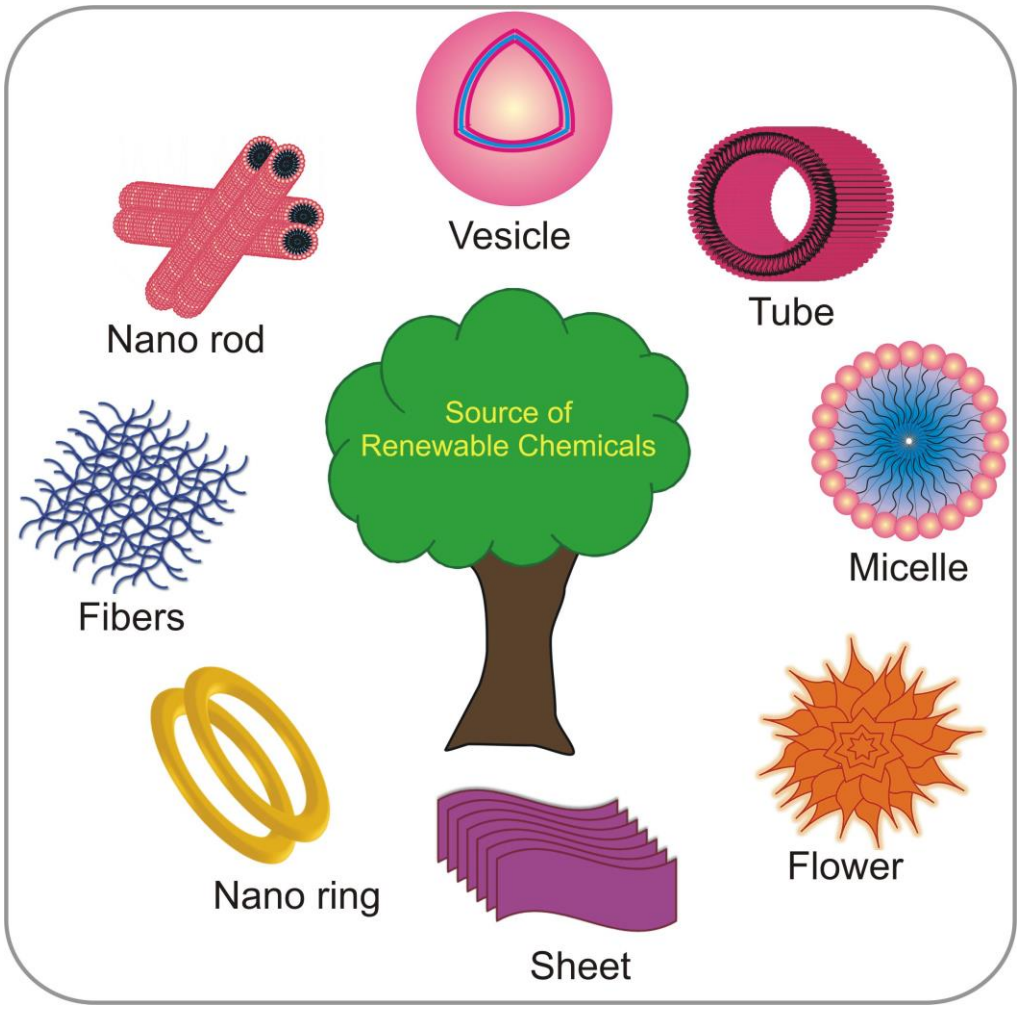
Chapter 1

General Introduction

Part A: Astavarga plants, terpenoids and sterol based natural products

Part B: Self-assembly and Supramolecular Gel



Part A: Astavarga plants and terpenoids and sterol based natural products

1.1 Astavarga plants

Thousands of years ago, an Ayurvedic health tonic, formulated by Ayurvedic wonder healers Ashwini Kumars could rejuvenate the ill, feeble and emaciated body of Rishi Chyavan and he got back his youth. Since then this Ayurvedic formulation has been known as *Chyavanprash* and it became an important and demanding health

Figure 1: Kakoli (*Roscoea purpurea*) plant

tonic for the Kings and the rich people. It contained a set of eight medicinal plants namely *Kakoli, Kshrikakoli, Jeevak, Rishvok, Meda, Mahameda, Riddhi* and *Vriddhi* collectively known as Astavarga plants that grow in small patches in particular ecological environments of Himalaya at the elevation of 1200 – 4000 m from the sea

