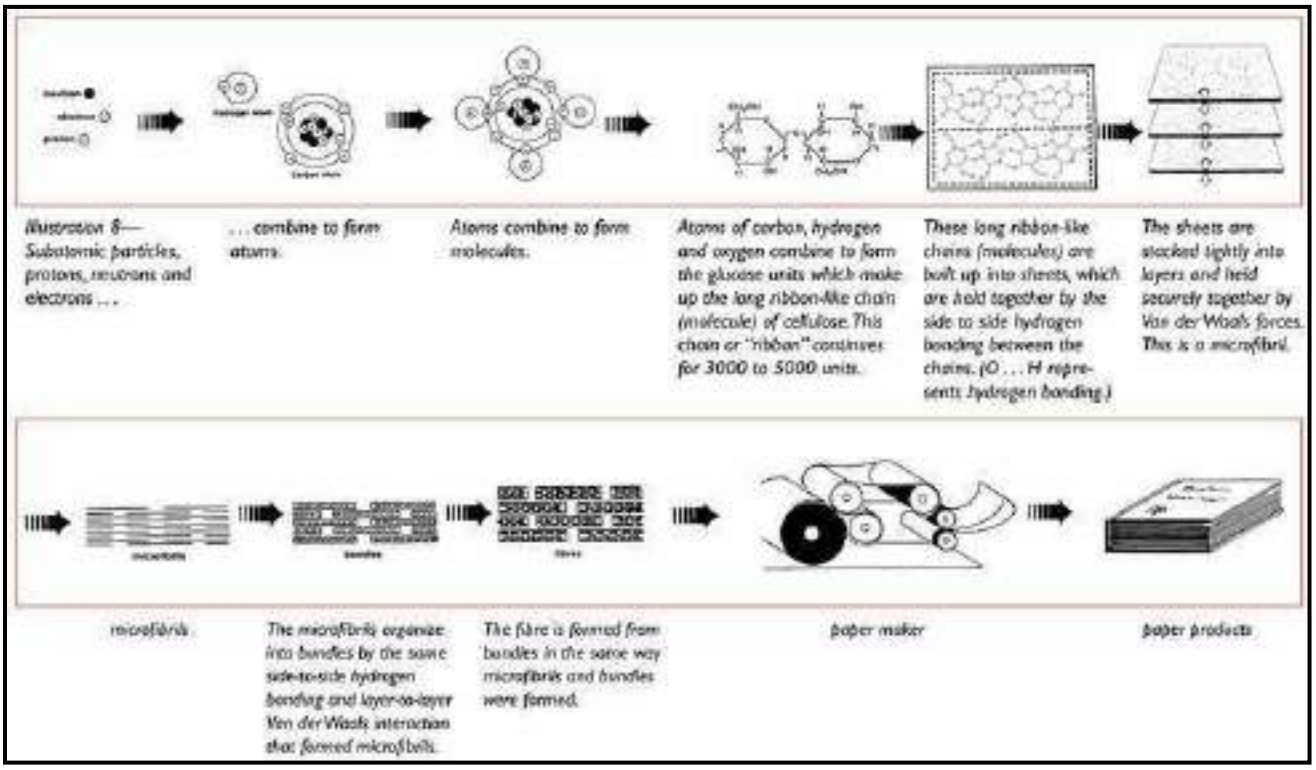


Figure 2: The process of Paper Manufacture

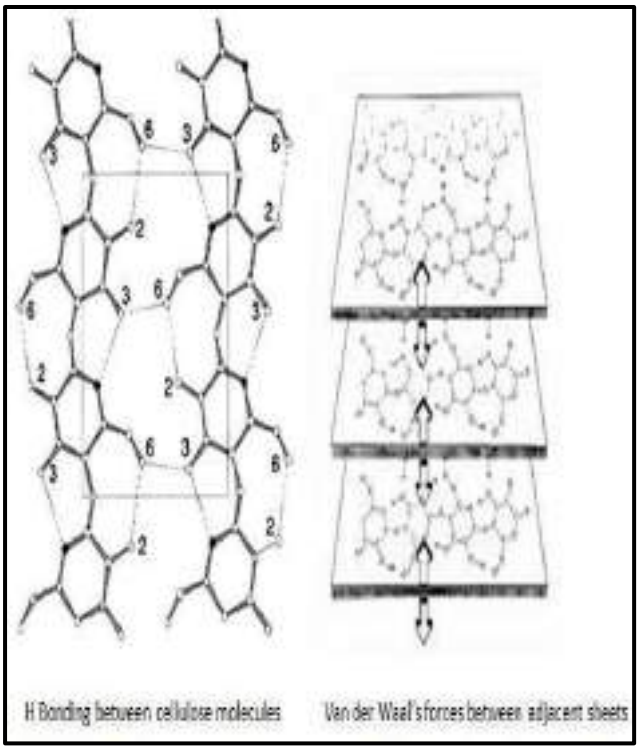


Figure 3: Secondary forces between cellulose.

The above points can be reinforced by closely studying the Page Equation, which gives the relation between various factors like the length and the diameter of a fiber and the eventual tensile breaking length, T, of the paper;

$$\left[\frac{1}{T}\right] = \left[\frac{9}{8Z}\right] + \left[\frac{(12g\ C^2)}{(P^2 \cdot l \cdot b \cdot RBA)}\right]$$

The Page Equation

$$RBA = \frac{(S_w - S)}{S_f}$$

l	= fiber length (length)
b	= fiber-fiber bond strength (N/m²)
RBA	= relative bonded area (unit less)
g	= gravitational constant -(length/second² = 9.8 m/s²)
T	= tensile breaking length (length)
Z	= zero span tensile (length)
C	= fiber coarseness (weight/length)
P	= fiber perimeter (length)

The relative bonded area (RBA) in Page's equation is a measure of the contact area between fi

This implies that,

- T α fiber length
- T α fiber-fiber bond strength
- T α contact area b/w fibers in the sheet
- T α 1/(fiber coarseness)

5. Tear Strength

- In a well bonded, refined fiber's paper, stress is localized at the point of propagation, whereas a moderately bonded paper will delocalize the stress.(Ref. US patent no. 4,609,432)
- Synthetic fibers have more tensile strength than cellulose fibers, and hence when the sheet is torn they do not rupture but come out of the sheet structure. (Ref. US patent no. 5,223,095)

The amount of synthetic fiber to be added has to be optimized; since more of it will decrease the tensile strength. Even the length should be optimized, as well as its diameter.

Hypothesis employed

a. Binding the fibers at the point of intersection/ overlap.
b. Providing reinforcement in the form of cotton gauze.
c. Increasing the H-bonding.

d. Using wet/ dry strength additives and binders in a blend.

6. Experimental Method

To test the above hypothesis the following experiments were performed. The detailed SOP of each experiment is followed by the relevant result. All the samples made were handmade and their data is compared to an untreated handmade paper; in both the cases all other conditions and procedures were same.

6.1. Experiment

The following is the SOP followed and standardized for making the handmade cotton rag paper.

1. Chop the cotton fibers and waste paper by scissors.
2. Weigh 9gm of fibers and 1gm of waste paper.
3. Take 1400ml of water in a beaker, add 14gm of NaOH and 14ml of Hydrogen Peroxide to it.
4. Heat till 75’C and keep adding the fibers and the waste paper while maintaining rigorous stirring.
5. Continue the heating for an hour.
6. Wash the fibers collected with water.
7. To refine this fibers further, ball mill them (we used Retsch Planetary Ball Mill, at 450 rpm, for 15mins).
8. Disperse half of the above fibers in water.
9. Pour this mix into a tub which has a stainless steel sieve kept in it; the same quality and size is used in every sample.
10. Let the fibers settle on the sieve for 3 hrs.
11. Remove the sieve from the tub and leave it to dry.
12. Peel the paper off the sieve.
13. To give the paper a smooth finish, hot press it for 10mins at R.T.

6.2. Experiment

Using aqueous polyurethane dispersion (PU) as a binder to test hypothesis no. 1 by coating the fibers and curing them before dispersing them in water. The PU aqueous dispersion used throughout is Drokyl 77106, supplied by DRC resins and coats.

After step 7, the fibers weredipped in the PU dispersion and left to cure in the oven for around 2 hours at 100”C.

The experiment was done for various concentrations of PU.

Sample No. 1: PU 20% w/w of pulp.

Sample No. 2: PU 40% w/w of pulp.

Sample No. 3: PU 80% w/w of pulp.

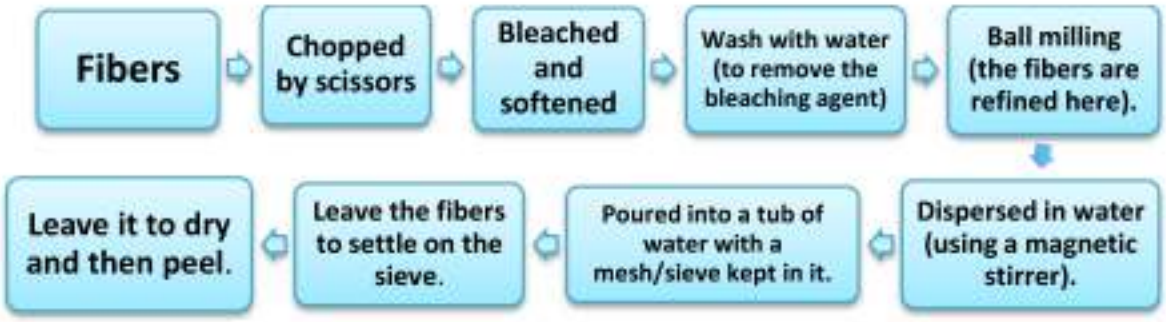
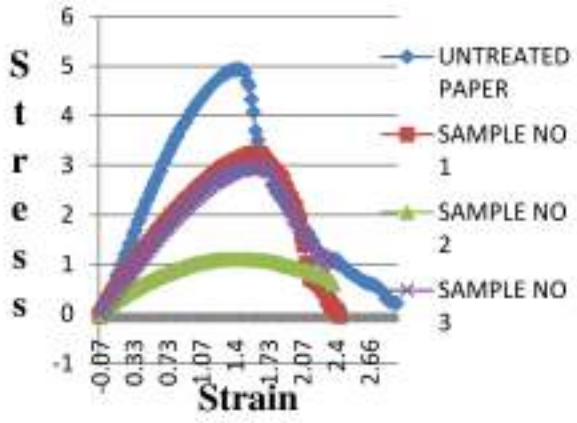


Figure 4: The process

As can be observed above, dipping the fibers in PU reduced the tensile strength of the paper. It is quite interesting to note that the tensile strength of 20% w/w PU and 80% w/w PU is almost the same whereas the 40% w/w PU has shown a considerable dip in the tensile strength.

The reason for this behavior may be attributed to the decrease in H-bonding between the fibers because of presence of inert PU. It may also be because of very less density of fibers in one layer (paper has multiple layers of cellulose fibers).

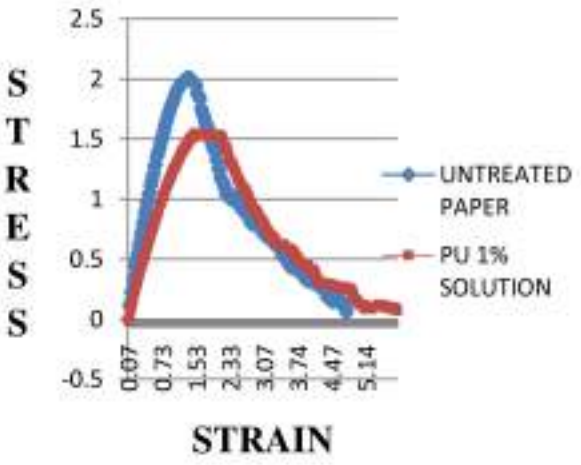


Graph 1: Stress v/s Strain plot for Paper strengthened with PU binder

6.3. Experiment

Using aqueous polyurethane dispersion (PU) as a binder to test hypothesis no. 1 by dispersing the fibers in PU solution.

In step 8, instead of water, a 1% PU solution (1% w/w of water) is used.



Graph 2: Stress v/s Strain plot for Paper strengthened with PU binder (1 % wt)

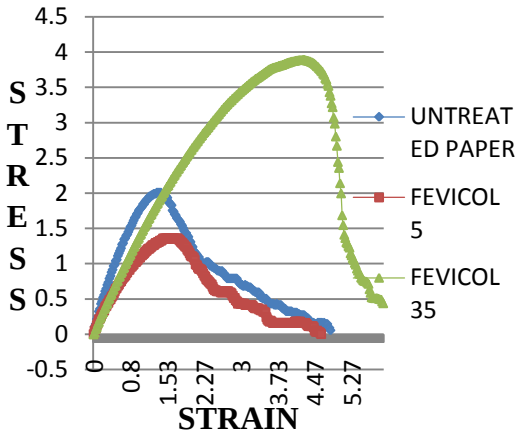
It is observed that there is no considerable change. The possible reason could be that since there are very few fibers in one plane (structure of the paper is multi-layered), the point of intersections/ overlap maybe very

few to affect the strength of paper considerably. It may also be possible that the resin did not bind with the fibers at all, and cross-linked with its own molecules. Possible diagnostic could be; if we wet press the paper, it may bring the fibers close enough to bind as desired; or other more interactive binders can be tested.

6.4. Experiment

Fevicol in various proportions was dispersed in water and then the fibers were dispersed in the solution.

Fevicol was dispersed in water in step 8 in various concentrations by weight of pulp.



Graph 3: Stress v/s Strain plot for Paper strengthened with fevicol (35%)

Low concentration of fevicol did not show any considerable change, though the 35% paper showed good improvement in the break stress of the paper.

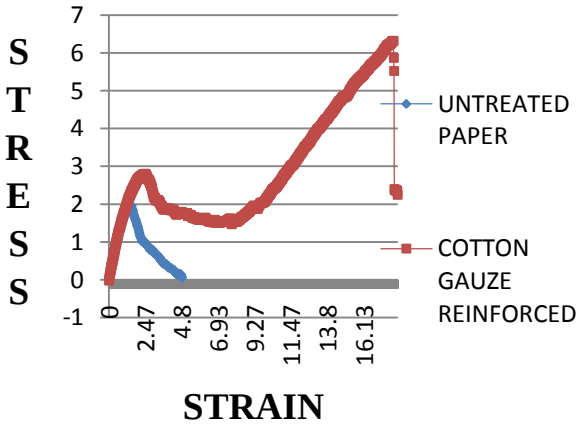
6.5. Experiment

Hypothesis No 2 was tested by reinforcing the paper with cotton gauze.

The cotton gauze was placed and stuck on the sieve, before placing it in water, and then the dispersed pulp was poured on it.

The reinforcement increased the stress as well as the strain to over 3 times.

To further improve upon this result, a gauge of very fine pores can be used, which will give us control over the opacity of paper, and may also increase the strength further. A binder can also be used in this system.



Graph 4: Stress v/s Strain plot for Paper reinforced with cotton gauge.

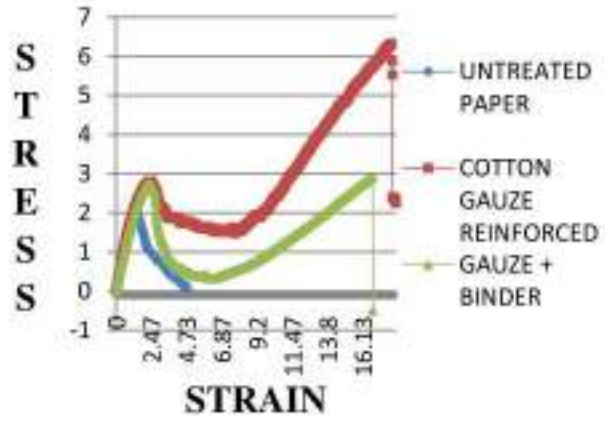
Though, using such reinforcement may cause a hindrance to security features such as those embedded in the paper, like the security thread or the watermark.

6.6. Experiment

6.7. Hypothesis No 2 was tested by reinforcing the paper with cotton gauze and adding a binder to it.

In step 8, PU dispersion was used instead of water, and a cotton gauge was also used.

Interestingly the polyurethane has not affected the 1st peak, i.e the peak which shows the tensile strength of the paper, whereas it has decreased the 2nd peak, i.e the peak which shows the tensile strength of the cotton gauze present inside the paper.

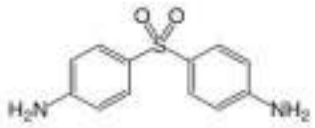


Graph 5: Stress v/s Strain plot for Paper strengthened with cotton gauge and PU binder.

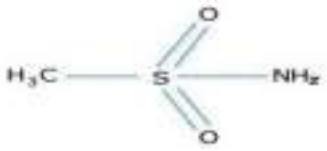
6.8. Experiment

4-Aminophenyl sulfone (Dapson) and methanesulfonamideare used to test hypothesis 3. They were dispersed in water in step 8.

4-Aminophenyl sulfone, provides for strong H-bonds since the lone pairs of N go into resonance and therefore making the N more electron deficient.

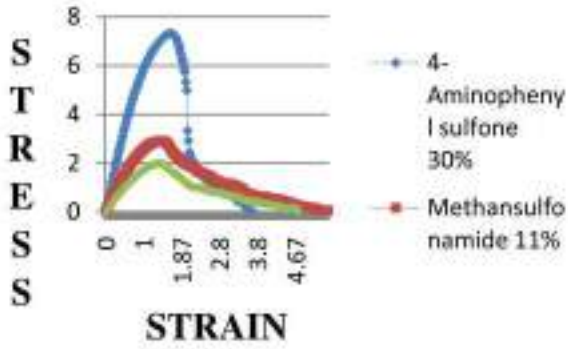


Methanesulfonamide also acts in the above manner, but since the SO_2 group is closer to the amino group, it makes the amino group more electron deficient, and should strengthen the H-bonding even more.



When 4-aminophenyl sulfone was used, stress increased to more than double its original value and for methanesulfonamide there was almost no effect in the properties of paper, which was unexpected.

The difference between them is that MS is



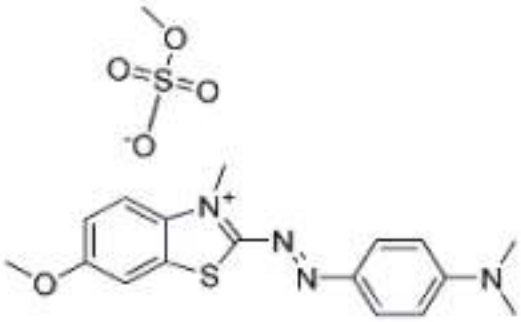
Graph 6: Stress v/s Strain plot for Paper strengthened with Dapson and Methanesulfonamide

water soluble whereas Dapson is only slighlty soluble; therefore it maybe deduced

that water soluble compounds remain dissolved in the water, and don't interact with the fibers.

6.9. Experiment

Using the dry strength additive cationic Polyacrylamide in various proportions.



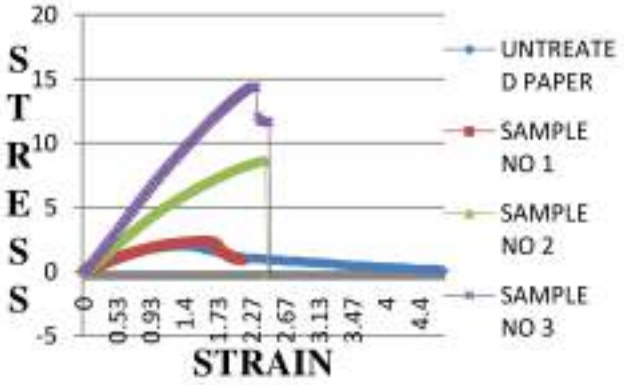
Cationic Polyacrylamide was added very slowly to the water while stirring vigorously taking care that lumps were not formed, and then the pulp was dispersed in this solution.

Cat. PAM was added in various proportions;

SAMPLE NO. 1 = 20% CPAM (w/w % of pulp)

SAMPLE NO. 2 = 33% CPAM (w/w % of pulp)

SAMPLE NO. 3 = 50% CPAM (w/w % of pulp)



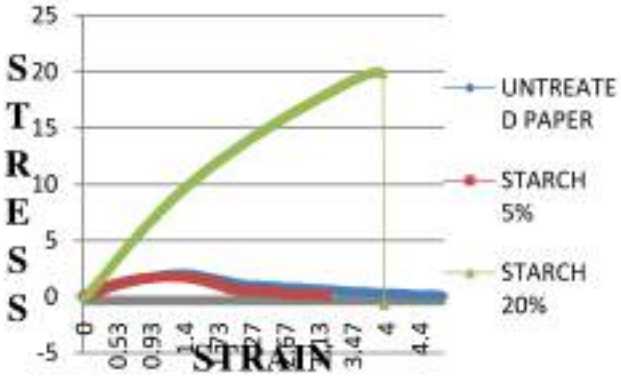
Graph 7 : Stress v/s Strain plot for Paper strengthened with Cationic Polyacrylamide

With increasing percent of CPAM, the break stress considerably increased, though the break strain remained constant.

6.10.Experiment

Using the binder starch in various proportions to test hypothesis 3.

Corn Starch was added very slowly to the water while stirring vigorously taking care that lumps were not formed, and then the pulp was dispersed in this solution.