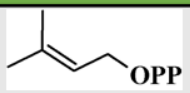
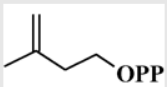
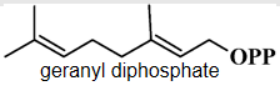
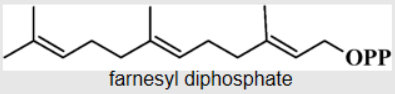
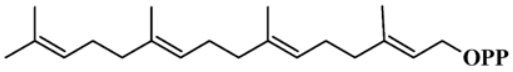
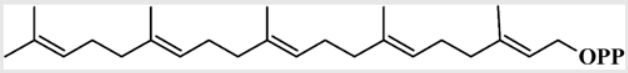
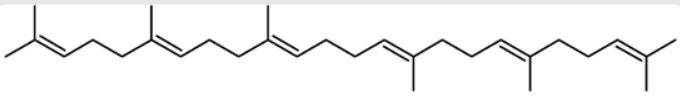
level. However, in early days, the knowledge of Ayurveda (a Sanskrit word, '*Ayus*' stands for life and '*Veda*' stands for science or knowledge) used to be taught orally through the lineages of sages (Rishis) that involved the use the inherent property of nature to maintain and prolong the life of a person via restoration of a balance among body, mind and spirit.[1] But, due to the lack of proper documentation and also because of the fact that the plants grow in the remote regions of Himalaya, the identification of the plants became illusory and difficult. However, recent investigations by a group of scientists and sages have led to the identification, botanical description and classification of all the eight Astavarga plants. Thorough literature search by us have revealed that there is no report of the active chemical constituents of most of the *Astavarga* plants.

Figure 2: Image of Jeevaka plant (*Crepidium acuminatum*)

Figure 3: Vriddhi (*Habneria edgowrthi*) plants and its dried tuber

Figure 4: Dried tuber roots of Mahameda (*Polygonatum verticillatam*)

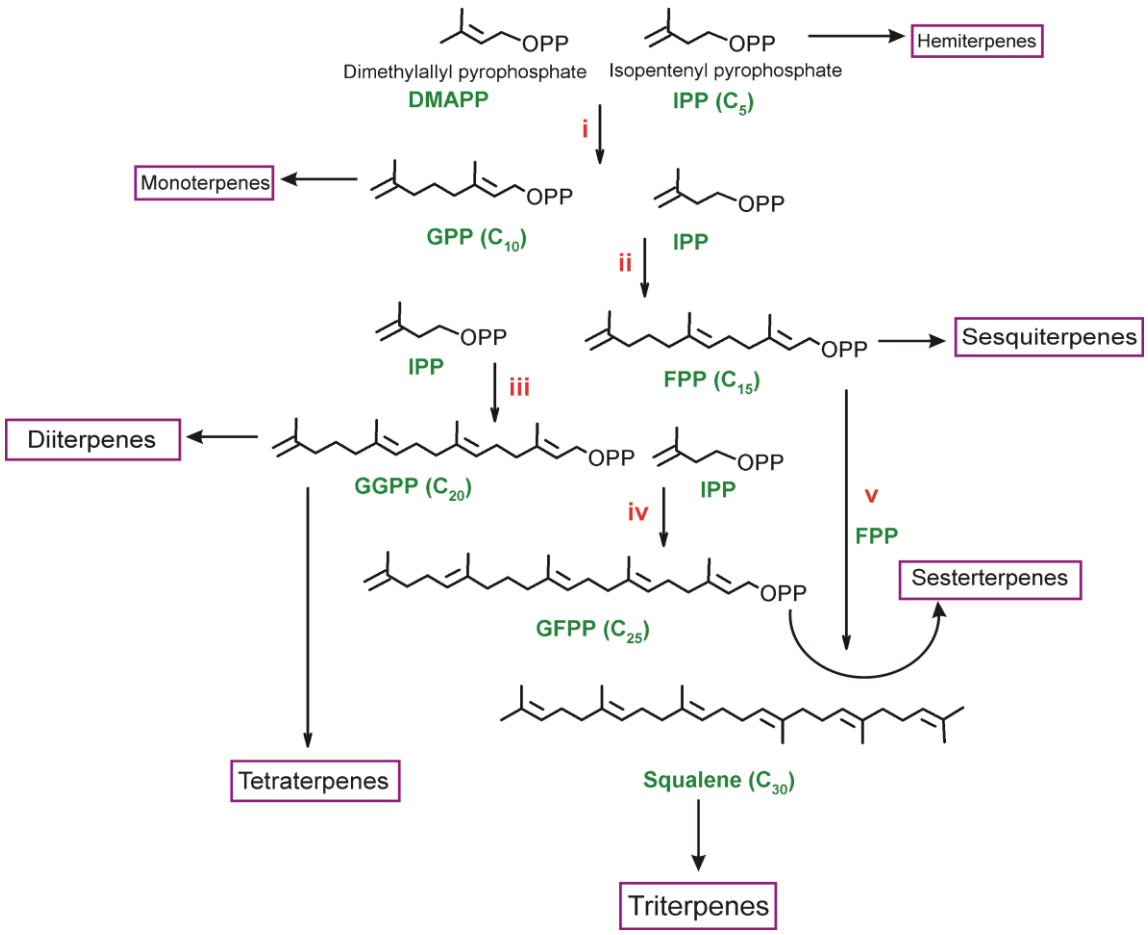
1.1.1 Conclusion: Isolation of phytochemicals from the Astavarga plants, elucidation of the chemical structures and study of the application of the phytochemicals in medicinal chemistry, materials research will open up a new frontier for society and mankind.