Graph 8: Stress v/s Strain plot for Paper strengthened with Starch

High % of CPAM and Starch both give a significant rise in the break stress of paper, whereas the low % compositions showed almost no effect at all.

Starch dissolves in water at higher temperatures, and even CPAM needs considerable stirring time to dissolve in water.It may be deduced from the above

results that when taken in high % composition, some of it is settles onto the pulp.

The low % composition gets dissolved completely and therefore does not settle onto the pulp and hence no change in properties.

7. Conclusion

Of all the compounds and materials tried above, 4-aminophenyl sulfone, cationic polyacrylamide, starch and reinforcing using gauze showed promising results, which can be further explored.

An interesting observation from the above experiments was that water soluble compounds/ polymers in low concentration did not show any considerable effect, whereas when used in a higher concentration they all showed promising results.

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8. Resveratrol

Review Article

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Abstract

Phytochemicals are being increasingly known as the "future prescription . Resveratrol is the most envisioned phytochemical belonging to family of phytoalexin polymers. Humans have been unknowingly consuming this compound since ancient times in the form of wine. It is widely distributed throughout the plant kingdom and has been recently considered for its role in health regulation and promotion. Various studies have been conducted on experimental animal models and some of them have been extrapolated on human subjects as well. It was observed that even though more than 70% was absorbed via oral route, its bioavailability was less due to rapid metabolism and excretion. The results can be used with a greater confidence level given the toxicity, absorption and metabolism of resveratrol is studied in detail. By increasing the bioavailability and optimizing the dosage this "red wine compound can open up new frontiers in the treatment of fatal diseases. This review is an attempt to promote application of resveratrol as a nutritional supplement by highlighting its health benefits.

1. Introduction

Phytochemicals are naturally occurring, biologically active chemical compounds in plants, acting as their natural defence system. These plant components offer discrete bio-activities that affect the human biochemistry and metabolism, and are increasingly being considered as nutritionally active ingredients. Such phytochemicals include terpenoids, phenolics, alkaloids and fibres. Resveratrol (3,5,4′-trihydroxystilbene) is a natural polyphenolic phytochemical. It is the parent molecule of viniferins, a family of phytoalexin polymers that prevent the progression of fungal infections in plants. Increased consumption of monomeric resveratrol and/or resveratrol-containing foods may be associated with health promotion. These health benefits are attributed to its diverse range of biological

activities. This review is an attempt to discuss various aspects of resveratrol including bioavailability, antioxidant capacity, cardio-protection, anticancer activity, anti-diabetic effects and other health benefits.

2. Occurrence

Resveratrol is found in at least 72 plant species and is formed via a condensation reaction between 3 molecules of malonyl CoA and 1 molecule of 4-coumaroyl CoA [11] (Figure 1). Resveratrol synthase facilitates this condensation reaction, which also produces 4 molecules of CO_2. Resveratrol exists in 2 structural isomeric forms, cis and trans (Figure 2), with the trans form being more biologically active. Polygonum cuspidatum, a weed, is one of the richest sources of this compound. The primary dietary sources which are a part of the human diet are blueberries, mulberries, peanuts, red grapes. Due to its presence in grapes, it is no surprise that resveratrol is also found in wines. The resveratrol concentration in wine varies, with grape variety and the growing conditions. Vinification which is the conversion of fruit juice to wine through fermentation also influences the resveratrol concentration. [11] The fermentation of grape flesh with the skin in red wine production allows red wines to have greater resveratrol concentrations than white wines, which are produced by fermentation of the flesh only. This is the reason why it is also referred to as the "red wine compound .

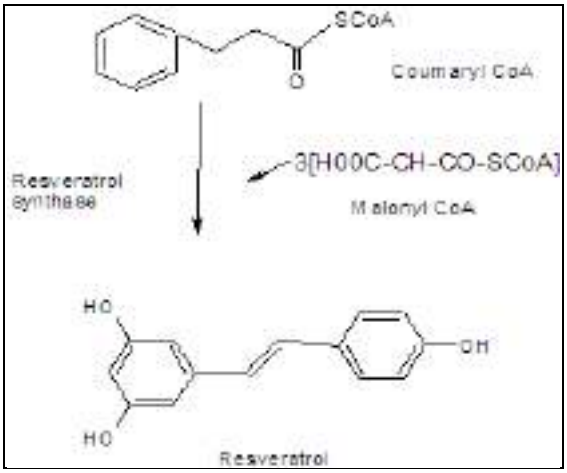

Figure 1: Synthesis of Resveratrol [11]

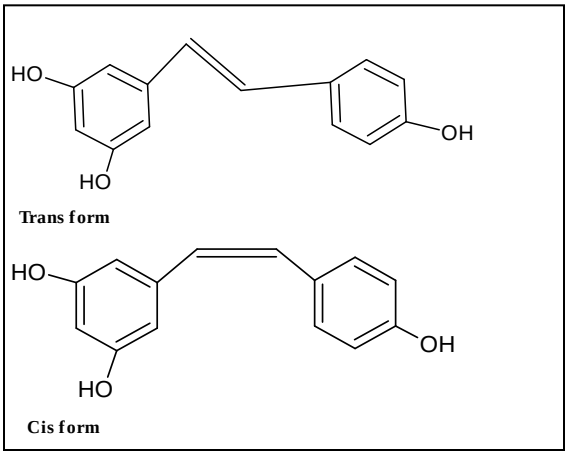

Figure 2: Resveratrol isomers [11]

3. Absorption Bioavailability & Metabolism

The oral absorption of resveratrol in humans is about 75% and is thought to occur mainly by trans-epithelial diffusion [13]. The food matrix affects absorption and bioavailability of resveratrol. Nano-encapsulated resveratrol, not being

metabolized in the gastrointestinal tract, is potentially absorbed through the intestinal wall in its active form. [33] Blood circulation and cell surface deposition is attained due to interaction of resveratrol with albumin. 50% to 98% of total resveratrol has been observed non-covalently bound to albumin, LDL (low density lipoproteins) and hemoglobin. Due to its lower molecular weight it has good skin penetration ability and this property of resveratrol is exploited in skin care and anti-ageing formulations. Resveratrol is well absorbed from oral mucosa, but undergoes significant first pass hepatic metabolism after absorption from the gastrointestinal tract. Blood levels indicate no more than 20% overall bioavailability. Resveratrol is metabolized within the intestinal lining to 3 dominant forms, reveratrol-4'-O-glucuronide (M1), resveratrol 3-O-glucuronide (M2) and resveratrol-3-O-sulfate.

A study published in the Journal of Food Science [11] states that following oral administration of pure resveratrol to human subjects, resveratrol glucuronide was the major metabolite detected in the plasma and urine. On the other hand when high oral doses of grape juice were administered, glucuronide and sulfate conjugates were detected in the plasma and urine of the human subjects. Resveratrol sulfate was the major metabolite found in human plasma. Sulfation may be the primary limiting factor in the bioavailability of resveratrol. Grape juice consists of essentially resveratrol glucosides, cis and trans-piceid (piceid is a stilbenoid glucoside and is a major resveratrol derivative in grape juices), with low amounts of the free resveratrol, indicating a lower bioavailability of the glucosides compared to the pure compound. [11] The kidney is the dominant excretion pathway with urinary and faeces recovery of total Resveratrol between 70 to 98% within 24h.

Information about the bioavailability of resveratrol in humans is critical because much of the basic research on resveratrol has been conducted in cultured cells exposed to un-metabolized resveratrol at concentrations that are often 10-100 times greater than peak concentrations observed in human plasma after oral consumption [10].

4. Health Benefits

4.1. Antioxidant Activity of Resveratrol

The antioxidant activity of resveratrol reduces damage to endothelial cells exposed to nitrite radicals and protects skin cells against damage caused by UV radiation. Lipoxygenase is a dioxygenase

with peroxidase activity involved in the synthesis of mediators in inflammatory, atherosclerotic, and carcinogenic processes. Resveratrol is a potent inhibitor of the dioxygenase activity of lipoxygenase, with an IC_{50} (half maximum inhibitory concentration) of 13 μM. Furthermore, oxidized resveratrol is as efficient a lipoxygenase inhibitor as in its reduced form. Resveratrol is able to prevent the increase in reactive oxygen species (ROS) following exposure to oxidative agents (i.e. tobacco-smoke condensate (TAR) and H_2O_2). Pre-treatment of cells with the phytochemical resveratrol resulted in less cellular damage caused by nitrite radicals (could cause atherosclerosis). Resveratrol increases the activity of superoxide dismutase (important antioxidant enzyme that neutralizes superoxide) and glutathione peroxidase (antioxidant enzyme that protects the cells from oxidative damage). It protects ultrastructure of the skin cells and also reduces lipid oxidation and oxidative stress. As per a study resveratrol was the most effective anti-oxidant in reversing cadmium-induced lipid peroxidation. [29]

4.2. Anti-Diabetic Effects of Resveratrol

Diabetes mellitus is a complex metabolic disease, classified into different types; type 1 and type 2 diabetes are the most frequent. Type 1 diabetes results from autoimmune destruction of beta cells. Patients with type 1 diabetes are dependent on exogenous insulin. Type 2 diabetes is characterized by defects in insulin secretion and action. The management of diabetes involves three main aspects: reduction of blood glucose, preservation of beta cells, and improvement in insulin action (type 2 diabetes).

Numerous studies on diabetic rats revealed the anti-hyperglycaemic action of resveratrol. The ability of resveratrol to reduce hyperglycaemia seems to be the best documented. [18] The anti-hyperglycaemic effect of resveratrol observed in diabetic animals is thought to result from its stimulatory action on intracellular glucose transport. A temporary inhibition of insulin secretion was reported to delay the progress of type 2 diabetes .According to some animal studies; experiments in vitro demonstrated the ability of resveratrol to reduce insulin secretion by freshly isolated rat pancreatic islets. The inhibition of insulin secretion caused by resveratrol was found to result from metabolic changes in beta cells. Resveratrol also ameliorates common diabetes symptoms, such as polyphagia, polydipsia, and body weight loss. In

human clinical trials conducted by Sirtris Pharmaceuticals, it was found that Resveratrol lowered blood sugar levels. [9]

4.3. Anti-Cancer Activity of Resveratrol

Resveratrol not only helps to prevent DNA damage but it also influences the transcriptions of genes responsible for redox metabolism and inhibits proliferation of cancer cells lines, including those from breast, prostate, stomach, colon, pancreas and thyroid.

- Resveratrol decreases tumour growth by inhibiting the enzyme cyclooxygenase-1, which converts arachidonic acid to substances that promote tumour growth. It antagonizes each stage of tumorigenesis and inhibits protein kinase C (PKC), a key mediator of tumour promotion.
- Resveratrol is more effective against tumours on which it can act directly like skin and gastrointestinal tract tumours.
- Resveratrol inhibits activity of certain cytochrome P450 enzymes which trigger carcinogens after metabolism. On the contrary it promotes expression of phase II enzyme (NAD(P)H:quinone) that eliminates potentially toxic chemicals. [10]
- Resveratrol induces apoptosis (Programmed Cell Death) in a number of cancer cell lines and regulates normal cell cycles. It reduces growth of invasive tumours by inhibiting angiogenesis (development of new blood vessels).
- Macrophages produce a cytokine (MIC-1) which has anti-tumorigenic activity. Resveratrol increased (MIC-1) gene expression in pancreatic cancer cells.
- The phytochemical reduced levels of steroid receptor coactivator-3 and growth factor signalling proteins causing reduced cell proliferation and increased apoptosisin colon cancer cell lines.

A study shows that the concentration of resveratrol inhibiting cell growth by 50% (IC_{50}) ranged from about 20 to 100 μM. Thus we can say that resveratrol increases the expression of genes responsible for cell survival, differentiation, proliferation inhibition and apoptosis. Resveratrol therefore has a chemo preventive and anticancer effect.

4.4. Cardiovascular Health and Resveratrol

Coronary heart disease (CHD) is one of the primary causes of death in developed countries and can be prevented by incorporating changes in one's lifestyle & diet. Resveratrol is often touted as the bioactive compound in grapes and red wine, and has particularly been associated with the so-called 'French Paradox'. Thephrase, coined in 1992 by Dr. Serge Renaud from Bordeaux University,

describes the low incidence of heart disease and obesity among the French, despite their relatively fat diet and levels of wine consumption.

Studies published in Annals of the New York Academy of Sciences showed [13] cardio- protective effects of resveratrol, and red wine with and without alcohol:

- Resveratrol inhibits oxidation of LDL, which is considered as the primary event in the initiation of atherosclerosis.
- Resveratrol suppresses proliferation of smooth muscle cells and pulmonary aortic endothelial cells. Migration and proliferation of smooth muscle cells in the intima of susceptible vessels is a requisite for atherogenesis (formation of plaques in the inner walls of arteries).
- Resveratrol is shown to inhibit platelet aggregation—platelets are actively involved in the process of haemostasis, by which injury in the vascular endothelium is rapidly repaired so that the fluidity of the blood is not compromised.

The most accepted mechanism of cardio protection by resveratrol is the inhibition of platelet aggregation. [35] Platelets can be activated by several different factors, including adenosine diphosphate (ADP), collagen, and thrombin. When activated platelets change morphology they aggregate and seal damaged blood vessels. Excessive aggregation can lead to the development of cardiovascular disease. Pre-treatment of platelets with resveratrol has been shown to inhibit lipopolysaccharide (LPS) and LPS + thrombin-stimulated platelet adhesion to collagen and fibrinogen in a non-dose-dependent manner. [37] Using in vitro and in vivo models, it has been [38] demonstrated that resveratrol inhibits ADP, collagen, and thrombin-stimulated human platelet aggregation in vitro.

The cardio protective effects of resveratrol are also attributed to its vasorelaxation properties. The vasorelaxation effects of resveratrol were examined on rat aortic rings with and without intact endothelium.[39] Pre-treatment with resveratrol resulted in a dose-related decrease in noradrenaline (NA) and phenylephrine (PE) induced contraction in endothelium intact rat aortic rings. Endothelium independent rings required higher concentrations of resveratrol before relaxation was observed. Therefore, resveratrol mediates vasorelaxation in endothelium intact and endothelium independent aortic rings via nitric oxide dependent and independent mechanisms, respectively.

The study published in Nutrition & Cardiovascular Diseases, [8] is the first

research to evaluate the acute effects of resveratrol supplementation on circulatory function, revealing that resveratrol improves flow-mediated dilation (FMD)-a marker of cardiovascular function. FMD of the branchial artery is a marker of blood vessel function and cardiovascular health, and is recognized as an independent risk factor for development of cardiovascular diseases (CVD).Impaired FMD is associated with several cardiovascular risk factors including hypertension, and obesity and is characterized by structural and functional changes to the blood vessel endothelium. The cardiovascular benefits of resveratrol include:

- Suppression of platelet aggregation
- Enhanced antioxidant status
- Increased NO(nitric oxide) availability

A key mechanism behind blood vessel endothelial dysfunction is suggested to involve the impaired release of NO causing blood vessels to constrict. Increased availability of resveratrol is suggested to increase NO production.

Due to all the above effects exhibited by resveratrol, it may be partially responsible for the correlation between increased wine consumption and decreased risk of CHDs.

4.5. Longevity

In 2003, David Sinclair and his team from Harvard added life extension to the list of possible benefits with his publication in Nature [9] resveratrol increased survival of yeast cells. Resveratrol protects cells and DNA against free radicals thereby slowing cell aging. Resveratrol was found to increase SIRT1, **(**SIRT1 is an enzyme that deactivates proteins that contribute to cellular regulation**)** activity 13-fold [34] .A study performed on short-lived seasonal fish having a life span of 13 weeks, revealed that if the fish received resveratrol in the early stages of life, their average and maximum lifespan increased considerably in a dose dependent manner [40]. An animal model study on mice was conducted, the results of which showed that when the high calorie fed mice reached old age (114 weeks), greater than 50% had died compared to less than 33% of the high calorie fed mice receiving Resveratrol. [22]

In a nutshell, resveratrol improves mitochondrial function and protects against metabolic disease by activating the SIRT1 enzyme which stimulates the generation of new mitochondria in other bodily tissue, boosting the body's metabolic rate and possibly slowing the effects of aging. From the above observations and, we infer that resveratrol extends life in multiple species. In mice, resveratrol prevents the early mortality

associated with obesity, but there is currently no experimental evidence to suggest that it can prolong life in lean, healthy animals. Although the mouse studies provide a good justification for studying the effects of resveratrol on human health, one cannot ignore the influence of factors such as interspecies differences in metabolism, genetic variation, diet, physical activity, disease, and mental health, when extrapolating from rodent models.

4.6. Anti-Toxic Effects

- Resveratrol protects liver cells from oxidative degradation caused by chronic alcohol consumption. It reduced hepatotoxicity and symptoms such as necrosis, fibrosis and inflammation were less developed.
- Resveratrol alleviated bleomycin, (a chemotherapeutic agent) induced lung injury.
- Resveratrol has a neuroprotective role against cognitive impairment and oxidative stress induced by the drug colchicine.

5. Conclusion

- Resveratrol is a polyphenolic substance possessing extensive physiological activities. However, its application is limited by light instability and poor aqueous solubility. But use of nanoemulsions based on soy lecithin/sugar esters and Tween 20/glycerol monooleate is known to increase physical & chemical stability.
- Along with peanuts and red grapes, chocolate and cocoa are said to be some of the newer sources of trans-resveratrol.
- Resveratrol exhibits antioxidant, cardio protective, chemopreventative, anti-diabetic, anti-toxic, life extension as well as other health benefits (anti-inflammatory effects, neuro-protective effects, estrogenic/anti-estrogenic properties, and modulation of cellular signal transduction pathways).
- Although well-absorbed by humans (via oral route), its bioavailability is relatively low because it is rapidly metabolized and eliminated.
- Its bioavailability is not enough to exhibit chemo preventative effects. Using piperine and deconjugation enzymes (β-glucuronidase and sulfatase) more resveratrol will be available in its free form.
- Further research should focus on translating in vitro findings regarding potential health benefits into in vivo models.

- Understanding the potential toxicity, health effects, bioavailability, and metabolism of resveratrol is necessary before dietary and supplement recommendations can be made.

- Nature-identical Resveratrol is now commercialized and marketed as nutritional supplement having anti-ageing and cardio-protective benefits by Companies like DSM Nutritional Foods under brand name Resvida.

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9. Self Assembly of Block Copolymers and its applications in Drug Delivery

Review Article

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Abstract

Block copolymers are polymeric molecules that impart strong surface activity and adsorption to the surface. They have received great attention in the past decade due to their versatile properties. Their tendency to self assemble on nanometer length scales, makes them ideal for emerging nanotechnologies. This review encompasses various aspects involved in self assembly of block copolymer micelle. Thermodynamics, phase behaviour and modelling of block copolymer micelle are main contents of the article. Final section describes about the application of these copolymers in controlled drug release.

Keywords**:** Block copolymer, self assembly, controlled drug release.

1. Introduction

Most of the industrial formulations are in form of suspensions (solid – liquid) or emulsions (liquid – liquid). An energy barrier between particles is required for the stabilization of these dispersions against flocculation or coalescence such that it prevents their close approach where the van der Waals attraction is large. The stabilization can be achieved in two ways. The first is electrostatic stabilization. It is based on charge separation and formation of electrical double layers, a surface charge compensated by unequal distribution of counter and co-ions. When two particles approach to a distance of separation h that is smaller than twice the double layer thickness, repulsion occurs due the double layers. At low electrolyte concentrations, the double layers are extended and the repulsive energy at intermediate distances becomes larger than the van der Waals attraction, producing an energy barrier that prevents approach of the particles or droplets. But as industrial

formulations mostly contain high electrolytes and the ionic surfactants, producing surface charge, do not adsorb on the surface efficiently. This stabilization can be effectively achieved by polymeric surfactants due to their self assembling tendency when dissolved in selective solvent such that it is good solvent for one block and poor for the other. Such type of stabilization is called steric stabilization. They have a strong 'anchor' chain and a 'stabilizing' chain that extends from the surface giving a layer thickness of several nanometers.