Carbon Atoms	Terpene prefix	Linear Isoprenoid Precursor
5	hemi-	 dimethylallyl diphosphate (DMAPP)  isopentenyl diphosphate (IPP)
10	mono-	 geranyl diphosphate (GPP)
15	sesqui-	 farnesyl diphosphate (FPP)
20	di-	 geranylgeranyl diphosphate (GGPP)
25	sester-	 geranylfarnesyl diphosphate (GFPP)
30	tri-	 squalene

1.2 Terpenoids and sterol based natural product

The isolation of natural products from plants plays an important role in organic and biological chemistry. The year 2017 is remarkable for scientific society for twentieth anniversary of terpenoid cyclase structural biology. One of the most complex chemical reactions in biology is terpenoid cyclases catalyze. More than 80000 natural products were characterized including steroids and carotenoids, the terpenome accounts for nearly one-third of all compounds in the Dictionary of Natural Products (http://dnp.chemnetbase.com).[2,3] In nature one of the most complex reactions are terpenoid cyclization reactions, during the course of a multistep cyclization reaction more than half of the substrate carbon atoms undergo changes in bonding, hybridization, and stereochemistry. Terpenoids are a large and structurally diverse group of natural products containing multiple of isoprene unit. All the

terpenoids and sterols are extractable from various natural sources. Monoterpenoids citronellol, nerol, geraniol and camphor were isolated from different plants such as *Rosa hybrid*,[4] *Pelarginium graveolens, Rosa dilecta*,[5] and *Salvia officinalis L.*[6] respectively. Sesquiterpenoid, fernisol and longifolene were isolated from *Candida albicans*[7] and Red Clover Leaves[8]. Diterpenoid, terpentetriene, crotocembraneic acid