The simplest type of a polymeric surfactant is a homopolymer (formed from same repeating units), but they show low surface activity at the oil/water interface. However, they have good adsorption capacity. A small variation in a typical polymer molecule opened door for wide range of applications. Attaching two different homoploymers in a certain fashion lead to synthesis of block copolymers. A block copolymer is a linear arrangement of blocks of varying composition (Figure 1):

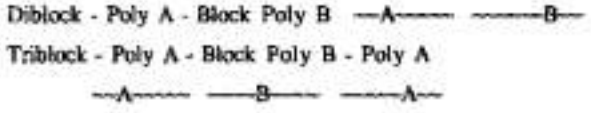

Figure 1[11]: Block Copolymers (A: Hydrophilic chain and B: Hydrophobic chain)

Since block copolymers are amphiphilic in nature, they aggregate in solution to form micelles. These molecules can be applied as emulsifiers and dispersants with the hydrophobic chain residing at the hydrophobic surface, leaving the hydrophilic chain dangling in aqueous solution (providing steric stabilization). They are widely used in dyestuffs, paper coatings, inks, agrochemicals, pharmaceuticals, personal care products, ceramics, and detergents.[1][2][3][12]

2. Synthesis and Properties

A triblock copolymer is synthesized by sequential addition of first the hydrophobic monomers and then hydrophilic part in presence of the alkaline catalyst. The cloud point, the temperature at which the copolymers phase separate from water, increases with increase in the hydrophilic content of the block copolymer. The rate of dissolution decreases as the hydrophilic content increases, due to increase in hydrogen bonding. Higher hydrophobic content leads to decrease in foaming ability. [4][7]

3. Micelle formation in Block Copolymer Aqueous Solutions

A micelle is an aggregate of surfactant molecule in a solution that is responsible for dispersion of two immiscible phases. Micellization or micelle formation is a

thermodynamically driven process. A typical block copolymer micelle consists of a core and a shell with core consisting of hydrophobic part and shell consisting of hydrophilic part. [4] (Figure 2)

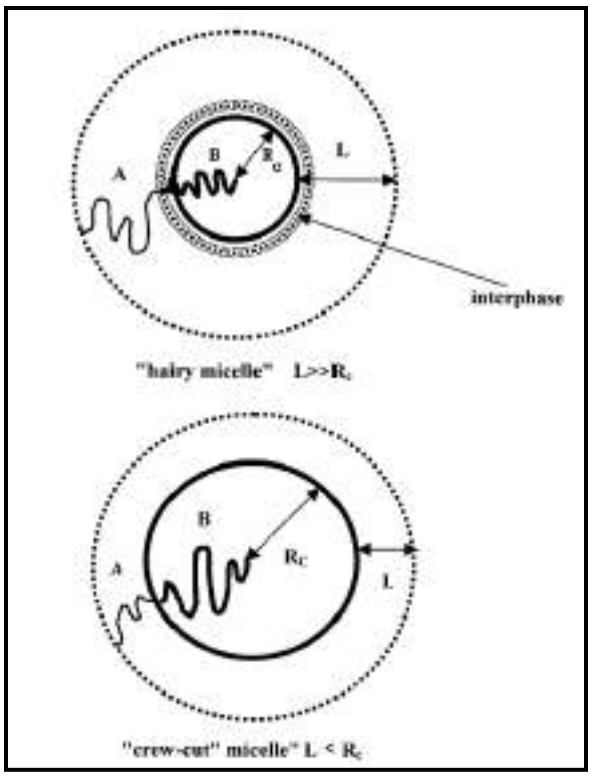

Figure 2 [7]: Schematic representation of AB diblock copolymer micelles in a selective solvent of the A block. Rc: core radius; L: shell (corona) thickness.

The critical micellization concentration (CMC), the amphiphile concentration at which micelles (thermodynamically stable polymolecular aggregates) start forming, is a parameter of great importance. The micellization of amphiphilic block copolymers is inherently more complex than that of conventional, low-molecular weight surfactants. The CMC of aqueous block copolymer solutions decreases with increasing temperature. Micellar growth increases with increase in the copolymer concentration at a particular temperature.

The CMC values of block copolymers (at a given solution temperature) decreased with increasing number of hydrophobic segments, indicating that polymers with a larger hydrophobic domain form micelles at lower concentrations. Higher temperatures resulted in lower CMC values. [4]

4. Thermodynamics

The block copolymers of the A-B or A-B-A type form micelles in selective solvents which are thermodynamically good solvents for one block and precipitants for the other. The standard free energy change for the transfer of 1 mol of amphiphile from solution to the micellar phase, ΔG (the free energy of micellization), in the absence of electrostatic interactions is given by:

$$\Delta G = RT \ln(X_{CMC})$$

where R is the gas law constant, T is the absolute temperature, and X_{CMC} is the critical micellization concentration in mole fraction units. This is the governing equation for micellization.

Obtaining ΔG, ΔH and ΔS values for certain block copolymers results that ΔH is a positive value, indicating that the transfer of unimers from solution to the micelle is

an enthalpically unfavorable endothermic process. The free energy, ΔG^0, is negative, since thermodynamically stable micelles are formed spontaneously. Thus, it becomes clear that a negative entropy contribution must be the driving force for micellization of the block copolymers. The traditional view of micelle formation is based on the "hydrophobic effect . The presence of hydrocarbon molecules in water causes a significant decrease in the entropy of the latter, suggesting an increase in the degree of structuring of the water molecules. When hydrocarbon residues aggregate in aqueous solution to form a micelle, the hydrogen bonding structure in the water is restored and the water entropy increases, overcoming the entropy loss due to the localization of the hydrophobic chains in the micelles. The entropy contribution usually dominates the micellization process in aqueous surfactant solutions, with the enthalpy playing a minor role. [4][7]

5. Phase Diagram

The phase behaviour of block copolymers dissolved in water was studied using small-angle neutron scattering and dynamic light scattering. At low temperature ($T \leq 15\ ^0$C) and low polymer concentrations, the unimers were fully dissolved gaussian chains with radius Rg = 1.7 nm. Close to ambient temperature, the hydrophobic part causes aggregation of the polymers into spherical micelles with core sizes of the order of 4-5 nm, somewhat temperature dependent. The core size increased with decreasing hydrophilic block size and with increasing temperature. The copolymer with the largest hydrophilic block aggregated in micelles with a core diameter which, within the whole temperature regime, was smaller than the length of a stretched hydrophobic chain. Micelles formed by copolymers of intermediate hydrophilic size had a core diameter which at high temperature approached the size of a fully stretched hydrophobic chain, thus causing an abrupt change from a spherical to a rod-like structure. The concentration of micelles increased roughly linearly with temperature until either saturation was reached (where all polymers were part of a micelle) or the volume density of micelles was so high that they "locked into a crystalline structure of hard spheres. In the 60-70 ^{0}C temperature range, the micellar structure changed from spherical form to prolate ellipsoid, leading to a decreasing intermicelle interaction. At high copolymer concentration this caused melting of the cubic lattice and led

successively to the formation of a rod-like structure with hexagonal symmetry. Large aggregates of block copolymers ordered in lamellae structure were formed close to 95°C, leading to an opaque suspension. The phase diagram of PEO-PPO-PEO copolymer in water is presented in Figure 3, in which the concentration-temperature regions where the different aggregates (discussed in the previous paragraph) exist are also depicted. [4]

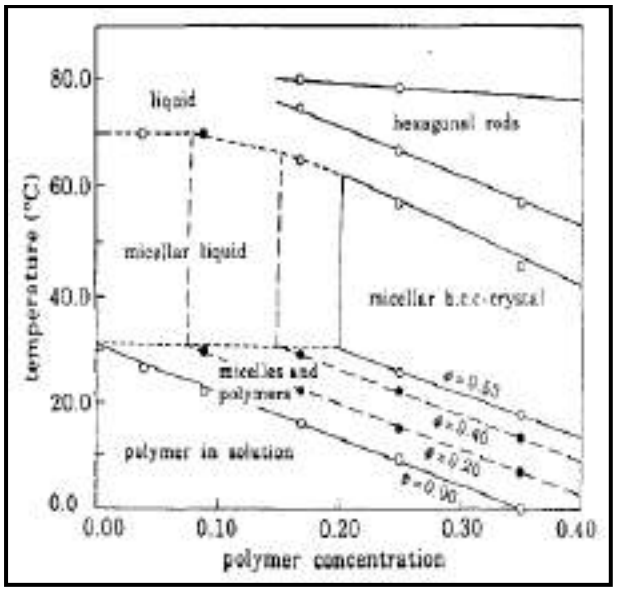

Figure 3[4]: Phase diagram of aqueous solution of bock copolymer, showing the fully dissolved polymers (unimers) at low temperatures and concentrations, the CMC-CMT line (¢=0), the $¢_o$=0.53 transition to b.c.c, micellar crystal, and the high temperature hexagonal phase (¢ is the hard-sphere micelle volume fraction). At T~ 60°C a liquid phase appears between the crystalline b.c.c, and hexagonal phases.

6. Modelling of self assembly

Block copolymer in the solution tend to self assemble and form microphases. In selective solvent, the bad solvency condition for one type of monomer and good one for the other type is important for self assembly along with repulsive interactions between monomers on different blocks. The structure and symmetry of micelles/ aggregates formed depends on relative strength of interactions as well as the composition and the architecture of block copolymer. Following is an overview of different theoretical treatments of the self assembly of block copolymers in selective solvent.

The concept of the scaling theories is to establish on the basis of simple model the correlations between the molecular characteristics of a given block copolymer, mainly diblock copolymers AB, and the characteristics, such as the core radius Rc ; the corona thickness L and the aggregation number Z of the resulting micelle in a selective solvent for one of the blocks. In this approach, monodispersed AB diblock copolymers are generally considered, where N_A and N_B are the number of A and B monomer units in the corresponding blocks.

Assuming uniformly stretched chains for the core radius Rc; with an aggregation number Z; the following relationships are predicted

$R_c \sim \gamma^{1/3} N_B^{2/3} a$

$Z \sim \gamma N_B$

where γ is the A/B interfacial tension and a the segment length.

In the second case, that of hairy micelles, the star polymer theory of Daoud and Cotton can be applied. These authors defined for star-like polymers in good solvents the segment density profile as a function of the distance of the core center. Their model predicts that the star polymer radius scales as

$R \sim N_A^{3/5} f^{1/5}$

where f is number of arms. As in a block copolymer micelle the number of arms corresponds to the aggregation number Z; it follows that $L \sim Z^{1/5} N_A^{3/5}$ with $Z \sim N_B^{4/5}$.

The application of the scaling concepts to the description of the polymer concentration profiles and free energy in micellar systems is largely restricted to long polymer chains in good solvents. In fact scaling models presented above are unable to include finite chain effects and polymer/solvent interactions. Furthermore, numerical values of the micellar characteristics are not directly accessible, as the scaling laws only predict the trends, e.g. how a given micellar parameter scales with a given copolymer parameter. The scaling models have thus to be complemented by more detailed mean-field calculations and molecular simulations.

The development of the self-consistent meanfield formalism provided the means to calculate the polymer concentration profiles in a relatively tractable form. Two approaches were considered, one by semi-analytical mean field models and other by numerical self-consistent mean field description. In the first approach, micellar characteristics were derived by minimizing the Gibbs energy of an isolated micelle using numerical values of the Flory–Huggins interaction parameters x; molecular weight and composition of the copolymer.

Complementary to these methods, computational simulations are used to study self assembly of block copolymers. The computer simulation proceeds with relatively very few approximations and without presumption of micelle geometry or chain conformation. It is possible also to vary intermolecular forces at will in a well-controlled manner. The main limitation of the simulation is the requirement for extensive computation and therefore simulations are mostly reported for 'short' block copolymers, e.g. with N_A or N_B from 2 to about 30. [2][4][7]

7. Application of self assembled Micelles in Drug Delivery

Many important therapeutic compounds exhibit poor aqueous solubility, rendering

delivery of those agents quite challenging. The development of effective delivery systems is crucial to the success of future drugs, which may include larger and more sophisticated synthetic compounds as well as complex natural molecules. The functional properties of micelles based on amphiphilic block copolymers render them ideal for encapsulation and delivery of hydrophobic drugs. During the micellization process, the hydrophobic blocks associate to form the core region, whereas the hydrophilic segments position between the core and the external aqueous medium. Hence, the hydrophobic core is stabilized by the hydrophilic shell, which serves as an interface between the bulk aqueous phase and the hydrophobic domain. This unique architecture enables polymeric micelles to serve as nanoscopic depots or stabilizers for poorly water-soluble compounds. These molecules are biologically stable. Nanocarriers with insufficient stabilities tend to break up and be removed rapidly from blood by kidneys. The molecular weight of polymeric micelles (10^6 g mol^{-1}) prevents renal elimination unless the micelle structure dissociates to unimers. Supramolecular structures with sufficient stability often end up accumulating in the liver and spleen due to a large size or protein adsorption, both triggering a rapid uptake by the reticuloendothelial system (RES). Delivery systems that are smaller than 200 nm have low uptake by RES and may circulate in blood for prolonged periods. Polymeric micelles usually range in size between 10 and 50 nm. Based on the results obtained for other colloidal delivery systems, the nanoscopic size is expected to facilitate the discharge of polymeric micelles at leaky sites of capillaries, e.g. tumours and sites of inflammation.

Sustained release of drugs for polymeric micelles can be achieved by chemical or physical means. The stability of the micellar structure is a prerequisite for control over the rate of drug release. For drugs physically encapsulated in stable structures of polymeric micelles, release is controlled by the rate of drug diffusion in the micellar core or break up of the micelles. The diffusion rate may be quite low if a favourable interaction exists between the solubilizate and the core-forming block in a rigid core. The physical state of the micelle core and encapsulated drug plays an important role. The localization of the solute in the core / shell structure, micellar size and molecular volume of the drug are among other factors influencing the rate of drug diffusion in the polymeric carrier. [5][6][8]

7.1 Micelle-forming for Drug Delivery

There are three different types of drug delivery systems:

7.1.1. Micelle-forming block copolymer–drug conjugates.

7.1.2. Micellar nano-containers.

7.1.3. Polyion complex micelles.

Micelles based on poly(ethylene oxide)-block- poly(L-aspartate), PEO-b-p(L-Asp), have been used extensively for drug delivery. Because of the carboxyl functionality, p(L-Asp) block, PEO-b-p(L-Asp) can be used for the chemical conjugation of drugs. This approach is appealing for the delivery of highly cytotoxic chemotherapeutic agents. In this case, polymer/drug conjugates are assembled into micelles, enabling drug molecules to be protected in the micelle core until the carrier vehicle accumulates at solid tumour sites. This strategy may minimize premature drug release and nonspecific action toward healthy cells, yet allowing for drug release in tumour tissues. [5] See Figure 4.

Micellar Nanocontainers

A more attractive approach is physical encapsulation of drugs within polymeric micelles since many polymers as well as drug molecules do not bear reactive functional groups, e.g. carboxyl, hydroxyl or amino groups, for chemical conjugation or the free functional site may be required for the pharmacological effectiveness of the drug.

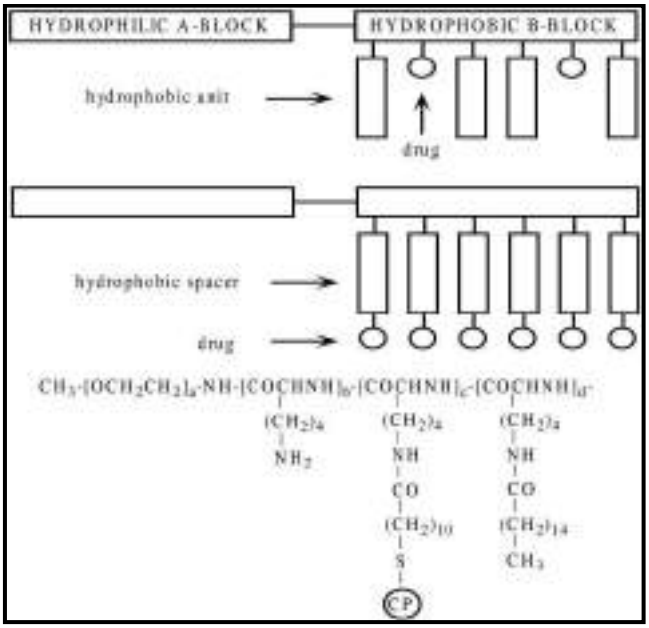

Figure 4[5]: First models of micelle-forming block copolymer–drug conjugate

In addition, conjugates of drugs may exhibit markedly dissimilar biological properties relative to parent drugs, leading to inherent difficulties in characterization and regulatory approval even for already approved drugs.

Physical encapsulation of drugs in the polymeric micelles is usually carried out through dialysis or O/W emulsion methods. In the dialysis method polymer and drug are both dissolved in an organic solvent. The solution is then dialyzed against distilled water to remove the free drug and organic solvent. In the O/W emulsion method, drug is dissolved in a volatile solvent, which is also immiscible

with water, such as chloroform, and added to an aqueous solution of polymeric micelles. The mixture is homogenized by sonication and chloroform is evaporated in an air open system. Free drug is removed by ultra-filtration. The choice of organic solvent and loading process seem to be important factors affecting micellar stability, size and extent of encapsulation. [5]

Polyion Complex Micelles

Depending on the type of amino acid, PEO-b- PLAA block copolymers may bear positive or negative charge at their side chains. Therefore, oppositely charged macromolecules such as DNA or peptides can form polyion complexes with the PLAA segment of the block copolymer, neutralize the charge and induce required amphiphilicity for micellization of the complex. The incorporation of DNA and peptides in polymeric micelles may lead to stabilization against digestive enzymes such as nuclease and facilitate their penetration in cells. [5]

8. Conclusion

The micellization of block copolymers is a unique example of self assembled nanoparticles. The phase behavior and aggregation properties of block copolymers in solution, as affected by the copolymer molecular composition and concentration, additives, and solution temperature, are important in understanding the mechanism of copolymer action underlying the various applications.

In drug delivery, polymeric micelles have huge potential. The thermodynamically stable micelle structure and its nanoscopic size are major advantages of block copolymers over other delivery agents. The effect of variations in chemical structure of polymeric micelle on drug delivery is currently the area of research.

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10. Polymers for Solar Energy Storage

Review Article

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Abstract

Energy storage is the need of the hour. We need to conserve our resources to avoid a "power-less future. The article discusses the use of polymeric materials for solar energy storage and release applications. Mainly focusing on Phase Change Materials (PCMs) as a reliable method of solar energy storage, some other methods have also discussed.

Keywords: Solar energy, PCMs, polymers

1. Introduction

Due to rapid world-wide development, and growing population, the increase in energy demand is inevitable. At the current global energy consumption rate, it is just a matter of time before our energy resources will be exhausted. The need of the hour is to develop high performance, low cost and environment friendly energy conversion and storage systems. This is where application of polymers steps in!

Energy is available in various forms. This energy has to be efficiently managed to make it available at appropriate times and locations, both to balance generation with consumption and to maintain the energy grid stability. For example, wind and solar power installations generate power only intermittently and with a highly variable output. When the wind is blowing or the sun is shining, excess power should be stored and made available during suboptimal generating conditions or during peak demand. This requirement has led to greater demand for alternative energy storage facilities to support the grid.

Thus, energy storage plays a vital role in preventing energy loss and also helps utilizing the energy more efficiently. Most applications require that the energy is stored only for a short period like few

hours; however, long term storage of a few months may also sometimes be needed.

When we talk of energy storage, the first thing that clicks, and rightly so, is the battery. This was invented in the 19th century, a time around which the electrochemical devices, fuel cells, were also developed. These fuel cells were light-weight, non-thermal sources of electricity that were required during manned spaceflights. With the passage of time these cells have been getting upgrades to increase the conversion efficiency of chemical energy to electrical energy. Several other technologies have also been investigated such as flywheels, which can store kinetic energy and compressed air storage, which can be pumped into underground caverns and storage. Another method of energy storage is the use of molten salts to store solar energy and deliver it as needed. The system pumps molten salt through a tower that is heated by the Sun's rays. There are a number of other such examples.

The conventional energy storage methods can be summarized as follows (Table 1), categorized based on the type of energy stored:

Table 1: Conventional Energy Storage Methods

Sr. No.	Energy	Storage-Types
1.	Chemical	Hydrogen/ Bio-fuels/ Liquid Nitrogen/ Oxyhydrogen/ Hydrogen Peroxide
2.	Biological	Starch/ Glycogen
3.	Electrochemical	Batteries/ Flow batteries/ Fuel cells
4.	Electrical	Capacitor/ Supercapacitor/ Superconducting magnetic energy storage
5.	Mechanical	Compressed air energy storage/ Flywheel energy storage/ Spring/ Gravitational Potential energy
6.	Thermal	Ice storage/ Molten salt/ Cryogenic liquid air or nitrogen/ Seasonal thermal store/ Solar pond/ Hot bricks/ Steam accumulator/ Fireless locomotive/ Eutectic systems

Taking into account today's increasing energy crisis situation, it becomes increasing necessary to tap the renewable energy sources. The fossil fuels are depleting rapidly. Also their use leads to emissions of large amounts of green house

gases have several global impacts. Renewable energy sources like solar energy, wind energy, tidal energy, etc. are all clean forms of energy. The problem is that their use is limited due to time dependence and hence storage is a must. Polymers have proven to be effective materials for energy storage and delivery. Besides renewable energy sources, energy from non-renewable sources can also be stored.

2. Polymers for Energy Storage and Delivery

The demand for increasing renewable energy resources implies that storage of energy is a must. The fossil fuel reserves are depleting rapidly and the need of the hour is not only harnessing of cleaner, greener fuels but prevention of loss of any form of energy. Thus, we need to capture and store energy as far as possible.

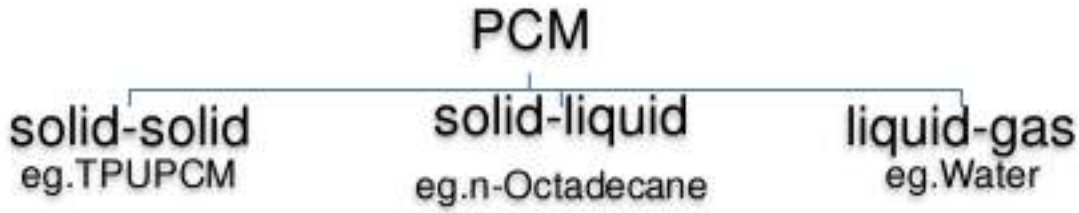
2.1. Solar Energy: The use of PCMs (Phase Change Materials)

Solar energy is one of the most and abundant widely available energy resources to man: available throughout the Earth's surface. This reserve needs efficient and economic tapping. The main problem with conventional solar energy harnessing methods is that the captured energy has to be used immediately. This limits the amount of energy that we can capture. However, with the development of effective methods to store this energy it is possible to increase the quantum of energy harnessed. By storage, we can capture solar energy during the day, when the radiant energy received is at its peak and the excess energy collected can be used at night, when the solar power received is negligible. The total rate at which energy enters the Earth's atmosphere is estimated at 174 petawatts. This is equal to the product of the solar constant, about 1,366 watts per square meter, and the area of the Earth's disc as seen from the Sun, about 1.28×10^{14} square meters, averaged over the Earth's surface, which is four times larger. (i.e. the area of a disc with the Earth's diameter, which is effectively the target for solar energy, is 1/4 the area of the entire surface of the Earth.) The solar flux averaged over just the sunlit half of the Earth's surface is about 680 W/m^2. This is a substantial amount of energy which can be efficiently used to reduce the energy crunch. Polymeric materials are well suited for this purpose, especially in the form of PCMs.