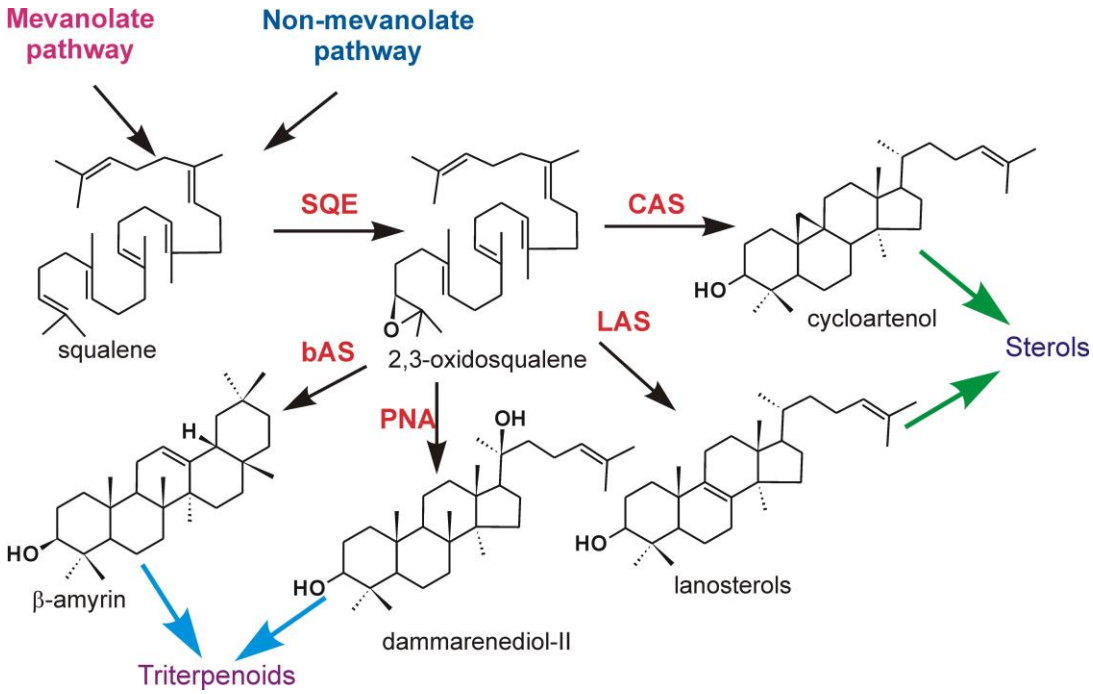


Scheme 1 Enzymes: i, geranyl diphosphate synthase; ii, farnesyl diphosphate synthase; iii, geranylgeranyl diphosphate synthase.

and cis-abienol were isolated from *Boswellia carteriia*[9], *Croton oblongifolious*[10] and *abies balsamea.elesabethatriene* respectively and labdanolic acid were isolated from the *Pseudopterogorgia elisabethae* and Resins from *Copaifera speciesrespectively*. Sesterterpenoid like genopolide and geranyl farnesol etc. were isolated from

Artemisia umbelliformis[11] and *Triticum aestivum*[12] respectively. Triterpenoid like arjunolic acid, betulin, betulinic acid, etc are isolated from *Terminalia arjuna*, *Betula papyrifera Ziziphus jujube*, etc.[13] Two sterols namely stigmasterol and β-sitosterol were isolated from the leaves of *Rubus suavissimus*,[14] campesterol was isolated from several natural sources such as banana, pomegranate, pepper, coffee, grapefruit, cucumber, onion, potato, and lemon grass.[15]

Since Ruzicka's proposal in 1953 of the biogenetic isoprene rule that '*all the triterpenoids are biosynthesized from the common precursor squalene'*, the triterpenoids containing 30C have been recognized as a very special class of natural products. According to the biogenetic isoprene rule, all the terpenes are synthesized from isopentenyl diphosphate (IPP) or its epimer dimethylalyl diphosphate (DMPP) (scheme 1 and 2). There are several sterols in nature which are also biosynthesized from the isopentenyl diphosphate (IPP) or its epimer dimethylalyl diphosphate (DMPP). Biosynthesis of terpenoid and sterol from mevalonate and deoxy-xylulose pathway was investigated in plants.[16] Linear, achiral C_{5n} isoprenoid diphosphates (n = 1, 2, 3, etc.) are formed by head-to-tail coupling reactions of 5-carbon precursors.[17,18] C_{5n} isoprenoid diphosphates are undergoes cyclization reactions to yield a myriad of products typically containing multiple fused rings and stereo centers.[19,20,21,22,23,24] For the cyclization of sterols the common precursor is 2, 3-oxidosqualene. In yeast and in mammals, ergosterol and cholesterol are biosynthesized via lanosterol and catalyzed by lanosterol synthase (LAS), ,[25,26] respectively. In higher plants, phytosterols like sitosterol and stigmasterol, are biosynthesized via cycloartenol and catalyzed by cycloartenol synthase (CAS) (scheme 2).[27,28]



Scheme 2: Triterpenoid and sterol biosynthetic pathway. After the cyclization of 2, 3-oxidosqualene catalyzed by OSC.

All the triterpenoids containing 30C's have nano-metric lengths and detailed computations have been carried out with 120 naturally occurring mono- (C10), sesqui- (C15), di- (C20), sester- (C25), tri- (C30), sesqua- (C35), and tetra- (C40) terpenoids using Gaussian 09 software with Gauss view 07 and molecular mechanics calculation using Serena software. Interestingly, monoterpenoid to tetraterpenoid all the terpenoids (except three monoterpenoid and one sesqui-terpenoid) have molecular lengths above one nanometer making them useful as nano-sized building blocks. Thus terpenoids are nature's renewable nano gift. As the terpenoids and sterols are extractable from plants, and terpenoids with one or more hydroxyl and/or carboxyl groups and a hydrophobic backbone, the terpenoids opened up a new era for the study of their self-assembly properties in different liquids. Similarly sterols

with one hydroxyl and a hydrophobic backbone opened up era for self-assembly study. Seven triterpenoids namely arjunolic acid,[29] betulinic acid[30], betulin,[31] oleanolic acid,[32] glycyrrhetinic acid,[33] ursolic acid[34] and α-onocerin[35] and one diterpenoid crotocembraneic acid[36] and one sterol stigmasterol have been isolated from plants and their self-assembly properties have been studied in different organic and aqueous binary solvent mixtures. Interestingly, all the above mentioned terpenoids and sterol spontaneously self-assembled in liquids yielding supramolecular structures of nano- to micrometer dimensions such as vesicles, fibers, flowers, tubules, etc. Study of the self-assembly of naturally occurring terpenoids is of special significance due to their green and renewable nature, large structural diversity and also because of their tremendous use in various facets of life. The self-assemblies have been utilized for the entrapment of fluorophores, pollutant capture, generation of hybrid materials, etc.[37]