2.1.1. What is a PCM?

PCM or Phase Change Materials are those materials which are capable of storing large amounts of latent heat energy with

little or no temperature change, and can also release this energy, in a suitable environment, when required. These PCMs can be of the following types:



PCMs use chemical bonds to store and release heat by phase transition. Liquid-gas PCMs are not of much practical importance due to large volume change on phase change. The solid-liquid PCMs are susceptible to leakage on phase change. Hence these are not very economical since they require containment- use containers or microencapsulations which are expensive. Properties of polymers making them good PCMs:

The phase transition temperatures for many polymers are viable for application in around the range of 15-90°C

- The quantum of latent heat stored is significantly high
- Show little or no volume change upon phase change
- The phase transition occurs at a constant temperature
- Have good reliability over several thermal cycles i.e. their thermal properties do not vary much over repeated cycling
- High energy storage density

Poly-ethylene glycol (PEG) is an attractive material to be used as a polymer backbone. It has a suitable phase change temperature, high latent heat capacity, melts uniformly at constant temperature, is non-toxic in, does not undergo super cooling, has low vapor pressure (leads to less loss) and has little or no volume change during the solid-liquid phase change. Depending on the molecular weight of PEG used, the thermal properties are seen to vary slightly. PEG can be used to synthesize thermoplastics, polyurethane solid-solid PCM, named as TPUPCM, one such of which is synthesized from THCD [bis(1.3-duhydroxypropan-2-yl)4,4' methylenebis(1,4-phenylene) dicarbamate].

The TPUPCM has good thermal and mechanical properties. The thermal stability is good, repeated thermal cycling studies [1] show very slight variation in the thermal properties over a thousand cycles. This indicates that it has great potential for thermal energy storage.

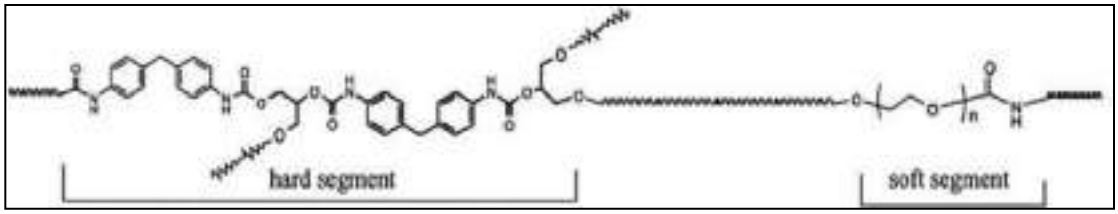


Figure 1: The structure of TPUPCM [1]

Table 2: Thermal Properties of TPUPCM

Sample	$\Delta H_{Heating\ Cycle}$ J/g	$T_{p,\ Heating}$ °C	$\Delta H_{Cooling\ Cycle}$ J/g	$T_{p,\ Cooling}$ °C
TPUPCM2000	97.5	56.3	88.9	31.8
TPUPCM4000	126.4	55.8	112.4	33.4
TPUPCM6000	137.4	57.1	127.6	31.2
TPUPCM8000	127.1	57.5	115.2	30.1

Trying to capitalize on the suitable properties of PEG, a number of PCMs are being developed. Polymer PCMs are now under use to store and release energy, by incorporating them in building structures, to improve the thermal comfort within [1]. PEG has a property: it can directly be incorporated into porous materials. Making use of this, PEG/ diatomite composite was synthesized and is seen to have good thermal properties for the above mentioned application. Diatomite is a white crumbly, porous rock. It is light weight, rigid and inert. These properties coupled with the fact that it is abundantly available and relatively cheap make it a very viable energy storage material. According to studies [2] carried out, this composite has good thermal properties and with very slight variation over 1000 thermal cycles.

Table 3: Thermal Properties of PEG/diatomite composite

Sample	$\Delta H_{Heating\ Cycle}$ J/g	$T_{p,\ Heating}$ °C	$\Delta H_{Cooling\ Cycle}$ J/g	$T_{p,\ Cooling}$ °C
PEG(50wt%)/ diatomite	87.09	27.7	82.22	31.8

This polymer composite can be applied for temperature regulation and reduce the overheating in buildings. This composite used as energy storing wallboard/ plaster

stores extra energy thus reducing cooling requirements in the premises.

Talking of energy storage applications in buildings, another set of polymers that have made their ground are those with a palmitic acid backbone. Palmitic acid has the advantage of a suitable phase change temperature in the range 58-64°C besides having other prerequisites. One such polymer is the palmitic acid/ expanded graphite composite PCM. It has high energy storage density with excellent thermal reliability as studies [3] suggest. For a Palmitic Acid/ Expanded graphite (80/20 w/w%) composite the thermal properties are in Table 4 below.

Table 4: Thermal Properties of PA/Eg composite with thermal cycling

Cycle No.	$\Delta H_{Heating\ Cycle}$ J/g	$T_{p,\ Heating}$ °C	$\Delta H_{Cooling\ Cycle}$ J/g	$T_{p,\ Cooling}$ °C
0	148.36	60.88	149.66	60.81
1000	147.39	60.85	146.55	60.98
2000	151.81	60.94	150.58	60.84
3000	140.38	60.78	139.97	60.98

In recent years, several other polymeric solid-solid PCMs have been synthesized for energy storage and release. Another such one is polystyrene-graft-palmitic acid copolymer. The advantages of these are as follows:

- Fairly good thermal properties (enthalpy and transition temperature)
- Good thermal conductivity (which would mean the device using this material will be more compact)
- Good thermal reliability and stability.

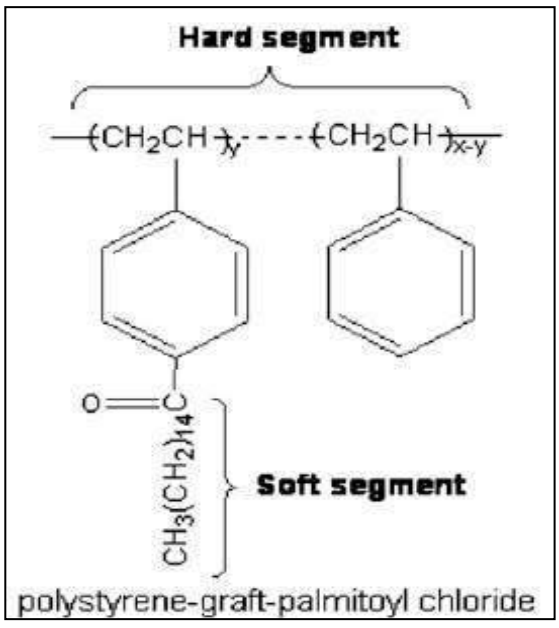


Figure 2: The structure of polystyrene-graft-PA-copolymer [4]

The thermal properties as reported in literature [4] are as in Table 4. The effect of varying the palmitic acid percentage on these properties can also be seen as follows:

Table 5: Thermal Properties of polystyrene-graft-PA-copolymer

Sample	$\Delta H_{Heating\ Cycle}$ J/g	$T_{p,\ Heating}$ °C	$\Delta H_{Cooling\ Cycle}$ J/g	$T_{p,\ Cooling}$ °C
25% mol PA	26.20	21.47	20.02	17.65
50% mol PA	31.16	18.72	28.04	18.51
75% mol PA	39.78	19.18	39.19	18.72

The excellent thermal reliability of these materials makes them ideal phase change materials. Even after 5000 thermal cycles, the variation in properties is minimal [4]. E.g. For 75% Palmitic Acid copolymer, less than 1% change was observed in the enthalpy value.

Almost all polymer material matrices could be tweaked so as to form a stable PCM. The electrospinning technique provides a viable and versatile method to prepare ultrafine fibers of diameters ranging in the nano-micrometer range (Provides for greater surface area for energy storage applications). New techniques such as coaxial electrospinning allow for fibers with a hydrocarbon core and a TiO_2 or polyvinylpyrrolidone sheath (McCann et al., 2006). An important example in this class of polymers is polyamide6 (PA6). This material has good properties such as high strength and toughness, low coefficient of friction and good chemical resistance. Lauric acid acts as a composite material for the PCM (taking into account that PA6 has a higher melting point than Lauric Acid, PA6 makes for a good supporting matrix). For fibers of approximately 1 micro meter diameter, studies [5] were carried out for various composition of the fibers and the results are tabulated as follows. The first column indicates the mass ratios of the components of the composite.

Thus PCMs find major applications in solar energy storage, waste heat recovery, building energy conservation [e.g. use of porous building materials to micro-encapsulate polymers for use as PCM (Li et al., 2009)], temperature control for greenhouses, in kitchen utensil for electric energy storage, clothing thermal insulation and so on. These are next generation materials for thermal energy storage and

retrieval. Below is a comparative plot for the various polymeric solar energy materials discussed. It summarizes their working temperature and enthalpy values.

Table 6: Thermal Properties of PA 6/LA composite fibers

Sample	$\Delta H_{Heating\ Cycle}$ J/g	$T_{p,\ Heating}$ °C	$\Delta H_{Cooling\ Cycle}$ J/g	$T_{p,\ Cooling}$ °C
LA/PA680/100	63.66	44.57	54.64	40.28
LA/PA6100/100	70.44	44.53	57.14	40.67
LA/PA6120/100	73.01	44.87	58.35	40.57
LA/PA6150/100	74.12	44.71	62.48	40.09

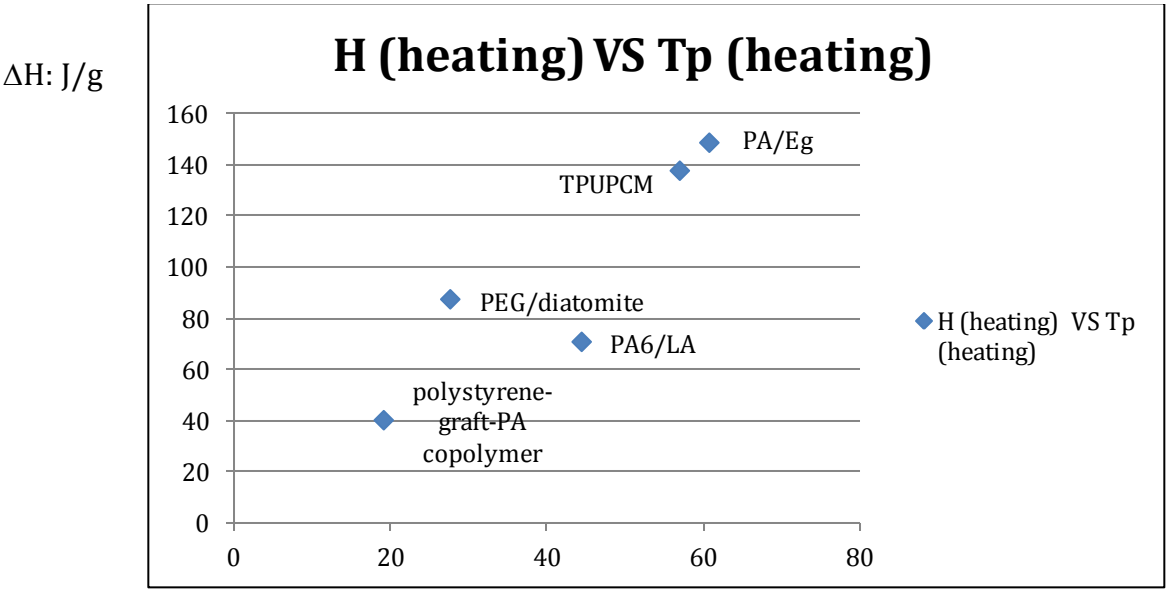


Figure 3: A comparison of PCMs [1],[2],[3],[4],[5]

After looking at PCMs, it is worthwhile looking at another class of polymers that are capable of solar energy storage but function differently. These polymers work using "Valence Isomerization.

This method has proven itself to be one of the most promising systems for the harnessing of solar energy – its conversion and storage. A good example of this is norbornadiene in polymer systems [6]. The wavelength of sunlight received by us ranges between 300-700 nm. This energy cannot be harvested directly by norbornadiene – we have to introduce auxiliary groups like chromophores, sensitizers etc. The structure variation during energy storage has been studied [7] and is in the figure below:

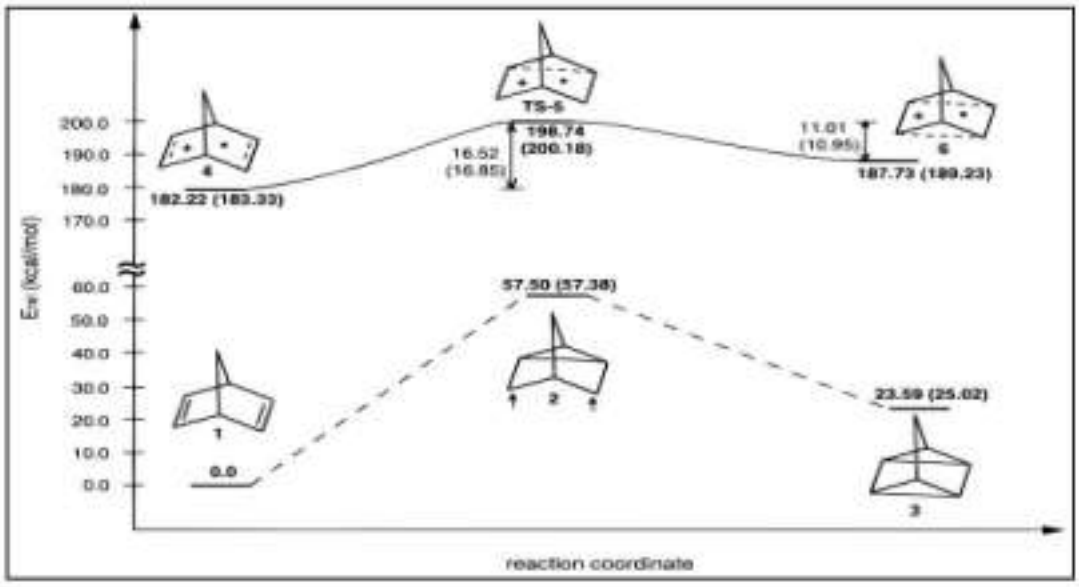


Figure 4: Valence Isomerization as seen in Norbornadiene[7]

3. Conclusions

Energy storage is the need of the hour. We need to conserve our resources to avoid a "power-less" future. As discussed and cited in the report, it is rather clear that polymeric materials are very capable materials for energy storage and release.

In solar energy harnessing, the implementation of proper energy storage remains crucial to achieve energy security and to reduce environmental impact. No because of the lower mass and volume of the system and the energy is stored at a relatively constant temperature and energy losses to the surrounding are lower than with conventional systems. These

phase transformation is associated with a significant volume change. This increases the working cost using these polymers as

single type of storage method can be used universally to store energy. For specific situations, geological locations (temperature being a major factor), and existing facilities, different storage methods are possible and must be considered. PCMs store latent heat within their structure. The quantum of energy stored for organic PCMs ranges from 10kJ/kg to 300kJ/kg. Energy storage in PCMs has a lot of advantages over conventional systems

materials are viable to use provided their peak phase change temperature is within practical limits (15-90°C). In certain PCMs (solid-liquid & especially liquid-gas), the

we need to take into account a variable volume system in our design. Liquid PCMs are susceptible to leakages –

microencapsulation technique can be applied to prevent the same. PCMs have varied applications from heat and coolness storage in buildings to thermal storage in satellites and protective clothing. Recently, many developments in latent heat storage have been observed, mainly because the utilization of solar and industrial heat is a promising method to save energy and reduce CO_2 emission. Incorporating these materials into building structures during construction phases can reduce the energy requirement for years together as solar heat stored can be used for electrical purposes or even for warming the structure during later hours of the day.

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Psychiatric Pharmacotherapy

A Review

Prof. (Mrs) Malathi R. Baichwal Visiting Fellowship Lecture

December 22, 2012

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Introduction

- Currently there is no cure for mental illness.
- Globally the estimated prevalence of Schizophrenia (about 1.5%), Depression (about 16%), and Bipolar (about 1.5%).
- Pharmacological treatment, in addition to being prohibitively expensive, is often complicated by serious side effects, drug-drug interactions, and drug-induced co-morbidities.
- Compliance is the single primary factor in successfully controlling the symptoms

Major Manifestations of Mental Disorder

- Schizophrenia
- Depression
- Bi-polar
- Anxiety Disorders

Global attitudes toward Mental Illness

Most of us are familiar with either a member of our family, or a neighbor who used to talk to themselves, or behave oddly in different ways; they were usually left alone and looked after as long as they are not a threat to themselves or others. Of course, the other aspect of this is mental illness is considered a stigma on the family, and most families try to hide it. Unfortunately that means the patient goes untreated, and eventually things would only get worse.

For about 30 years, Americans have been exporting their ideas of mental illness; their definitions and treatments have become the international standards.

The Diagnostic and Statistical Manual of Mental Disorders (DSM), published by the American Psychiatric Association provides a common language and standard criteria for the classification of mental disorders.

In 1994, DSM-IV was published**,** listing 297 specific mental disorders in 886 pages. DSM – V is scheduled for publication in May 2013.

DSM IV Multi-axial system

The DSM-IV organizes each psychiatric diagnosis into five dimensions (axes) relating to different aspects of disorder or disability. For example,

Axis I – Major Mental Disorders	Axis II – Major Personality Disorders
Schizophrenia	Paranoid personality
Depression	Schizoid personality
Bipolar	Borderline personality
Anxiety	Antisocial personality
ADHD	Narcissistic personality
Autism	Histrionic personality
Anorexia	Avoidant personality
	Dependent personality
	Obsessive-compulsive personality

Axis III: Medical / Neurological such as brain damage, HIV etc.

Axis IV: Psychosocial stressors (death in the family, unemployment, etc.)

Axis V: Current highest level of functioning.

The Nature of Mental Illness

Neurotransmission

The nervous system is composed of billions of specialized cells called neurons. Efficient communication between these cells is crucial to the normal functioning of the central and peripheral nervous systems. Neurons allow the brain to communicate with the rest of the body through the propagation of waves of depolarization, known as action potentials.

The transmission of information is accomplished in two ways:

Electrically: the neuron is directly adjacent to other neurons. As the action potential reaches the end of the axon, the depolarization continues across the membrane to the postsynaptic neuron directly.

Chemically: there is a space (the synaptic cleft) between the axon terminus and the adjacent neuron. As the action potential reaches the end of the axon, a chemical is released that travels across the synaptic cleft to the next neuron to alter its electric potential.

With very few exceptions, mammalian organisms use chemical means to transmit information.

Conduction

To begin conduction, an action potential (an electrical signal in which ions, which are electrically charged particles, move across the neuronal membrane) is generated near the cell body portion of the axon.

An action potential ends at the axon terminals. Axon terminals are where neurotransmission begins.

At electrical synapses, the OUTPUT will be the electrical signal itself. At chemical synapses**,** the OUTPUT will be neurotransmitter.

Neurotransmission

Neurotransmission (or synaptic transmission) is communication between neurons, accomplished by the movement of chemicals across a synapse.

A resting neuron has a negative charge. That is, there are more negative ions inside the axon than outside the axon. (Ions are molecules with an electric charge.) In contrast, the fluid outside the

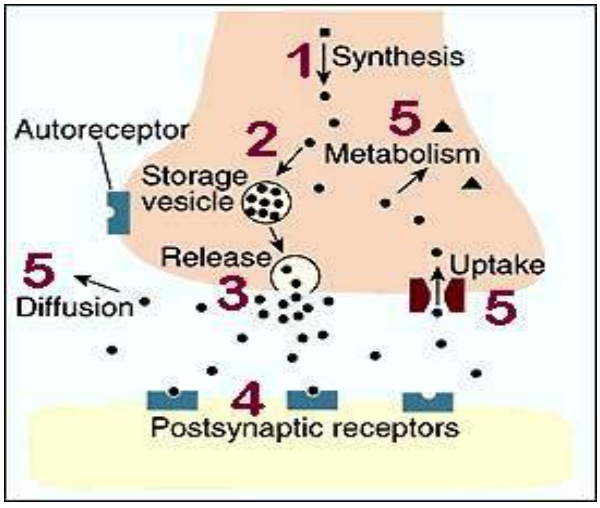
axon has a positive charge. Because the outside and inside of the axon have different charges, the axon is said to be polarized.

When a neuron is excited or fires, several events take place to create an electrical impulse. Sodium ions, which have a positive charge, enter the axon. This depolarizes the axon-that is, changes the electrical charge inside the axon from negative to positive. This change starts at one end of the axon and continues all the way to the other end. In response to this electrical impulse (called an action potential), the vesicles swarm to the very edge of the axon and release neurotransmitters into the synapse.