1.2.1 Conclusion

Terpenoids and sterols are a diverse group of natural products in plants and have a wide range of applications in food, cosmetics, pharmaceutical industries and biotechnology sectors. Biosynthesis of terpenoids and sterols has so far been reported to be mainly from the classical mevalonate pathway**.**

PART-B

Self-Assembly and supramolecular Gel

1.3 *Self-Assembly*

1.3.1 Introduction

Molecular self-assembly is the assembly of molecules into an organized structure with the aid of multiple intermolecular forces including relatively weak non-covalent interactions, such as hydrogen bonding, co-ordination, electrostatic, π-π stacking, van-der Waals and ion-dipole interactions. Nature's power to create many diverse complex biological functions which are based on various non-covalent interactions of covalently prefabricated building blocks including bi-layered protein tertiary structures, DNA double helices, lipid liposomes, as well as the complex biological process. The molecular self-assembly is ubiquitous in nature and has recently emerged as a new approach in chemical synthesis, nanotechnology, biotechnology. There are innumerable example of multicomponent self-assembly in nature which inspired the scientific community to create artificial highly ordered, complex, and dynamic protein and peptide-based nanostructures utilizing the self-assembly process.[38] Self-assembly of multicomponent compounds can produce a broad range of more complex feasible structures,[39] provide the possibility to enhance flexibility,[40] and allow the capacity for temporal control and a greater tenability of properties.[41]

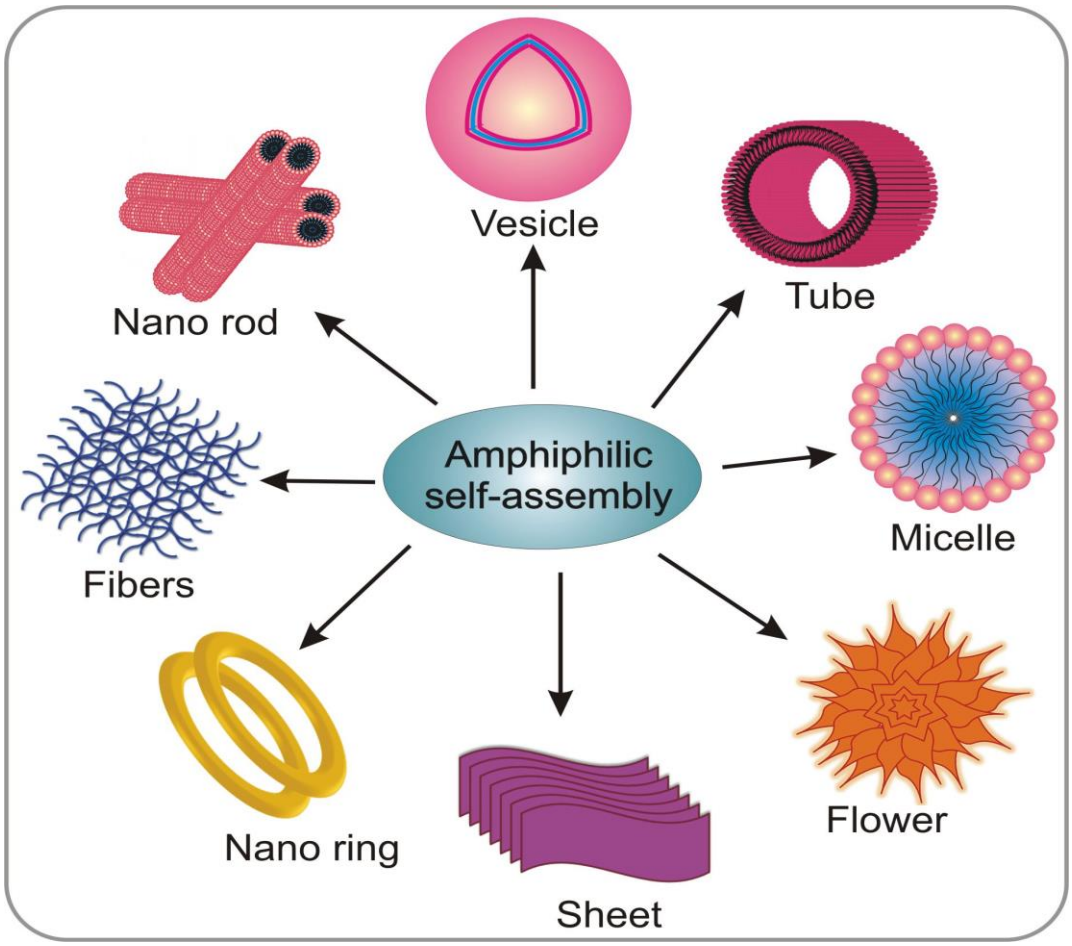


Figure 5: Self-assembly of amphiphiles into different

1.3.3 Self- assembly of amphiphiles

Amphiphiles are synthetic or natural molecules possessing both hydrophilic and hydrophobic properties. According to Tanford[42], two opposite forces are responsible for the formation assemblies. The effect of hydrophobic part of the hydrocarbon chains provides the impetus for self-organization whereas electrostatic forces and steric factor into the polar head groups stop the phase separations.[43] The self-assembly of amphiphile molecules yield a variety of supramolecular architecture like micelles, tubes, vesicles, fibers, and flower (Figure 5). By using properly designed amphiphiles having perfect functional groups and varying the experimental condition, the self-assembled morphologies can be controlled.[37]

1.4 Gel

1.4.1 Introduction

Jellyfish is an elegant example of a hydrogel. Millions of years ago, nature created this living organism containing only 1% of organic matter in 96% water.[44] For the development of new materials, chemists try to mimic nature in the laboratory in different forms.[45] Molecular gels are related with the formation of space arching structures constructed by self-assembly of low molecular weight molecules that affiliated through hydrogen bonding, van der Waals interactions, acid base or π-stacking etc.

Supramolecular or low-molecular weight gels are most fascinating and convenient class of gel. Self-assembly of small molecules into a network prevent the flow of the solvent. These novel materials are becoming increasingly common and offer a scope to prepare interesting, designed, and responsive systems. In recent years, study of gels has become an area of an immense interest for an better

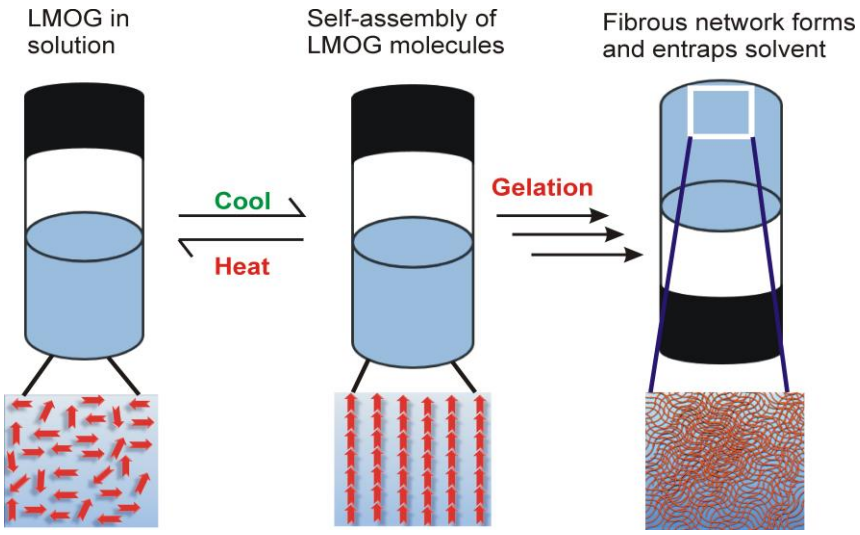


Figure 6: Schematic representation of formation of gel via self-assembly of LMOG's (Low molecular weight gelators)

understanding of the self-assembly process in a medium and also because of their potential applications in drug delivery, optical device, tissue engineering, light harvesting system, removal of toxic chemicals, waste water treatment, cosmetics etc.