After the neurotransmitters are released, potassium ions flow out of the axon. Potassium ions have a positive charge, so their absence restores the negative charge inside the axon. The neuron is again polarized and at rest, waiting to fire another impulse.

In chemical neurotransmission, the pre-synaptic neuron and the post-synaptic neuron are separated by a small gap — the synaptic cleft. The synaptic cleft is filled with extracellular fluid (the fluid bathing all the cells in the brain). Although very small, typically on the order of a few nanometers (a billionth of a meter), the synaptic cleft creates a physical barrier for the electrical signal carried by one neuron to be transferred to another neuron. In electrical terms, the synaptic cleft would be considered a "short in an electrical circuit. The function of neurotransmitter is to overcome this electrical short. It does so by acting like a chemical messenger, thereby linking the action potential of one neuron with a synaptic potential in another.

Chemical neurotransmission



Steps involved in chemical neurotransmission

Step 1: Neurotransmitter Biosynthesis

Step 2: Neurotransmitter storage

Step 3: Neurotransmitter release into synaptic/junctional cleft

Step 4: Interaction with neurotransmitter receptors

Step 5: Termination of neurotransmitter action (uptake, metabolism)

Synaptic vesicles contain Neurotransmitters (chemical substances which ultimately cause postsynaptic changes in the receiving neuron). Common neurotransmitters include:

- Acetylcholine
- Dopamine
- Nor-epinephrine (a.k.a., nor-adrenaline)
- Serotonin (5-HT)

Whether due to genetics, drug use, aging process, or other various causes, Biological dysfunction at any of the four steps of synaptic transmission often leads to imbalances and is the ultimately source of conditions such as schizophrenia, Parkinson's disease, and Alzheimer's disease.

Schizophrenia

Schizophrenia is a heterogeneous, psychotic thought disorder characterized by a mix of symptoms including delusions, hallucinations, disorganized speech or disorganized / catatonic behavior.

It is characterized by Positive Symptoms and Negative Symptom.

Positive Symptoms (symptoms that are present, but should not be)**:**

- Suspiciousness
- Hyperactivity
- Delusions (fixed, false beliefs, beliefs without a basis in reality)

- Hallucinations (hearing, feeling, tasting, or smelling things that are not there; and very commonly, hearing voices
- Conceptual disorganization (difficulty in speaking or organizing thoughts and paying attention

Negative Symptoms (symptoms that are absent, but should not be):

- Alogia: **(**poverty of speech, inability to speak because of mental deficiency, mental confusion
- Avolition**:** (literally meaning "poverty of will or motivation", characterized by general lack of drive, or motivation and may sit still for long periods of time)
- Anhedonia**:** (inability to take pleasure in usually pleasurable activities. Nothing is fun — not eating, playing, socializing or having sex)
- Flat Affect**:** unable to express emotions.

Epidemiology

The prevalence of schizophrenia among adults globally is about 1% - 3%.

Etiology

Contributing factors are:

- Genetics: First degree biol. relative - 10X higher risk.
- Environmental stresses**:** Low socioeconomic class, living in an urban area, stress, being born in winter (!).
- Neuro-developmental: Neurotransmitter imbalance**,** Upper respiratory tract infections in the second trimester of pregnancy, neonatal hypoxia, low birth-weight, intrauterine trauma.
- AT scans and MRI studies have shown brain asymmetry, and an overall decrease in brain size, and an increase in the ventricle size.

Treatment Options

Non-Pharmacological (Psychosocial rehabilitation): Oriented toward improving the patient's adaptive functioning, and includes:

- Case management

- Disease-education
- Cognitive behavioral therapy
- Social skills training
- Basic education
- Work programs
- Supported housing and financial support (very difficult)

Although well directed non-pharmacological efforts contribute significantly (up to 50%) to the overall treatment goals, unfortunately, it is almost impossible to implement these without concurrent antipsychotic medication regimen.

Pharmacological

Antipsychotics are generally classified as: Typical (1st generation) and Atypical (2nd generation) These are distinguished by unique receptor binding profiles with Dopamine and Serotonin (5-HT) receptors.

Side Effects (Adverse Drug Reactions, ADRs)

Since the treatment with antipsychotics tend to be long term, the serious side effects of both the first and second generation medications present challenges in their management as well as in life style changes.

For all antipsychotics FDA requires the following black-box warning:

"Increased Mortality in Elderly patients with Dementia-Related Psychosis

When used in adjunctive therapy for depression (MDD), the risk of suicidality increases in children, adolescents, and young adults.

- CNS effects: Sedation, Seizures, Weight gain
- Autonomic: Anticholinergic (dry mouth, constipation, urinary hesitancy, blurred vision.)
- Anti-adrenergic: Cardiovascular effects (Orthostatic hypotension, QTc prolongation)
- Endocrine effects: Hyperprolactinaemia / Galactorrhea / amenorrhea / Hyperlipidemia
- Extra Pyramidal Syndrome (EPS) Symptoms:

  Dystonia (muscle spasms of head, neck, limbs or trunk)

Akathisia (restlessness, inability to stay still / calm)

Parkinsonism or Pseudo-parkinsonism (decreased motor activity, mask-like face, resting tremor, rigidity, pill rolling, and drooling (Sialorrhea)**)**

- Tardive dyskinesia (Involuntary movements of face (tics, blinking, grimacing), tongue,chewing, protrusion), lips (smacking, puckering, pursing), toe tapping). This could be a long-term side effect; however, it is not seen much today due to the newer anti-psychotic medications.
- Neuroleptic Malignant Syndrome (NMS): (Rare but serious)

  Hyperpyrexia

  Muscle rigidity

  Altered mental status

  Irregular pulse or BP

  ECG Changes, tachycardia

  Cardiac dysrhythmia

  Diaphoresis (sweating)
- Urinary effects: Hesitation, retention, incontinence
- Sexual dysfunction **:** Erectile dysfunction and Anorgasmia
- Weight gain
- Ophthalmic effects: Cataracts. (And rarely narrow angle glaucoma, opaque deposits in cornea and lens, pigmentary retinopathy with Thioridazine))
- Hematologic effects: Transient leucopenia, Agranulocytosis
- Dermatologic effects: Photosensitivity, Rashes

First Generation Antipsychotics (FGAs)

- Efficacy is primarily related to their binding to Dopamine D2 receptors.
- High potency agents have the highest affinity for D2 receptors, and are effective at relatively lower doses (more EPS related side effects).
- Low potency agents have lower D2 affinity and require larger doses (less EPS related side effects).
- More effective on positive symptoms.

First Generation (Typical) Antipsychotics (FGAs)

Low Potency:

- Chlorpromazine (Thorazine)
- Mesoridazine (Serentil)
- Thioridazine (Mellaril)

Mid Potency:

- Loxapine (Loxitane)
- Molindone (Moban)
- Perphenazine (Trilafon)

High Potency:

- Fluphenazine (Prolixin)
- Haloperidol (Haldol)
- Pimozide (Orap)
- Thiothixine (Navane)
- Trifluoperazine (Stelazine)

Drug	Sedation	EPS	Anticholinergic	Prolactin	Orthostasis	Seizures	Wt. gain
FIRST GENERATION (TYPICAL) ANTIPSYCHOTICS							
Chlorpromazine (Thorazine)	++++	+++	+++	+++	++++	+++	++
Thioridazine (Mellaril)	++++	+++	++++	+++	++++	++	+
Loxapine (Loxitane)	++	+++	++	++	++	++	++
Molindone (Moban)	+	+++	++	+++	++	++	+
Perphenazine (Trilafon)	++	+++	++	++++	+	++	+
Fluphenazine (Prolixin)	+	+++	++	++++	++	++	+

Haloperidol (Haldol)	+	+++ ++	+	++++	+	++	++
Thiothixine (Navane)	+	+++ +	++	++++	++	++	++
Trifluoperazine (Stelazine)	+	+++	++	+++	++	+++	++

Notes:

- Inexpensive
- Low potency agents have higher sedation, orthostasis, anticholinergic effects and lower EPS
- May be as effective as some atypicals for positive symptoms
- Thioridazine has the FDA mandated Black Box Warning regarding QT prolongation
- Concomitant anti-cholinergics could be used to reduce EPS

.

2nd Generation (Atypical) Antipsychotics (SGAs or Atypicals)

The characteristics that define "atypicality are not all agreed upon, but in general they all share at least three characteristics:

- Lower risk of EPS than with typical antipsychotics at usual clinical doses
- The risk of tardive dyskinesia is reduced
- The ability to block 5-HT2 receptors, which improves activity for the negative symptoms

Atypical Antipsychotics (SGAs)

- Aripiprazole (Abilify)
- Asenapine (Saphris)
- Clozapine (Clozaril, Fazaclo)
- Lurasidone (Latuda)
- Olanzapine (Zyprexa)
- Quetiapine (Seroquel)
- Risperidone (Risperdal)
- Iloperidone (Fanapt)

- Paliperidone (Invega)
- Ziprasidone (Geodon)

Drug	Sedation	EPS	Anticholinergic	Prolactin	Orthostasis	Seizures	Wt. gain
SECOND GENERATION (ATYPICAL) ANTIPSYCHOTICS							
Aripiprazole (Abilify)	+	+	+	+	+	++	+
Asenapine (Saphris)	+	++	+ / -	+	++	++	+
Clozapine (Clozaril)	++++	+	++++	+	++++	++++	+++ +
Lurasidone (Latuda)	++	+	++	+	+	++	++
Olanzapine (Zyprexa)	++	++	+++	+	++	++	+++ +
Quetiapine (Seroquel)	+++	+	++	+	++	++	++
Risperidone (Risperdal)	+	++	++	++++	++	++	++
Iloperidone (Fanapt)	+	+ / -	+++	+	++	++	++
Paliperidone (Invega)	+	++	++	++++	++	++	+++
Ziprasidone (Geodon)	++	++	++	+	++	++	+

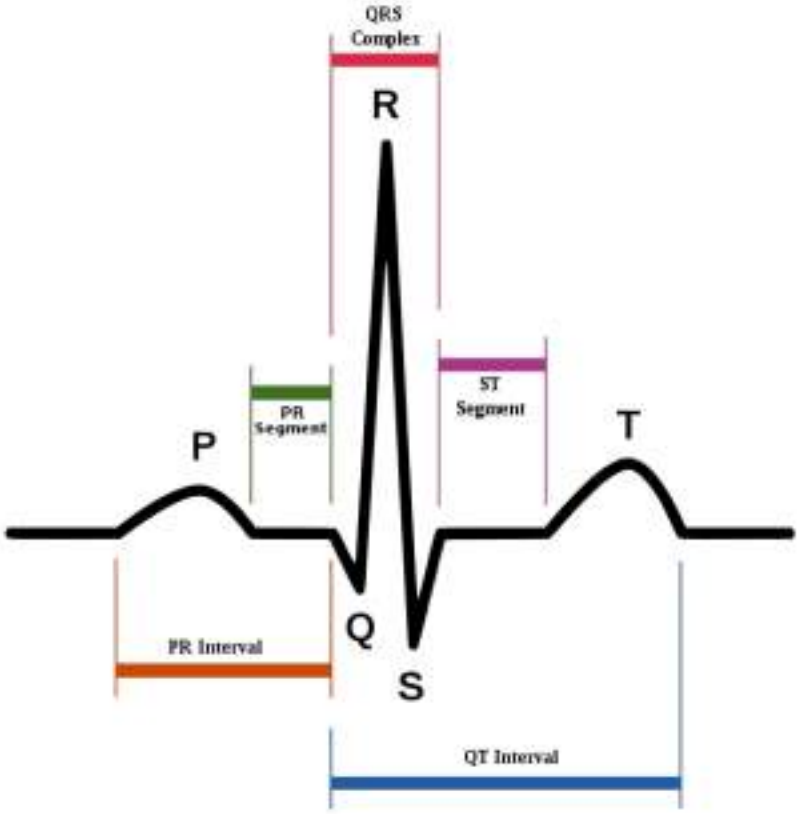
Special Problems:

- Orally Disintegrating Tablets for Cheeking, Acute conditions (Asenapine), Dyspahagia (Asenapine)
- Long acting (Depot) injections for Cheeking & better compliance: Haloperidol decanoate, Fluphenazine decanoate, Risperdal Consta.
- For acute agitated behavior: Ziprasidone (Geodon) I

- Other injectables: Invega sustenna, Zyprexa relprev

QT Interval

Since 2005, the FDA has required that nearly all new molecular entities are evaluated in a Thorough QT (TQT) study to determine a drug's effect on the QT interval. The TQT study serves to assess the potential arrhythmia liability of a drug.



Schematic ECG Trace

In cardiology, the QT interval is a measure of the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle, and represents electrical depolarization and re-polarization of the left and right ventricles.

A lengthened QT interval ("normal" QTc ≤440 msc, where the "c" is a constant, to adjust for heart rate) is a biomarker for ventricular tachy-arrhythmias like torsades de pointes (a lethal form of ventricular tachycardia) and a risk factor for sudden death.

Prolongation of the QT interval may be due to an adverse drug reaction. Many drugs, such as Haloperidol, Thioridazine, Ziprasidone, Asenapine, Lurasidone, and Iloperidone, can prolong the QT interval.

Clozapine

It is considered the "last resort SGA for 20 - 30% of patients who are RESISTANT TO TREATMENT.

(Treatment Failure is defined as failure to respond to two or more antipsychotics (one of which should be an atypical) when given at an adequate dose of 6-8 Wks.)

- Clozapine is the only antipsychotic that can effectively reduce both the positive and negative symptoms. It also reduces suicidality.
- Causes agranulocytosis, sedation, seizures, myocarditis, Orthostasis, Hyperlipidemia; however, decreased risk of EPS / TD.
- The patient, the physician, and the pharmacy must use a Clozaril registry.
- MUST BE MONITORED WEEKLY. For Clozapine: weekly WBC for 1st 6-months, then biweekly for 7 through 12 months. To Start: WBC > 3500 nm3 and ANC > 2000 nm3;
- DC Clozapine if WBC < 2000 nm3 or ANC < 1000 nm3.

Pharmacokinetics of Antipsychotics

Pharmacokinetics: How the body acts on drugs, to absorb, distribute, metabolize, and then excrete them.

Pharmacodynamics: How drugs act upon the body, especially the brain

Pharmacokinetics/Drug – Drug Interactions (DDIs)

Pharmacokinetic actions are mediated predominantly through hepatic drug-metabolizing system known commonly as Cytochrome P450 enzyme system. There are several known cytochrome P450 systems. Five of these are most important for psychotropic drug metabolism. These are: 1A2, 2D6, 2C9, 2C19, and 3A4.

Drugs that inhibit these enzymes increase the levels of the antipsychotics in the blood.

- 1A2 inhibited by SSRI (Fluoxamine) - Raises the levels of Clozapine and Olanzapine
- IA2 also inhibited by grapefruit juice, Ciprofloxacin, Smoking
- 2D6 is inhibited by SSRI (antidepressants like Paroxetine, Fluoxetine, and Sertraline) --- Raises the levels of Risperidone, Clozapine, Olanzapine.
- 3A4 inhibitors would raise the levels of Clozapine, Quetiapine, and Ziprasidone, which could cause QTc interval prolongation, which in turn could result in cardiac arrhythmias.

Depression

Depression (Major Depressive Disorder, MDD) is a serious, yet often overlooked or under-recognized psychiatric disorder, often described as a disturbance of mood associated with changes in behavior, with a life-time prevalence of about 16%.

Many with this illness do not seek treatment or are unaware of the presence of this potentially debilitating condition. If left untreated, the effects of Clinical Depression, MDD, can range from loss of productivity and motivation to thoughts and attempts at suicide.

Epidemiology

- Depression, along with Bipolar Disorder, is classified as a Mood Disorder.
- Depressive disorders are common during adolescence and are frequently associated with substance abuse, suicide attempts, and poor academic performance.
- Episodes of MDD are often precipitated by a severe psychological stressor (divorce, death in the family, loss of livelihood, etc.)

Etiology

Includes several factors:

- Genetic predisposition
- Psychological stressors, acute and chronic.
- Patho-physiological factors
- Decreased levels of 5-HT, NE, and DA
- Dysregulation of neurotransmitters

These theories have been supported by the mechanism in which the current antidepressants work – they increase synaptic monoamine (5-HT, DA, NE) concentrations.

Non-pharmacological Therapy

1. Psychotherapy, cognitive therapy, behavioral therapy, interpersonal psychotherapy are all equally effective, supplemental to antidepressant therapy.
2. Electroconvulsive Therapy (ECT): Safe and effective option for patients requiring a rapid response, and patients with a history of poor response to antidepressants. Administered 2 to 3 times weekly for 6 to 12 weeks. Results seen in 10 to 14 d. Must be supplemental to antidepressant therapy.
3. Bright light therapy: Patient gazes into a 10,000-lux intensity light, daily. Effective for treating Seasonal Effective Disorder (SAD). Well tolerated. Frequent eye exams recommended (seen mostly in people living in northern and southern latitudes, like Scandinavia or Southern Argentina)

Pharmacological Therapy

In October 2004, the FDA directed manufacturers of all antidepressants to include a boxed warning detailing the risk of suicide in children and young adults (18 to 24 years of age) with MDD and othe depressive disorder.

Main types of antidepressants:

1. Non-selective antidepressants (Tricyclics)
2. Selective Reuptake Inhibitors
    - Selective Serotonin Reuptake Inhibitors (SSRIs)
    - Serotonin and Norepinephrine Reuptake Inhibitors (SNRIs)
    - Selective Noradrenaline Reuptake Inhibitors (NARIs)
    - Norepinephrine and Dopamine Reuptake Inhibitors (NDRIs)
3. Receptor blockers
    - Noradrenergic and Specific Serotonergic Antidepressants (NaSSAs)
    - Serotonin Receptor Blockade with Serotonin Reuptake Inhibition (SARIs)

In general, the older antidepressants (Tricyclics) are just as effective as the newer ones (SSRIs) but, have more side effects and can be lethal in OD, and are not so dangerous if someone takes an overdose.

Mode of Action

For reasons that are not completely understood, depressives often have low levels of certain neurotransmitters.

Antidepressants work by increasing the amount of neurotransmitters and making them flow normally in the brain. Neurotransmitters cannot be taken orally or intravenously because they cannot pass blood-brain barrier.

Tricyclic antidepressants and SSRIs stop the nerve cells from reabsorbing neurotransmitters

NOTE: About 25% of the patients experience a hyper-stimulatory response to antidepressants, especially when therapy first starts, which can be confused with a worsening of the anxiety symptoms; especially with Prozac & Venlafaxine.

Antidepressants – Side Effects

Agent	Nausea GI	Agitation Insomnia	Sexual Dysfunction	Weight Gain	Notes
SSRIs					
Citalopram (Celexa)	++	+	++	---	QTc issues
Escitalopram (Lexapro)	++	++	++	---	
Fluoxetine (Prozac)	++	++	+++	---	Least risk of
Fluvoxamine (Luvox)	++	+	+++	++	
Paroxetine (Paxil)	+++	+++	+++	+	EPS, Sedation
Sertraline (Zoloft)	++	++	++	---	Diarrhea
SNRIs					
Duloxetine (Cymbalta)	++	++	+	---	Also for Fibromyalgia
Venlafaxine (Effexor)	++	++	++	---	HBP, Needs Dose titration
SARIs					
Trazodone (Desyrel)	+	---	++	+	Priapism,

Antidepressants – Side Effects (Cont.)

Agent	Anticholinergic	Sedation	Orthostatic Hypotension	Wt. Gain	Notes
NDRIs					
Bupropion (Wellbutrin)	+	---	---	---	Also for smoking cessation
TRICYCLICS					
Amitriptyline (Elavil)	++++	+++	+++	+++	Lowers seizure
Clomipramine	++++	+	++	++	Lowers seizure
Desipramine	++	---	+	+	Insomnia
Doxepin (Sinequan)	+++	++++	+++	++	
Imipramine (Tofranil)	+++	+	++	++	Lowers seizure
Nortriptyline	++	+	+	+-	Lowers seizure
TeCAs / NaSSAs					
Mirtazapine	+	+++	++	++	

Bipolar Disorder

- Bipolar disorder is a cyclic, mood disorder characterized by recurrent fluctuations in mood, energy, and behavior ranging from major depression to mania.
- Bipolar disorder differs from recurrent major depression in that a manic, hypomanic, or mixed episodes occur during the course of the illness.
- Patients with Bipolar disorder have a high risk of suicide.
- Lifetime prevalence rates of psychiatric comorbidity co-existing with bipolar disorder are 42% to 50%.

There are 3 types of bipolar disorder:

Bipolar I patients have full-blown manic and/or mixed episodes often followed by a full depressive episode.

Bipolar II patients are characterized by at least one hypomanic episode and one full depressive disorder.

Cyclothymic disorder is characterized by mood swings less severe than full mania and full depression, but still waxing and waning above and below the boundaries of normal mood (rarely suicidal but usually treatment resistant).

Etiology

It is thought to be a complex genetic disease that is environmentally influenced and caused by a wide range of neurobiologic abnormalities.