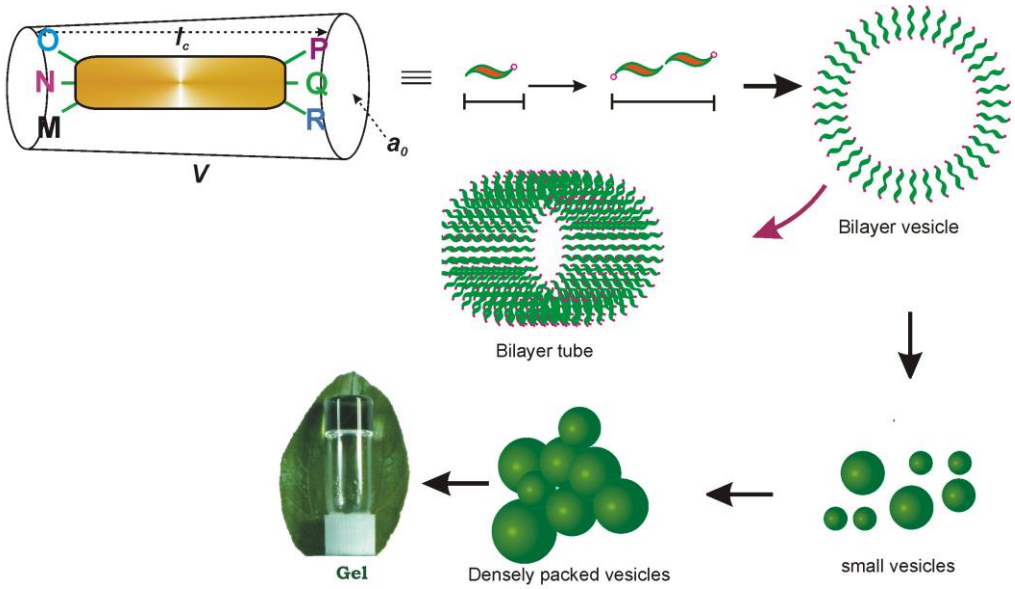


Figure 7: LMOG's yielding vesicles in aqueous binary liquid mixtures via self-assembly, mechanism of the formation of bilayer vesicular and tubular self-assembly and gel (the inverted vial with a leaf of *Terminalia arjuna* in the background contains a supramolecular gel of arjunolic acid in ethanol-water.[37]

Gels obtained from low molecular mass molecules are usually prepared by heating or stirring the molecules in solvent and cooling the resulting saturated solution at room temperature. A simple ''inversion test'' simply identifies a gel, i.e., when a vial is turned upside down the flow of the solvent is stoped under gravitational force. Gel systems contain two contemporary phases; a solid network which immobilizes the flow of solvents and a large amount of liquid phase.[46] Gels are broadly classified into two categories on nature of liquid phase: hydrogels and organogels.[47,48]