Pathophysiology: Neurotransmitter / neuroendocrine abnormality, such as : dysregulation between excitatory (NE, DA, glutamate and aspartate,) and inhibitory (5-HT and GABA) neurotransmitter systems (excess of catecholamines (NE & DA) cause mania.)
Several classes of drugs can induce mania (Anticonvulsants, Antidepressants, Antimicrobials, Anti-Parkinson's Drugs, Anxiolytics/ hypnotics, AAPs, CNS stimulants, Drugs of Abuse, Sympathomimetics, Herbals, etc.

Non-pharmacological treatments

- Adequate nutrition, Sleep, exercise and stress reduction
- Mood charting to help detect early signs and symptoms of mania and depression
- Psycho-educational programs
- Supportive counseling
- Family therapy
- Crisis intervention plan
- Cognitive behavioral therapy
- Electroconvulsive therapy is the application of prescribed electrical impulses to the brain (inducing controlled seizures) for the treatment of severe depression, mixed states, psychotic depression, and treatment-refractory mania. May be used in pregnant women who cannot take carbamazepine, lithium or divalproex.

Pharmacological Treatment

Pharmacotherapy is the cornerstone of acute and maintenance treatment of Bipolar Disorder. Mood stabilizing drugs are the usual first choice of treatment.

FDA-Approved Medications for Bipolar Disorder				
Drug	Mania	Mixed	Depression	Maintenance
Carbamazepine (ER	X	X		
Lamotrigine				X
Lithium	X			X
Divalproex Sodium	X	X		
Aripiprazole	X	X		X
Olanzapine	X	X		X
Quetiapine	X		X	
Risperidone	X	X		
Ziprasidone	X	X		

Lithium (Gold standard for mood stabilization)

- The first-choice drug for the classic presentation of bipolar disorder.
- Effective for both the manic and depressive components of bipolar and reduction in suicides.
- Only lithium is FDA-approved in children /adolescents as young as 12.
- It is a unique agent (an element that behaves like an electrolyte) which has almost no psychotropic effect in normal individuals; it is not a Sedative, depressant or euphoriant.
- It takes about 2 weeks to see the full effects of Lithium; hence interim use AAP and/or benzodiazepines is recommended.
- Lithium toxicity which can occur at serum concentrations over 2mEq/L, can be severe and life-threatening requiring ICU admission and a push of IV fluids and possible renal dialysis.

Lithium - DDIs

- Common and Significant drug interactions involve drugs that increase lithium levels, and very commonly prescribed drugs such as thiazide diuretics, NSAIDs (non-steroidal anti-inflammatory agents), and ACE inhibitors.
- If a diuretic must be used, a loop diuretic such as furosemide is less likely to increase lithium retention (but must be given with lot fluids and potassium to avoid dehydration and replace potassium)

- ACE inhibitors should be avoided at any cost since they can abruptly increase serum lithium with the potential for acute and fatal toxicity.
- Drugs that reduce lithium levels include Caffeine and theophylline.
- Drugs that increase CNS toxicity include Carbamazepine, phenytoin, and neuroleptics.

Lithium Side Effects - ADRs

- GI upset: Dose related. Nausea, dyspepsia, and diarrhea
- Tremors
- Kidney function: Causes increases in frequency and Volume of urine, and increase in thirst. (Diabetes insipidus, inhibits kidney's ability to concentrate urine).
- Thyroid function: Lithium induced hypothyroidism
- Cardiac effects (EKG changes & Cardiac arrest, due to inhibition of Potassium reuptake at cellular level resulting in intracellular hypokalemia & extracellular hyperkalemia)
- Dermatological effects such as rashes & psoriasis
- Weight gain
- Metallic taste
- Reduced libido and sexual dysfunction

Alternatives and Adjuncts to Lithium

- Antipsychosis

Aripiprazole, Olanzapine, Quetiapine, Risperidone, Ziprasidone are all approved for Bipolar Mania, Quetiapine is approved also for Bipolar Depression, and Aripiprazole and Olanzapine for maintenance therapy.

- Benzodiazepines

High potency agents such as Clonazepam and Lorazepam are helpful acutely for agitation, insomnia, and hyperactivity

- Valproate (Depakote)

Primarily an anticonvulsant, but also for mood stabilization, especially good for rapid cyclers. FDA approved for the treatment of manic episodes or bipolar disorder (great for use if the patient is not able to tolerate or respond to Lithium.)

- Lamotrigine (Lamictal)

DDI: Carbamazepine / Phenytoin / Phenobarb / Valproate

- Carbamazepine (Tegretol) for both acute and prophylactic management.
- Oxcarbazepine (Trileptal) Effective substitute for carbamazepine. Much better tolerated, less monitoring required.(No CBC, WBC, platelets, LFTs, and eye exams).

Anxiety Disorders

Generalized anxiety disorder: 6 months or more of excessive worry or anxiety, generally with an unidentified cause.

Panic disorder: discrete periods of sudden, intense fear or terror and feelings of impending doom lasting about 10 min.

Obsessive-compulsive disorder: Obsessive or intrusive thoughts that cannot be controlled, and involve repetitive ritualistic behavior, such as washing hands, combing hair, cleaning house, etc.

Post-traumatic stress disorder: follows a traumatic event

Social anxiety disorder

Pharmacotherapeutic options

Benzodiazepines

These drugs have anxiolytic properties, and some have preventive efficacy for panic attacks.

Pharmacologically all these agents share, to various degrees, five properties:

a) anxiolytic, b) hypnotic, c) muscle relaxation, d) anticonvulsant, and e) amnesic

AGENT	HALF-LIFE	DOSE (mg)
Alprazolam (Xanax)	6-12	1
Chlordiazepoxide (Librium)	5-30	25
Clonazepam	20-50	0.5
Diazepam (Valium)	20-100	10
Lorazepam (Ativan)	10-18	1

Short half-life / high potency: Rapid acting, tolerance prone, withdrawal problems. For acute management

Long half-life / low potency: Long lasting, less pronounced withdrawal symptoms. They can accumulate in elderly patients.

The primary issue associated with Benzos is tolerance and dependence.

Generalized Anxiety Disorder: Antidepressants (SSRIs), Benzodiazepines, Buspirone

Panic Disorder: Antidepressants (SSRIs), Benzodiazepines.

Obsessive Compulsive Disorder: Serotogenic agents such as SSRIs, especially in combination with Atypical antipsychotics.

Post-traumatic Stress Disorder: Sertraline and Paroxetine. Valproic acid has been used effectively to control and reduce aggression and anger.(Benzos are not effective and should be avoided).

Role of Pharmacy and Pharmacists in Psychiatric Treatment

Pharmacy and Pharmacists become critical players in treatment because Pharmacological treatment:

- Is prohibitively expensive (help with generic substitution)
- Needs Medication Reconciliation (such as drug-drug interactions, adjustments in the dose or how to take the medication, etc., becomes necessary due to number of medications taken by the patient, medications to treat the main condition, medications to treat the side effects, and other over-the-counter medications
- Is often complicated by (help by consultation):
    - ✓ Long term, often lifelong treatment
    - ✓ Need to modify changes in life style
    - ✓ Serious side effects
    - ✓ Drug-drug interactions,
    - ✓ Drug-induced co-morbidities.
    - ✓ Denial
    - ✓ Non-compliance

The Following Sources are gratefully acknowledged:

- Psychiatry Textbooks and Handbooks
- Articles from Psychiatry Journals
- Manufacturers' Drug Information Brochures / Drug Seminars
- Practical Knowledge from the practice of psychiatric pharmacy and
- The collective practical knowledge of the Psychiatric Pharmacy Staff.

12. Free Convection in Non-Newtonian Fluids from Heated Objects

Professor B D Tilak Distinguished Lecture (2012-13)

April 3, 2013

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Abstract

This paper presents an overview of our research activity in the field of free convection in non-Newtonian fluids from variously shaped heated objects. In particular, consideration is given to two broad classes of fluids, namely, power-law fluids (shear-thinning and shear-thickening type) and Bingham plastic fluids. We have sought numerical solutions to the coupled momentum and energy equations within the framework of Boussinesq approximation to capture the temperature-dependence of the liquid density; all other thermo-physical properties are, however, assumed to be independent of temperature within the narrow range of temperature differences imposed in the system. The present results span wide ranges of Grashof number, Prandtl number and power- law index for a range of shapes including a sphere, a horizontal cylinder, elliptic cylinders of various cross-sections, a semi-circular cylinder and a square bar maintained at a constant temperature which is greater than that of the surrounding liquid. Extensive results on isotherm contours and streamline patterns and on Nusselt number are presented to delineate its scaling with Grashof number, Prandtl number and power-law index. Finally the present results are shown to be in good agreement with the scant experimental results available in this field. The paper is concluded by elucidating the role of shape and orientation of the heated object on free convection. The universal appeal of a composite parameter, akin to the Rayleigh number, in correlating the Nusselt number results for a wide variety of 2-D axisymmetric shapes is demonstrated. Finally, additional challenges posed by the Bingham plastic fluids are briefly discussed by way of free convection from a heated cylinder submerged in quiescent Bingham plastic fluids.

1. Introduction

Whenever there exists a temperature gradient in a fluid, heat transfer occurs by conduction and convection from the region of high temperature to that of low temperature. In the absence of a mechanical device to facilitate fluid motion, temperature-dependent density of the fluid gives rise to the buoyancy-induced flow which, in turn, transfers heat by the so-called free convection. Conversely, this contribution, howsoever small, is always present in most heat transfer applications. Of course, as the strength of the forced convection diminishes (indicated by vanishingly small values of the corresponding Reynolds number, Re), the contribution of free convection progressively increases. The relative importance of the free and forced convection mechanisms is quantified in terms of the familiar Richardson number, Ri, which is defined as, $Ri = Gr/Re^2$. Here, the Grashof number is a measure of the strength of the buoyancy-induced flow and the Reynolds number is that of the forced convection. Naturally, the pure forced convection limit is characterized by $Ri = 0$ ($Gr = 0$) whereas $Ri \rightarrow \infty$ corresponds to the pure free convection limit ($Re = 0$). Suffice it to add here that $Ri \sim O(1)$ corresponds to the conditions when the buoyancy-induced velocity is comparable to the imposed velocity. Thus, the contribution of the free convection to the overall heat transfer increases with the increasing value of the Richardson number. Notwithstanding the fact that free convection is always present, typical examples include heat losses from pipes carrying hot process streams like steam or water, storage tanks, high-temperature process equipment like distillation columns, reactors, etc., all of which are exposed to ambient conditions. In addition to such overwhelming pragmatic significance of free convection, the momentum and energy equations are coupled via the body force term and therefore the study of buoyancy-induced transport also constitutes an important sub-class of problems within the realm of transport phenomena. Consequently, over the years, much progress has been made in this field as far as the free convection transport in simple Newtonian fluids like air and water is concerned for most geometric configurations of practical interest. Excellent treatises are available on this subject (Martynenko and Kharmastov, 2005).

In contrast, it is readily conceded that most fluids of macromolecular (polymeric melts and solutions, protein solutions) and of multiphase nature (foams, emulsions, suspensions, for instance) encountered in a broad spectrum of industrial settings including polymer, food, pharmaceutical, personal and health care products, lubricants like grease, drilling muds,

biological fluids do not conform to the simple Newtonian postulate. Instead, such "structured" fluids exhibit a range of rheological complexities including shear-thinning and shear-thickening viscosity, yield stress, visco-elasticity, thixotropy, etc. (Chhabra, 2006; Chhabra and Richardson, 2008). Naturally, it is not possible to consider all these aspects simultaneously and, in order to keep the level of complexity at a tractable level, it seems reasonable to begin with the simplest and possibly also the commonest type of non-Newtonian aspect, namely, shear-thinning and shear-thickening fluid behaviour which is usually approximated by the simple two-parameter power-law model which is written, in simple shear flow, as follows:

$$\tau = m(\dot{\gamma})^n \quad \ldots\ldots\ldots \quad (1)$$

In eq. (1), τ is the shear stress produced in the fluid when it is sheared at the rate of shear, $\dot{\gamma}$. The pre-factor, m, is known as the consistency index and it is a measure of the fluid consistency. Conversely, it can be viewed as the value of the fluid viscosity at a shear rate of $\dot{\gamma} = 1\,s^{-1}$. The index, n, is known as the power-law index. Evidently, $n < 1$ indicates shear-thinning behaviour whereas $n > 1$ corresponds to the so-called shear-thickening behaviour. Of course, n = 1 denotes the standard Newtonian fluid behaviour. From a practical standpoint, many polymeric fluids and suspensions exhibit values of the power-law index in the range $\sim 0.2 \leq n \leq 0.6$. On the other hand, thick pastes and suspensions (corn flour in water, starch in water, for instance) exhibit values of $n > 1$ thereby leading to shear-thickening behaviour. So the major thrust of this work is on studying the laminar free convection from variously shaped heated objects in quiescent power-law media.

Next, we provide a brief introduction to the additional challenges posed by the so-called yield-stress fluids which exhibit elastic solid-like behavior below a threshold stress level (yield stress) and hence the flow domain is spanned by fluid-like and solid-like zones (Bird et al., 1983; Barnes, 1999). The simplest viscosity model to capture the yield-stress behaviour is the so-called Bingham plastic model which, in simple shear, can be written as:

$$\tau = \tau_0 + \mu_B(\dot{\gamma}) \quad \text{for } |\tau| > |\tau_0| \quad \ldots\ldots\ldots \quad (2a)$$

$$\dot{\gamma} = 0 \quad \text{for } |\tau| < |\tau_0| \quad \ldots\ldots\ldots (2b)$$

In eq. 2(a), τ_0 is the so-called yield stress and μ_B is the Bingham plastic viscosity. Whether the true yield stress exists or not has been a matter of debate (Barnes, 1999), the flow behaviour of many practical materials is conveniently approximated by eq. 2, Intuitively, it appears, with these fluids, convection will dominate the fluid-like regions whereas the unyielded (solid-like) regions permit heat transfer only by conduction thereby lowering the overall rate of heat transfer. In this talk, we present an overview of our recent work in this field which has been reviewed extensively elsewhere (Chhabra, 2011)

2. Analysis and Dimensional Considerations

Undoubtedly, the major thrust of research in this field is on the prediction of the heat transfer coefficient in a given application where free convection is the sole mechanism of heat transfer. From a theoretical standpoint, the momentum and energy equations are coupled via the buoyancy term and therefore, these need to be solved simultaneously. This aspect precludes the possibility of general rigorous solutions even for Newtonian fluids (Martynenko and Kharmastov, 2005). Therefore, early attempts at such analysis of free convection are based on the solution of the boundary layer equations which implicitly assume infinitely large values of the Grashof number and/or Prandtl number so that the thin boundary layer assumption can be justified as well as the curvature effects can be neglected. Admittedly this approach has led to reliable scaling of the skin friction and Nusselt number with Grashof and/or Prandtl number, it does not capture the wake region. Nor does it help delineate the values of the Grashof number and/or of the Prandtl number beyond which this analysis is applicable. Notwithstanding these limitations, this approach has been widely used for axisymmetric shapes like sphere, cylinder, spheroids, and of course, plane surface, etc. The other limiting case of the vanishingly small values of the Grashof number is treated via the asymptotic expansion technique such as that used by Singh and Hasan (1983) for a sphere and Nakai and Okazaki (1975) for a cylinder. Therefore, neither of these approaches is valid at finite values of the governing parameters (Grashof number and/or Prandtl number) and, more importantly, these can be only employed for axisymmetric shapes which are free from geometric singularities like a square or a triangular prism. Indeed the results for Newtonian fluids based on the numerical solution of the complete momentum and energy equations even for regular shapes like a plate or a cylinder or a sphere have been reported during the past 25-30 years only (Martynenko and Kharmastov, 2005). Suffice it to say here that based

on a combination of the analytical, numerical and experimental studies, reliable methods are now available for the estimation of skin friction and Nusselt number in the free convection regime over most ranges of practical interest in Newtonian fluids, at least for the regular shapes of spheres, cylinders or plates.

For a given geometric configuration, most of these results are expressed by the following generic form:

$$Nu = f(Gr, Pr) \qquad \ldots\ldots\ldots (3)$$

Where the general definition of Nusselt number, Nu = hd/k. Here h is the convective heat transfer coefficient, d is a characteristics linear dimension like diameter or radius for sphere and cylinder and k is the thermal conductivity of the fluid. Similarly, the Grashof number (Gr) and Prandtl number for a Newtonian medium are defined as:

$$Gr = \frac{\rho^2 d^3 (g\beta\Delta T)}{\mu^2} \qquad \ldots\ldots\ldots (4)$$

$$Pr = \frac{C_p \mu}{k} \qquad \ldots\ldots\ldots (5)$$

where ρ is the fluid density; g is acceleration due to gravity; β is the coefficient of expansion and ΔT is the temperature difference between the ambient fluid and the heated object; μ is the Newtonian viscosity; C_p is the thermal heat capacity.

The actual functional relationship embodied in eq. (3) depends upon many other aspects including the nature of the boundary condition prescribed on the surface of the heated object (constant temperature or constant heat flux), laminar or turbulent flow conditions, viscous dissipation, temperature-dependence of the physical properties, etc. Extensive compilations encompassing wide ranging shapes and conditions are available in the literature (Martynenko and Kharmastov, 2005).

The analogous literature for power-law fluids is neither as extensive nor coherent as that for Newtonian fluids (Shenoy and Mashelkar, 1982; Chhabra, 2006). Early pioneering effort in this field is due to Acrivos (1960) who presented limited results for the laminar free convection in

power-law fluids from axisymmetric shapes including the case of a sphere, a cylinder and a plate. Subsequently, this work has been confirmed by the other studies (Stewart, 1971; Meissner et al., 1994). However, as noted previously, such an analysis neither accounts for the wake region nor is applicable at finite values of the Grashof and Prandtl numbers. Therefore, one must resort to the numerical solutions of the complete momentum and energy equations to circumvent these limitations.

During the past five years or so, reliable numerical predictions of the detailed kinematics of flow (streamline and isotherm contours), distribution of Nusselt number along the surface of heated objects and the overall mean Nusselt number have been reported for power-law fluids. Prahashanna and Chhabra (2010, 2011) studied laminar natural convection from an isothermal sphere and cylinder over wide ranges of power-law index (n) and Grashof and Prandtl numbers. Subsequently, analogous results have been reported for elliptical cylinders (Sasmal and Chhabra, 2012b), square and rotated square cylinders (Sasmal and Chhabra, 2011, 2012a) and semi-circular cylinders in different configurations (Chandra and Chhabra, 2012; Tiwari and Chhabra, 2013). This paper provides an overview of our work in this field.

Dimensional considerations as applied to the appropriate momentum and energy equations together with the relevant boundary conditions lead to the following definitions of the Grashof number and present emerge power-law fluids

$$Gr_p = \frac{\rho^2 (d)^{n+2} (g\beta\Delta T)^{2-n}}{m^2} \qquad \ldots\ldots\ldots (6)$$

$$Pr_p = \frac{\rho C_p}{k}\left(\frac{m}{\rho}\right)^{\left(\frac{2}{1+n}\right)} (d)^{\left(\frac{1-n}{1+n}\right)} \left(dg\beta\Delta T\right)^{\frac{3(n-1)}{2(n+1)}}$$

……(7)

Note that much more unwieldy forms of the Grashof and Prandtl numbers for power-law fluids, albeit these do reduce to their limiting forms, as given in eq. (4) and eq. (5) for n = 1. In addition, the power-law index, n, is a dimensionless parameter in its own right. Thus, for a power-law fluid, the functional relationship denoted by eq. (3) is reformulated as follows:

$$Nu = f_1(Gr_p, Pr_p, n) \qquad \ldots\ldots\ldots (8)$$

Both the experimental and numerical approaches have been used to establish the functional relationship implicit in eq. (8) using Gr_p, Pr_p and n. However, our recent experience suggests that the following composite parameter, Ω, is rather more effective in consolidating the results for a range of geometric shapes studied thus far than the Grashof number and Prandtl number. It is defined as:

$$\Omega = Gr_p^{\frac{1}{2(n+1)}} Pr_p^{\frac{n}{3n+1}} \qquad \text{..... (9)}$$

For n = 1 (Newtonian fluids), the composite parameter, Ω, is identical to the $Ra^{1/4}$ where the so-called Rayleigh number, Ra, is defined as $Ra = Gr \cdot Pr$. The available experimental and numerical results for laminar free convection in Newtonian fluids conform to $Nu \propto Ra^{1/4}$, i.e., $Nu \propto \Omega$. By analogy, one can thus re-cast the relationship of eq. (8) as follows:

$$Nu = a\Omega^b \qquad \text{.....(10)}$$

Naturally, eq. (10) is applicable for a fixed geometric configuration. Indeed, Table 1 presents a summary of the currently available results on laminar free convection in power-law fluids thereby showing the universal appeal of the composite parameter.

Broadly speaking, all else being equal, shear-thinning fluid behaviour (n < 1) promotes heat transfer over and above that seen in Newtonian media. Of course, shear-thickening behaviour impedes it. Indeed, it is possible to realize up to 100% augmentation in Nusselt number in shear-thinning fluid under appropriate conditions. Also, the fact that $b \approx 1$ in almost all cases summarized in Table 1 demonstrates the universal appeal of the composite parameter, Ω, at least in conformity with the scaling suggested by the boundary layer considerations. The effect of geometry reflected by the value of a is seen to be significant. Before closing this sub-section, it is important to reiterate here the assumptions inherent in the numerical studies which form the basis of the results reported in Table 1. Firstly, these results are based on the assumption of constant physical properties and negligible viscous dissipation effects thereby limiting their validity to the situations wherein ΔT is not excessive and one can thus evaluate k, C_p, m, n etc. at the mean film temperature. Secondly, only the results corresponding to the constant wall temperature conditions are included in Table 1. Lastly, the flow is assumed to be laminar in all cases.

Table 1: Values of ^{a} and ^{b} in Eq. (10) for different shapes.

Reference	Shape	Linear dimension	a	b
Prhashanna & Chhabra (2010)	Sphere	R	2.00	0.72
Prhashanna & Chhabra (2011)	Horizontal circular cylinder	d	1.19	0.89
Sasmal & Chhabra (2012b)	Elliptic cylinder ($0.2 \le E \le 5$)	2a	0.83	0.89
Chandra and Chhabra (2012)	Semi-circular cylinder (flat base upward)	d	0.93	0.79
Tiwari and Chhabra, (2013)	Semi-circular cylinder (flat base downward)	d	0.72	0.90
Sasmal & Chhabra (2011)	Square cylinder	B	0.60	0.92
Sasmal & Chhabra (2012a)	Tilted square cylinder ($\alpha = 45^{\circ}$)	B	0.76	0.92

Next, we turn our attention briefly to the case of free convection in Bingham plastic fluids where the fluid behaviour is characterized in terms of a yield stress (τ_0) and plastic viscosity (μ_B), as suggested by eq. 2. Since once the prevailing stress level exceeds the yield stress, τ_0, such a material behaves like a Newtonian fluid with viscosity μ_B, one can use the same definitions of the Grashof number and Prandtl number, as that given by eq. (4) and eq. (5) for Newtonian fluids, or one can work in terms of the Rayleigh number, $\mathrm{Ra} = \mathrm{Gr} \cdot \mathrm{Pr}$. However, an additional dimensionless group, namely a Bingham number emerges in this case. For free convection, it is defined as:

$$\mathrm{Bn} = \frac{\tau_0}{\mu_B}\sqrt{\frac{\mathrm{d}}{\mathrm{g}\beta\Delta\mathrm{T}}} \qquad \text{.....(11)}$$

Intuitively, it appears that with the increasing value of the yield stress, i.e., the Bingham number, the flow domain is increasingly dotted by the unyielded (solid-like) regions where heat transfer occurs only by conduction. This point is illustrated by showing some results from a heated circular cylinder which is submerged in a body of Bingham plastic fluid (at a lower temperature than the cylinder) filled in a square duct whose walls are also at the same temperature as the fluid (Fig. 1). These results have been recently reported by Sairamu et al. (2013). Figure 2 shows typical results on the so-called yielded and unyielded (shaded) regions. Evidently, there are regions which act like a solid material and this has an adverse influence on the overall heat transfer. Fig. 3 shows that beyond a limiting value of the Bingham number (Bn_{max}), the Nusselt number is constant which is identical to the corresponding conduction limit (Sairamu et al., 2013).

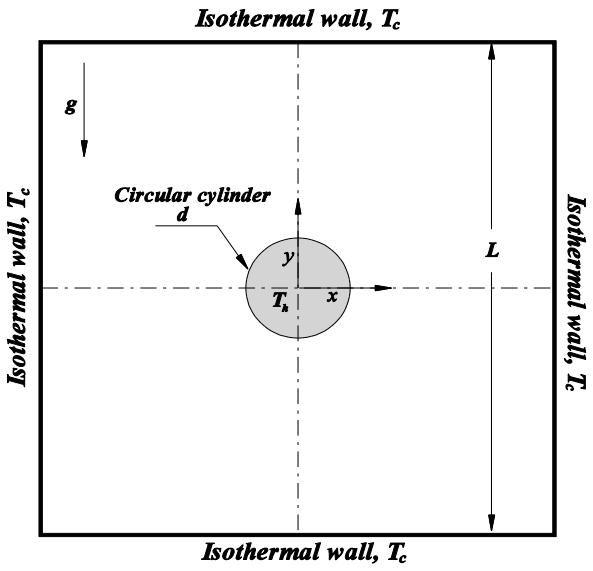

Figure 1: Schematics of the flow configuration

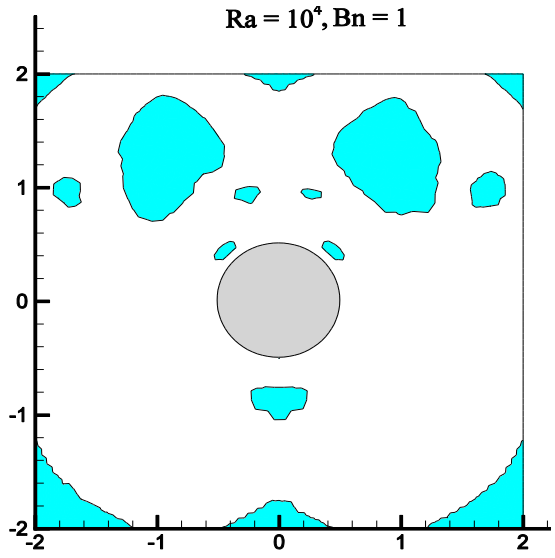

Figure 2: Typical structure of yield/un-yielded regions

In this case, it is naturally advantageous to correlate the Nusselt number results by two expressions. Thus, for instance, Sairamu et al. (2013) reported the following equations:

$$Nu = 2.585 \qquad \text{for } Bn \geq Bn_{max} \quad \ldots.(12a)$$

$$Nu = 2.585 + 0.0095 Ra^{1/4} (Bn_{max} - Bn)^{2.3} \quad \ldots\ldots\ldots\ldots(12b)$$

Thus, heat transfer in such fluids not only tends to be inherently poor, but its prediction also necessitates knowledge about the value of Bn_{max} a priori. Indeed, Bn_{max} shows an intricate dependence not only on the geometrical configuration but also on the type of boundary conditions as well as on the range of Rayleigh numbers of interest.

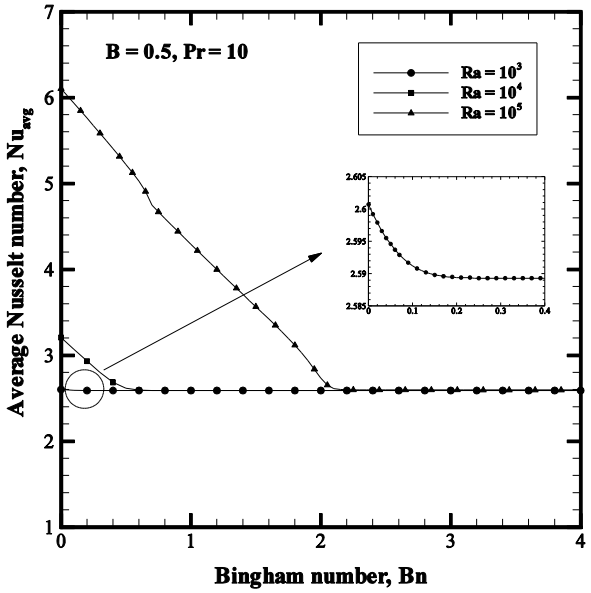

Figure 3: Dependence of average Nusselt number with Bingham number and Rayleigh number.

3. Concluding Remarks

In this work, an attempt has been made to provide an overview of the nature of buoyancy-induced free convection in two types of non-Newtonian fluids, namely, power-law fluids and Bingham plastic fluids. Reliable results available in this field are not only rather scant but these are also of very recent vintage. Broadly, shear-thinning and shear-thickening fluids behave in a qualitatively similar fashion as Newtonian fluids. However, shear-thinning behaviour can enhance the rate of heat transfer by up to 100% under appropriate conditions whereas the shear-thickening fluid behaviour somewhat impedes the rate of heat transfer. Analogous buoyancy-induced flow in yield stress fluids has been studied even less extensively and the field is still in its infancy. In this case also, there are parts of region which are dominated by conduction thereby lowering the overall rate of heat transfer. This rugged terrain of non-Newtonian transport phenomena deserves more attention that it has received thus far.

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Harnessing seabuckthorn (Hippophae L.) resources of Himalayas to provide nutritional and health security to India

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1. Introduction

Many children, women and poor people suffer from nutrient deficiencies in India and other developing countries. Further modern medicines or aliphatic drugs are quite expensive for them, besides sides effects (WBRMI, 2009). Seabuckthorn (Hippophae sp. family Elaeagnaceae), a multipurpose plant of cold desert can be a solution to above problems to some extent. Seabuckthorn, a nitrogen fixing, deciduous plant, a thorny, nitrogen-fixing, deciduous shrub (2-5 m) or small tree (5-9 m) grows widely in dry temperate regions of Indian Himalayas. Fruits and leaves are a rich source of vitamin C, E, K, carotenoids, flavonoids and sterols etc., therefore it has high potential in health food, pharmaceutical and cosmetic industries. It has also been widely planted for the control of soil erosion in mountainous lands (Lu Rongsen, 1992). Seabuckthorn grows naturally in cold countries Europe, Asia, Russia, China and Himalayas. In India, seabuckthorn grows in high altitude areas of Himachal Pradesh, Jammu and Kashmir, Uttrakhand, Sikkim and Arunachal Pradesh. In Himachal Pradesh, seabuckthorn grows (2400-4300 m asl) on river sides and sun facing slopes in Lahaul-Spiti and Kinnaur (Singh et al., 1995). There are 6 species globally, among 3 found in Indian Himalayas, Hippophe rhamnoides, subspecies turkestanica is the most dominant and widely distributed plant (2-4 m tall), followed by H. salicifolia (4-9 m tall) and H. tibetana (0.2-0.6 m tall). Male and female plant are separate. Plant bears foliage from April to November, flowers in June or July for a week and fruit from mid-August to April. Female plant bears red, yellow or orange coloured fruits (10-20 g/100). They are picked up in September or October (Singh et al., 1995). It can be propagated from seeds as well as stem cuttings. It is a quality fodder (Singh et al., 1999) and fuelwood of the region. Raina et al. (2011) conducted survey on genetic diversity in seabuckthorn in Himalayas

and confirmed occurrence of three species of seabuckthorn. There are about 15,000 ha under seabuckthorn in Indian Himalayas.

Traditionally fruit of seabuckthorn has been used in Amchi System of Medicine, a part of Indian System of Medicine, Tibetan and Mangolian medicines since ancient times (Singh, 2006). Seabuckthorn medicinal properties and its application were recorded in "the RGyud Bzi (The Four Books of Pharmacopoeia) dated to the times of Tang Dynasty (618–907 AD) as early 8th century (Lu Rongsen, 1992). The present paper reviews the nutritional and medicinal potential of seabuckthorn.

2. Nutritional Values of Fruits

Fruit is a very rich source of vitamin C (300-1,600mg/100g), 4-100 times higher than any vegetable or fruit (Table 1.) Owing to this its juice and jam or recommended for weak children, pregnant and old women. High contents of vitamin C, organic and tannic acid make seabuckthorn an ideal choice for the beverage industries. The fruit oil is quite rich in vitamin E and K, carotenoids, flavonoids and sterols. Vitamin E is much higher than many other oils (Table 2). Bal et al. (2011) have recently reviewed the nutritional values of seabuckthorn.

Table 1: Vitamins in seabuckthorn as compared with other fruits and vegetables

Plant	**Vitamin (mg/100g)**				
	A	**B_1**	**B_2**	**K**	**C**
Seabuckthorn	11.0	0.04	0.56	100-200	300-1600
Amla					600
Kiwi	-	-	-	-	100-470
Orange	0.5	0.08	0.03	-	50-68
Tomato	0.3	0.03	0.02	-	12
Carrot	0.3	0.02	0.05	-	8

(Source: Lu Rongsen, 1992)

Table 2: Oil soluble vitamins in seabuckthorn and other edible oils

Vitamins	Seabuckthorn Seed oil	Seabuckthorn pulp oil	Olive oil	Walnut oil	Wheat embryo oil	Corn oil
Carotenoids (mg/100 g)	78.2	373.2	11.9	0.36	0.4	50.9
β-carotene (mg/100 g)	2.5	82.2	nil	0.17	nil	0.29
Vitamin E (mg/100 g)	206.6	213.0	6.0	21.3	144.5	34.0

(Source: Lu Rongsen, 2013).

The nutritional composition of seabuckthorn fruit vary considerably among different subspecies, with the place of origin, climate, harvesting time and method of processing.

Fruit nutrients: The content of moisture of fruit may be as high as 80–87% as reported by Lõugas et al. (2006) and as low 20–32% as reported by Sabir et al. (2005) for the Pakistani varieties. TSS of pulp is in the rage of 26.2–27.9% for Indian seabuckthorn (Arimboor et al., 2006). The TSS of juice from fruit is 10.7–13.2 (Arimboor et al., 2006). The fruit of seabuckthorn has organic acids mainly malic and quinic acids together making around 90% of all the fruit acids in different origins. Russian berries showed relatively lower values of total acidity (2.1–3.2 g/100 ml), Finnish seabuckthorn were medium with a range of 4.2–6.5 g/100 ml, while Chinese seabuckthorn showed the maximum values of organic acid with a range of 3.5–9.1 g/100 ml (Zhang et al., 1989; Kallio et al., 1999). Among the sugars, glucose, fructose and xylose are important ingredients of sea buckthorn berries. Total soluble sugars in Chinese seabuckthorn ranged from 5.6 to 22.7% in raw juice (Kallio et al., 1999; Zhang et al., 1989). Glucose is a major sugar part in all seabuckthorn forms. Fruit juice of seabuckthorn is quite rich in various free amino acids, as a total of 18 out of 22 known amino acids have been estimated in seabuckthorn fruit (Mironov, 1989; Zhang et al., 1989). The values of vitamin C in seabuckthorn fruit has been found to be higher than strawberry, kiwi, orange, tomato, carrot and hawthorn (Bernath and Foldesi, 1992). Vitamin C content in Russian forms are lower (50-250 mg/100g) than Chinese and Indian seabuckthorn (Singh et al., 2011). The vitamin C varies in fruits from low values of 360 mg/100g for the European subspecies rhamnoides (Yao et al., 1992) to 2500 mg/100 g of berries for the Chinese subspecies sinensis (Zhao et al., 1991).

Fatty acids: Chinese seabuckthorn fruits are low in oil (2-3%) (Lu Rongsen, 1992), Indian seabuckthorn medium (2-4.5%) (Singh et al., 2011), whereas Russian forms are very rich in oil (5-8%) (Kalinina, 1988). The proportions of linoleic and α-linolenic acids in seed oil of different origins are usually 30-40% and 15-40%, respectively (Yang et al., 1992; Moravcov et al., 1995). In pulp oil of Indian Himalayas, Palmitoleic acid was the major unsaturated fatty acid, being maximum in ssp. turkestanica, (46.4% of the total). Linoleic acid, an important fatty acid varied from a maximum of 15.0% in H. salicifolia, followed by pulp oil of ssp. mongolica (10.1□). α-Linolenic acid, being also maximum in H. salicifolia (1.3%), 2011). In seed oil also, Linoleic acid is the most abundant in ssp. turkestanica (23.8-39.8). Similarly, α-Linolenic acid values were higher in ssp. turkestanica. Seeds of H.salicifolia were most rich in Palmitoleic acid (8.1%) (Singh et al., 2011).

Vitamin E: The vitamin E content in seabuckthorn oil is higher than many crops like the oils of maize, wheat embryo, safflower and soybean (Bernath and Foldesi, 1992). The content in seabuckthorn fruit is 160 mg/100 g (Wahlberg and Jeppsson, 1990; Zhang et al., 1989). The vitamin E in the juice is 162–255 mg/100 g for Chinese seabuckthorn (Zhan et al., 1989) and pulp 481 mg/100 g for Pakistani varieties (Zeb, 2004a). Seeds of Chinese varieties contains vitamin E 40.1–103.0 mg/100 g (Ma et al., 1989). The seed oil of ssp. turkestanica growing at Leh, India, contains high content of tocopherols (140 mg/100 ml). Vitamin E was found maximum during early collection in the pulp oil of H. rhamnoides from arid Spiti (3063 ppm), India, which decreased during maturation of fruit. Among the tocopherols, in our study, α-tocopherol is the dominant one, followed by α-tocotrienol (Singh et al. 2011).

Flavonoids: Flavonoids in seabuckthorn fruit and leaves are well known to improve the functioning of cardiovascular system. According to Chinese studies, flavonoids are higher in contents than lower altitude areas, the average content in fresh fruit is 354 mg/100 g (Yuzhen and Fuheng, 1997). However, leaves are a better source of flavonoids than fruits. Value of flavonols in pulp oil ranged from 153 to 350.3 mg/Kg (Singh et al., 2011).

Carotenoids: Among the carotenoids, fruit of seabuckthorn is very rich sources (10-30 mg/100g) of many carotenoids (Total 39 identified), particularly B-carotene, one of the most active bioactive substances and a precursor of vitamin A. Carotenoids are effective remedies against burn, frostbites, ulcers and various gyniecological problems. Pulp and fruit oils are of yellowish colour, as it a good source of carotenoids, which vary from 900–1000mg/100 g for

Pamirs seabuckthorn (Mironov, 1989) to 314 to 2139 mg/100g for Chinese seabuckthorn (Zhang et al., 1989). In Himalayn seabuckthorn, total carotenoid value ranged from 15.67 to 323.7 mg/kg in pulp oil (Singh et al., 2011).

Phytosterols: Phytosterols are plant sterols, with structures similar to cholesterol, which on consumption are capable of lowering the plasma cholesterol in humans (Thurnham, 1999). The total sterol content in oils of the soft parts varies from 1% to 3%, depending on the source of raw material and method of oil isolation (Lagazidze et al., 1984;, Xi et al., 1997). The total sterol content in the seed oil varies in the range of 1-2% (Xi et al., 1997, Yang et al., 2001). The major phytosterol in seabuckthorn oil is sitosterol (β-sitosterol) followed by 5-avenasterol.

Other compounds: Radical-scavenging proanthocyanidins are also found in the seed (Fan et al., 2007). Seabuckthorn fruit is also rich in several other vitamins, including B1, B2, K and bioflavonoids (Bekker and Glushenkova, 2001). There is 0.09-0.36 % betaine compound (methylating product of glycine) which has very strong anti-ulcer activity and also curative effect on arteriosclerosis. Chlorogenic acid and other phenol compounds stimulate gastric secretion and take part in the diuretic function. The leaves and fruits contain certain coumarins, which strengthen the function of blood circulation in capillaries, anti-vitiligo, anti-tumorigenesis and regulate the disorders of gall bladder. It has the effect similar to adrenocortical hormone (ACH). It cures bronzed skin, health wounds, ulcer and inflammation (In Mingyu et al., 1998).

Nutrients of leaves: Seabuckthorn leaves contain mainly include flavonoids, carotenoids, free and esterified sterols, triterpenols, and isoprenols. The leaves are also rich in important antioxidants including _-carotene, vitamin E, catechins, elagic acid, ferulic acid, folic acid and significant values of Ca, Mg and K. The polyphenolic compounds in the leaves are represented by flavonols, leucoanthocyanidins, (−) epicatechin, (+) gallocatechin, (−) epigallocatechin and gallic acid. In the study by Shipulina et al. (2005), the tannin fraction was isolated from leaves and identified strictinin, isostrictinin, casuarinin, casuarictin. Upadhyay et al. (2010) estimated some of its bioactive phenolic constituents, such as quercetin-3-O-galactoside, quercetin-3-O-glucoside, kaempferol and isorhamnetin in aqueous and hydroalcoholic seabuckthorn leaf extracts.

3. Pre-clinical Studies

Anti-oxidant: Oxidative damage to cells has been found to be the main cause of pathological disorders and range of diseases (Halliwell, 1987). Geetha et al., (2002a) used alcoholic leaf extract of H. rhamnoides (500 g/ml), which inhibited chromium induced free radical production, apoptosis and restored the antioxidant status and mitochondrial transmembrane potential to that of control cells. Geetha et al., 2005) also suggested that seabuckthorn has significant immunomodulatory activity and specifically activates the cell-mediated immune response. Ting et al. (2011) observed that seabuckthorn seed oil showed strong inhibition of oxidative damage induced by CCl4 on mice, increased the activities of antioxidant enzymes and decreased the lipid peroxidation in liver. Ju Haisong et al. (1989) observed that total flavonoids of Hippophae (TFH) could significantly prohibit the chemiluminescence of the human polymorphonuclear leukocytes, induced by Phorbol Myristate Acetate (PMA) and neutralized the super-oxide free radicals.

Anti-stress and adaptogenic activity: The plant preparations, which improve physical endurance, mental function and non-specific resistance of the body to stress have been defined as adaptogens (Brekhman, 1980). Saggu et al. (2007) administered the leaf aqueous extract orally in rats at a dose of 100 mg/kg BW both in single and five doses, 30 min before C–H–R exposure and found significant anti-stress and adaptogenic activity.

Cardioprotective effects: Polyphenols in seabuckthorn fruit and leaves are well known to improve the functioning of cardiovascular system. Wang Bingwen et al. (1993) observed that TFH strengthens the function of cardiac or myocardial contraction and diastrolization, regulate blood pressure, and decreases the peripheral resistance in white rats. Further studies showed that TFH could improve the contractility of the extracorporeal papillary muscles of guinea pigs. Chai Qiuyan et al. (1989) found that seabuckthorn oil could decrease cholesterol, triglyceride and β-lipoprotein (LP) and counteract hyperlipemia induced by the use of high fat diet in white rats. Flavonoids of seabuckthorn were shown to decrease the production of pathogenic thromboses in mouse (Cheng et al., 2003). In a study using rabbit as an animal model, administration of seed oil along with high cholesterol diet restricted further rise of total cholesterol and caused a significant decline of triglyceride and LDL-cholesterol (Basu et al., 2007). Pang et al. (2008) showed the antihypertensive effect of total flavones at the dose of 150 mg/kg/day. Malik et al.