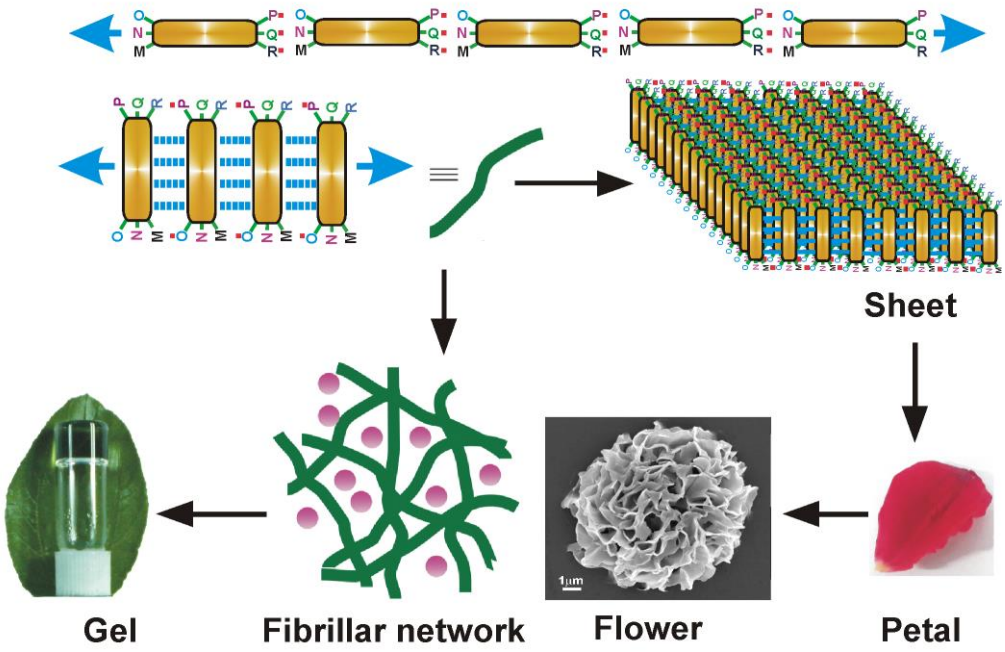


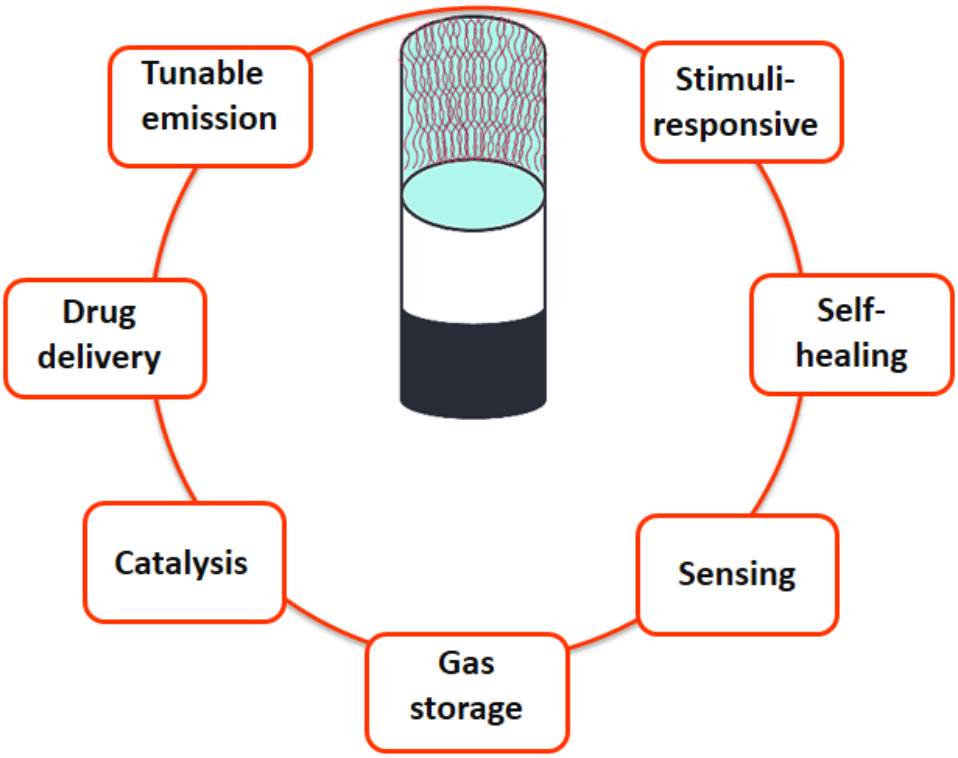
Figure 8: LMOG's yielding flowers, petals and fibrillar networks. Schematic representation for 1D growth, fibrillar network and gel. The inverted vial with the leaf of *Ziziphus jujuba* in the background contains a gel of betulinic acid in o-dichlorobenzene.[37]

1.4.4 Characterization techniques used to study supramolecular gels

Supramolecular gels have many fascinating properties due to their dynamic and reversible nature. The supramolecular gelator molecule can self-assemble in different solvent to form nano to micro-scale superstructures, such as vesicles, fibers, sheets, tubes etc. (Figure 7 and 8). The morphology of the self-assemblies were studied by different techniques such as computational techniques, UV-visible spectroscopy, NMR spectroscopy, FTIR spectroscopy, microscopy techniques, x-ray diffraction techniques, dynamic light scattering, rheology and thermal analysis.

1.4.5 Application of Gel

LMOGs are recognised as viscoelastic, thermo-reversible and solid like material. When the solvents molecules are entrapped within the 3D network under relevant condition it forms a gel. LMOGs can find use in diversified area of applications like separation technology, catalysis, cosmetics, sensors, pharmaceuticals and food. Hydrogels of synthetic and natural polymers are being broadly explored as media for tissue engineering.[49] Supramolecular gel can be used as a drug delivery vehicle. Self–assembled nano structures are favourably valuable for delivery and therapeutic



applications because the drug molecules is low aqueous solubility and for this reason a solubilising delivery system is required for deliver the drug molecules at appropriate site. LMOGs has been used extensively to remove toxic dyes from aqueous solution.[50] Malik et al. reported that an adenine based hydrogel can be used for the purification of water containing organic dye molecules such as rhodamine-

6G, methylene blue and crystal violet.[51] Recently, LMOGs has been utilized for the recovery of oil spillages and safer disposal of used domestic oils. [52,53]

1.4.6 Conclusion

Molecular self-assembly is a modern approach for constructing supramolecular architectures yielding novel materials. Self-assembly phenomenon has drawn the attention of scientific community because of its usefulness in the construction of novel materials from simple molecules. Newer self-assembling systems are being developed from short peptides, polymers to complex DNA structures, lipids and proteins etc. These self-assembled materials have a wide range of applications in diversified fields. Once the building blocks are obtainable from renewable resources then the resulting materials will led to a sustainable development without compromising the needs of the future

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