(2011) standardized the fruit pulp oil of ssp. turkestanica growing in Himalayas at 20 ml/kg per day, which significantly modulates hemodynamic and anti-oxidant derangements in rats.

Anti-diabetic activity: Sharma et al. (2011) evaluated the antidiabetic and antioxidant effect of seabuckthorn (Hippophae rhamnoides L.) in streptozotocin-nicotinamide induced type-2 diabetic rats. Sea buckthorn produced a significant ($p< 0.05$) reduction in blood glucose levels and TBARS levels in the STZ-diabetic rats. GSH, reduced significantly ($p< 0.05$) in diabetic rats, was brought back to near normal levels by co-administration of sea buckthorn. The results of the study indicate the role of oxidative stress in the induction of diabetes and suggest a protective effect of sea buckthorn in this animal model. Similar findings were observed by Dubey et al. (2011). Wang et al. (2011). Reviewed the literature on the anti-diabetic properties of seabuckthorn.

Anti-bacterial and anti-viral effects: Hiporamin, a purified fraction of polyphenol fraction, was found to possess a very strong anti-virus activity and wide range of action against Influenza and Herpes viruses (Shipulina et al., 2005). Hiporamin was observed by its inhibitory effect on the viral neuraminidase in case of influenze. It also showed inhibitory effect in a HIV infection in the cell culture. Jain et al., (2008) showed that seabuckthorn leaf extract also has a significant anti-dengue activity. The aqueous extract of seabuckthorn seeds was evaluated for antioxidant and antibacterial activities (Chauhan et al., 2007). Upadhyay et al. (2010) observed aqueous and hydroalcoholic leaf extracts showed growth inhibiting effect against Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis.

Healing of chronic wounds: Xu Mingyu et al. (1993) observed that seabuckthorn fruit oil has obvious effects on the anathrepsis, eliminating inflammation and slough. L.D.Lebedeva et al. (1989) observed that seabuckthorn pulp oil decreased the inflammation, which was induced in the subcutaneous tissue of the mouse. Upadhyay et al. (2011) observed that topical application of seabuckthorn increased neovascularization, collagen synthesis and stabilization at wound site. Gupta et al. (2006) obtained positive healing effect of topical application of seabuckthorn flavone (isolated from fruit pulp) (1.0%, w/v) on dermal wounds in experimental rats.

Upadhyay et al. (2009) demonstrated the safety and wound healing efficacy of seed oil on experimental burn wounds in rats. Treatment promotes significant re-epithelialization and wound closure and enhances formation of granulation tissue and collagen biosynthesis in burn wounds. Seed oil absorbs strongly in the UV-B range (290–320 nm) and can be used as a natural sun burn

protector (Beveridge et al., 1999). Jiang Zhenyi et al. (1989) observed that healing effect of seabuckthorn seed oil on the white rats' gastric ulcer, induced by acetic acid and chronic reserpinization was better than the cimetidine. They isolated the active principle from seabuckthorn seed oil and found that β-Sitosterol- β-D-Glucosid was a compound responsible for healing the gastric ulcer. Dogra et al. (2011) found seabuckthorn seed oil @5ml/animal in group 3 orally two times a day till endoscopic evidence of healing of the GUE lesions. Wu Airu et al. (1992) studied the effect of seabuckthorn oil on the chronic cervicitis. Oil embolus with seabuckthorn compounds had the general curative effect up to 97%.

Seabuckthorn leaf extract countered the radiation damage to hemopoietic system and restored the ferric reducing activity of plasma (Goel et al., 2002). More than 50% protection was estimated at 15–40 mg/kg BW given intra-peritoneal. It was reported that seabuckthorn extracts act as an antioxidant preventing cellular and mitochondrial free radical generation that could contribute to its ability to inhibit radiation induced apoptosis and cytotoxicity (Agrawala and Adhikari, 2009).

Hepatoprotective activity: Contaminated food, environmental pollutants and drugs are known to badly affect, damage and weaken the liver, causing the hepatitis or cirrhosis (Zimmerman and Ishak, 1994). The hepatoprotective activity of seabuckthorn leaves and seed oil was studied (Geetha et al., 2008), and it was found that seabuckthorn leaf alcoholic extract and seed oil ameliorated CCl4-induced liver injury as evidenced by both histological and biochemical observations. Maheshwari et al. (2011) identified some of the phenolic constituents of leaves, like gallic acid, myricetin, quercetin, kaempferol and isorhamnetin in the phenol rich fraction (PRF) by RP-HPLC. Oral administration of PRF at dose of 25–75 mg/kg BW significantly protected from CCl4 induced elevation in aspartate aminotransferase, alanine aminotransferase, glutamyl transpeptidase and bilirubin in serum, enhanced the hepatic antioxidants.

Cancer: Seabuckthorn acts directly as well as indirectly on cancer cells and by improving general immunity and neutralizing the carcinogens. Early investigations on anti-cancer effects of seabuckthorn have concentrated on extracts from bark (Ambaye et al., 1962) and 5-hydroxy tryptamine (5-HT) has been suggested to be the main responsible component. Peizhen et al. (1989) studied on the mice with transplanted tumors, including sarcoma (S180), lymphatic leukaemia (P388) and B16 and observed that both intraperitoneal injection and oral administration of seabuckthorn oil inhibited the tumor from developing. Seabuckthorn juice could both kill the cancer cells of S180 and P388 and inhibited growth of the cell strains of the

human gastric carcinoma (SGC7901) and lymphatic leukaemia (L1200). Yang Jianping et al. (1989) found that seabuckthorn oil and fruit residual, inhibiting the Ellis ascites carcinoma in vitro in the mice. It was found that it could prolong the life of the mice with Ellis-ascites carcinoma.

Padmavathi et al. (2005) found that fruit extract also had a positive effect on all antioxidant enzymes, and decreased the lipid peroxidation, showing reduced levels of cellular oxidation processes. The study also found that it decreased tumor incidence of skin and forestomach papillomagenesis in mice. Yasukawa et al. (2009) isolated from the active fraction of the 70% ethanol extract of seabuckthorn three phenolic compounds, (+)-catechin, (+)-gallocatechin, and (−)-epigallocatechin and a tritepenoid, ursolic acid which exhibited remarkable anti-tumor activity. A comparative activities of seabuckthorn fruit extracts having varying compositions against cell proliferation in the Caco-2 (colon) and Hep G2 (liver) cancer cell lines. It was found that ethyl-acetate soluble extract showed the strongest antiproliferative effects on Caco-2 cells. The ursolic acid was much higher in this extract than the others (Grey et al., 2010).

4. Clinical Studies

While traditionally seabuckthorn has been used to treat various human diseases, only few clinical studies have been reported. Wang (1995) treated 30 patients suffering from partial erosion of the cervix with topically applied seabuckthorn seed oil. All the patients were cured after 90 days of treatment. Seabuckthorn seed oil was applied topically (3–4 times a day) to treat 60 children (aged 4 months–12 years) with ulcerative stomatitis; 55 cases were cured after 3-5 days of treatment and two severe cases were cured after 8 days of treatment (Wang, 1992). Yang et al. (2000) used seabuckthorn seed oil to treat atopic dermatitis, which led to increased level of alpha-linolenic acid in plasma phospholipids. Eccleston et al. (2002) found that seabuckthorn juice affects the risk factors (plasma lipids, LDL oxidation, platelet aggregation and plasma soluble cell adhesion protein concentration) for coronary heart disease in humans possibly due to the high antioxidant levels. In one study, patients with ischemic heart disease were given total flavonoids of seabuckthorn, 10 mg, 3 times day for 6 weeks (Chai et al., 1989). The patients showed a decrease in cholesterol level and improved cardiac function.

5. Studies on Safety and Toxicity

Seabuckthorn fruit extract has a significant protective role against arsenic-induced oxidative injury (Gupta and Flora, 2006). All the biochemical parameters related to fuel metabolism, liver function and renal function and haematological parameters remained within normal limits following acute or subacute (30 days) administration of the seabuckthorn leaf aqueous extract. In sub-acute toxicity studies of 10 and 20 times of maximal effective dose, administration for 14 days, the body weight gain and biochemical parameters related to toxicity namely serum bilirubin, creatinine, were unaltered and comparable to controls (Saggu et al., 2007). Upadhyay et al. (2009) also studied safety and toxicological studies of CO2-SFE extracted seabuckthorn seed oil acute and sub-acute oral toxicity studies, no adverse effects were observed in any of the groups administered with SBT SFE-seed oil.

6. Global Trade

The first major seabuckthorn company "Altavitamina was launched in Russia, producing variety of seabuckthorn food products, oil and cosmetics. However, China has emerged as global leader on seabuckthorn, which has established over 500 seabuckthorn based industries producing over 200 food, cosmetics and medicines. Germany is emerging fast on seabuckthorn cultivation and commercial production of seabuckthorn. Presently, over 40 countries are engaged in commercial cultivation and production of seabuckthorn products. There is a annual global trade of about 2 billion USD, dominated by Chinese, Russian and German companies. Presently, in India, there are 4-5 small seabuckthorn companies, producing 5-7 seabuckthorn food and cosmetics for local consumption. However, there is much more potential in this area once commercial cultivation of seabuckthorn pick up in cold desert areas of Himalayan states.

It can be concluded that seabuckthorn fruit oil and leaves are quite rich in variety of nutrients with medicinal values. Its efficacy has been proven by preclinical trials in many areas like anti-oxidant, adoptogen, wound healing, skin and cardio-vascular diseases. More evidences are required in the area of cancer treatment. Further, there are needs for the clinical trials particular on characterized and cultivated species of the plant.

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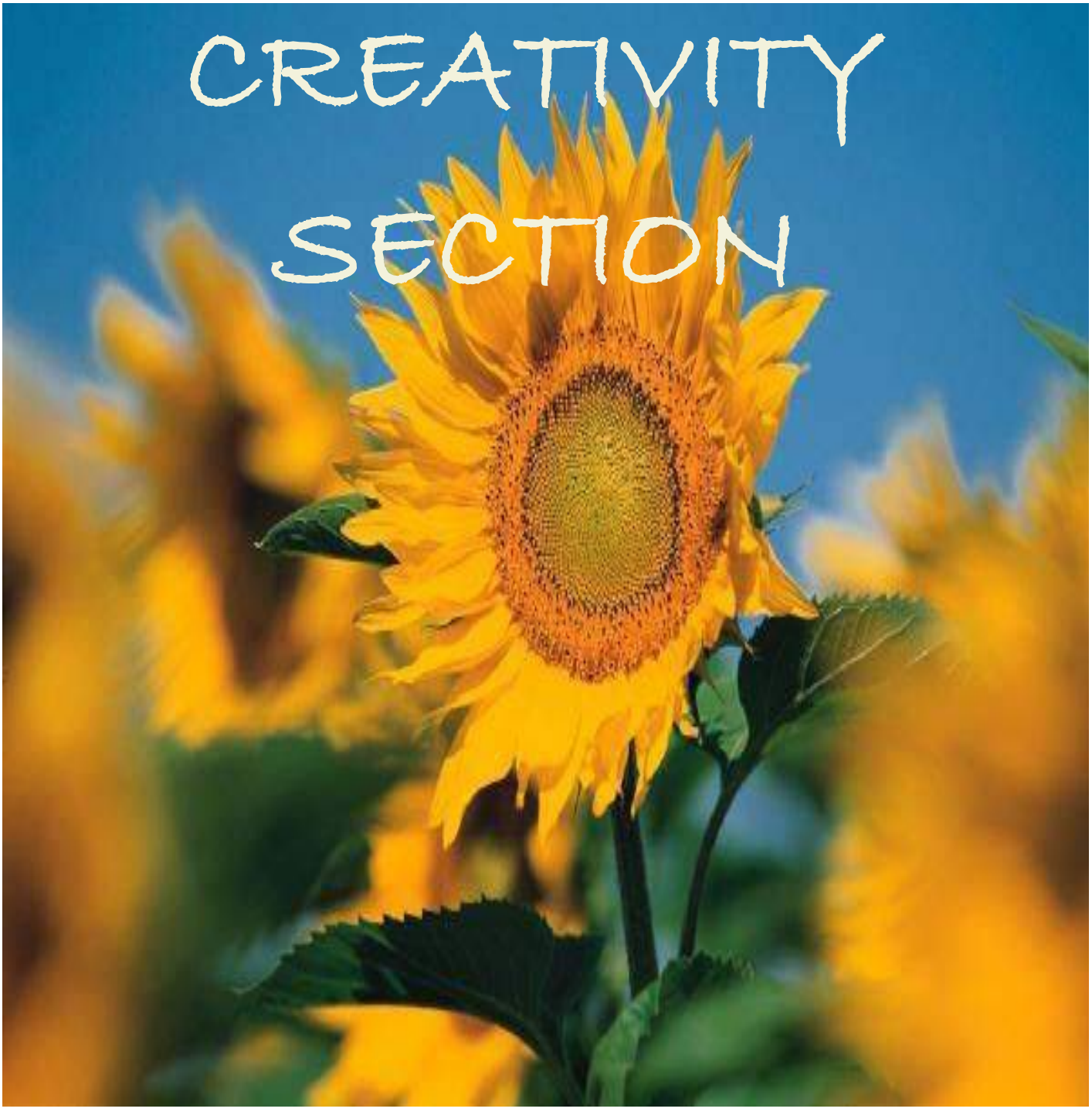

PHOTOGRAPHY

Here is the Photography of the winners of '**Photomania**' – online photography competition conducted by Art Club of Institute of Chemical Technology, Mumbai:

***Name**: Shruti Thakur*

***Photo**: Parrots and Squirrel*

***Rank**: 1st position*

***Name**: Divya Agrawal*

***Photo**: Oleander Hawk Moth*

***Rank**: 2nd position*

***Name**: Anand V Patwardhan*

***Photo**: Turning Path*

***Rank**: 3rd position*

***Name**: Aman Tandon*

***Photo**: Sunset At The Nilgiris*

***Rank**: 4th position*

***Name**: Ghata Nirmal*

***Photo**: Tricoloured Chrysanthemum*

***Rank**: 5th position*

Painting

Rashmi Garg (T.Y.B.Tech.Textiles)

Sketch

Sanket Sabnis (Final Yr. Chem. Engg.)

Poetry

गर्जा महाराष्ट्र माझा

आता पुन्हा तुमची तळपती तलवार
हवी राजे.. !!!
राजे असा कंटाळा करून चालणार नाही
माझ्याशिवाय तुमच्याशी
खरे कुणीच बोलणार नाही
'गाईड'होण्याची संधीही
मी कशाला हुकवतो?
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
सुरुवात शिवनेरीपासून?
की,रायगडापासून करायची?
उलटी की सुलटी?
कोणती मळवाट धरायची?
असे कोड्यामध्ये पडू नका,
कुणालाच उपदेश नको,
"आपापसात लढू नका"
तेव्हाही पटले नाही,
आताही पटणार नाही.
मरतील पण सवयीपासून
मागे कुणी हटणार नाही.
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
शिवबा घडवायचा असेल तर
त्यासाठी जिजाऊ असली पाहिजे
शहाजीच्या मनामध्ये
ही आस ठसली पाहिजे.
पण आजकाल हे सारे
घडताना दिसत नाही
तुमचे चरित्र वाचायला गोड वाटते
पण पचनी पडताना दिसत नाही.
पोकळ मराठी बाणा तर
बघा स्वत:लच फसवतो
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
ते बघा नवे देशमुख,
ते बघा नवे देशपांडे,
वतनदारीसाठी टपलेले आहेत
त्यांच्या आतली काळी माणसं
खादीमध्ये लपलेले आहेत.
पराक्रमाला तोड नाही
कर्तुत्वाला जोड नाही
महाराष्ट्राच्या भल्याची
स्वार्थापुढे ओढ नाही
म्हणूनच तर सह्याद्री
आपला माथा झुकवतो
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
ज्यासाठी जान की 'बाजी' लावावी
तोच खिंडीमध्ये गाठतो आहे
पिसाळांचा सूर्याजी तर
इथे दर फुटा-फुटाला भेटतो आहे.
"आधी लगीन कोंढाण्याचे"
म्हणण्याची
आज तानाजीत हिंमत नाही
बापजाद्यांच्या पराक्रमांची
आज रायबाला किंमत नाही.
इतिहास राहिला नाही
जो तो सोईप्रमाणे
आज इतिहासाला वाकवतो.
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
आपलेच आपल्याला लुटायला

लागले
परक्यांची आवश्यकता नाही
परक्यांनीच लुटले पाहिजे
हा काही त्यांचाच मक्ता नाही.
डोळे मिटलेल्या मांजरीचे
सारे नखरे कळत आहेत
मनातल्या मनात शायिस्तेखानाची
बोटे अजून वळवळत आहेत.
आपलाच गनिमी कावा
बघा आपल्यालाच कसा चकवतो?
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
तो बघ प्रतापगड सांगतोय,
इथेच पराक्रम घडला होता.
अफ़जुल्याचा कोथळा
बाहेर पायथ्याशी पडला होता.
अजूनही अफ़जुल्या
तो पराक्रम सांगतो आहे.
आम्ही आमची अक्कल
उगीच वेशीला टांगतो आहे.
मेलेल्यांशी वैर धरून
कुणी अफ़वा इथे पिकवतो
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
तो बघा ज्याचा प्रदेश,
तिथेच त्याचा किल्ला आहे.
इष्टप्रधान मंडळाचा
फ़ायदेशिर सल्ला आहे.
ज्याचा त्याचा झेंडा आहे
ज्याची त्याची राजमुद्रा आहे.
सुखी माणूस तोच,
ज्याच्या अंगी खादीचा सदरा आहे.
डोक्यावरून पाणी चाललेय
जो तो आपल्यापुरते चुकवतो
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...

शिवा काशिद,मुरारबाजी,
नेताजी,हिरोजी आणि मदारी
आता भेटाण्याची आशा नाही.
जीवाला जीव देण्याची,
मावळ्यांना आता नशा नाही.
खूप झाल्या सेना,
खूप झाले सेनापती,
सैनिका-सैनिकांची वाटणी आहे.
वाईट वाटण्याचे कारण नाही,
आजच्या राजकारणाची
हीच धाटणी आहे.
निष्ठा दाखवायची खुमखुमी येता
पटकन डिजिटल ब्यानर डकवतो
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
पुरंदरच्या तहाची परंपरा
आजकाल जोरात पाळली जाते.
परस्परांचा फ़ायदा असेल तर
राजकीय लढाईही टाळली जाते.
राजकीय मांडवली झाली की,
पाच वर्षापुरते तरी भागते.
राजे,छाव्याला जामीन ठेवायला,
वाघाचे काळीज लागते.
दुसर्याच्या जळत्या घरासामोर
आज आम्ही आपले कपडे सुकवतो.
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
राजे,यांना शहाणे समजू नका,
हे तर चक्क बावळे आहेत.
तुम्हाला केलेय देव त्यांनी,
तुमची इथे देवळे आहेत.
लाज वाटते म्हणून सांगतो,
आम्ही पदरचे रेटत नाही.
तुळजा भवानीने तलवार दिलीच कशी?
आमच्या बंडखोर मनाला पटत नाही.
खरा इतिहास राहिला बाजूला,

ओळखा कोण ह्या कंड्या पिकवतो?
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
शेतकर्यांची अवस्था अशी की,
जसा वेढ्यामध्ये पन्हाळा आहे.
बारा महिने तेरा त्रिकाळ
त्यांच्या आयुष्य़ात उन्हाळा आहे.
राजे,चूकुनही बघू नका
त्यांची अवस्था कशी आहे?
विषासाठी पैसा नसेल तर
घराच्या आढ्याला फ़ाशी आहे.
व्याजाने व्याज वाढत जाते
तरीही विचारतात,
हसा का थकवतो ?
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
सरकार म्हणाले शिका,
पोरं इथले शिकले आहेत.
शिक्षणाची दुकाने तर
वढ्या-वघळीला टाकले आहेत.
सिंहगडाखालचा पराक्रम तर
खरोखरच बघण्यासारखा होता.
रेव्ह पार्टीच्या थोबाडावर
उच्चभ्रुपणाचा बुरखा होता.
येतील तसे दिवस
आपला महाराष्ट्र धकवतो.
राजे, चला तुम्हाला तुमचा महाराष्ट्र
दाखवतो...
जसे राजकारणाचे,
तसे साहित्याचेही झाले आहे.
सहकाराला स्वाहाकाराचे
बकासुरी रूप आले आहे.
आया-बहिर्णींच्या इज्ज्तीची
समस्या तर जटील आहे.
नाक्या-नाक्यावर उभा
जणू रांझ्याचा पाटील आहे.
लोकशाहीचा पाईक मी,
तुमच्या राजेशाहीसमोर
माझा माथा टेकवतो.
राजे, चला
मी तुम्हाला
तुमचा महाराष्ट्र दाखवतो....

- Shrinath Ghadge (S.Y.Chem. Engg.)