Studies on Photochemical and Biological Properties of Metal Nanoparticle Encapsulated Dendritic Macromolecules and Supramolecular Host Systems

Introduction
Dendritic polymers are a relatively new class of promising polymeric materials with a regular and highly branched three dimensional architecture and numerous potential applications. The properties of this novel class of polymers are drastically different from linear, cross linked and long branched polymers. The branching of branches is the key feature of these polymers and is responsible for their unique properties and applications\(^1\). Dendritic polymers, the fourth major architectural class of macromolecules can be divided into three subclasses according to the degree of structural perfection: (a) dendrimers, (b) hyperbranched polymers, and (c) dendrigraft polymers. Hyperbranched polymers have a statistical structure, dendrigraft polymers have semi-controlled structure and dendrimers have controlled structures. Among these hyperbranched polymers and dendrimers are extensively used\(^2\).

Dendrimers are a unique class of polymers with a very well defined chemical structure which consists of three major architectural components: core, branches and end groups\(^1\). Dendrimers are produced in an iterative sequence of reaction steps, in which each additional iteration leads to a higher generation dendrimer. Two different synthetic approaches are known depending on the starting point: a divergent one, building the dendrimer layer-by-layer from the central core to the periphery and a convergent one, where the dendrimer grows from the periphery towards the central core\(^3,4\). One of the most appealing aspects of dendrimers is that it is relatively easy to control their size, composition and chemical reactivity very precisely. The versatility of dendrimers, in terms of variety of structures and chemical and physical properties makes them potentially suitable for a huge number of applications. Dendrimers has found applications in the field of medicine, host-guest chemistry, catalysis, drug delivery\(^5\) etc.

Unlike dendrimers, which are perfectly branched macromolecules, hyperbranched polymers are nonperfect structures which are synthesized via one-step reactions\(^6\). However, hyperbranched polymers and dendrimers built from AB, functional monomers have many common features because of their similarity in branching. Compared with dendrimers, hyperbranched polymers possess economical attraction due to the much cheaper one-step synthesis. They are promising products for industrial applications. Hyperbranched polyglycerol is one of the most extensively studied hyperbranched polymer. It is an aliphatic polyether with large number of peripheral hydroxyl groups. It is synthesized by the ring opening polymerization of glycidol\(^7\). Compared to other hyperbranched
polymers, it has got a more perfect dendritic structure. This polymer is soluble in water and other polar solvents, biocompatible and is having very low polydispersity. Owing to their unique properties and easier availability in comparison to dendrimers, hyperbranched polyglycerols have attracted increasing interest.

Nanotechnology involves the harnessing of unique physical, chemical, and biological properties of nanoscale materials in fundamentally new and useful ways. It is the understanding and control of matter at dimensions of 1 to 100 nanometers, the size scale between individual atoms and bulk materials. A nanometer is one billionth of a meter and nanotechnology involves imaging, measuring, modeling and manipulating matter at this size scale. At the nanoscale, the physical, chemical and biological properties of materials can differ in fundamental and useful ways from the properties of individual atoms and molecules or bulk matter.

In materials science, nanocomposites with nanoscale dispersed phases and nanocrystalline materials in which the very fine grain size affords quite different mechanical properties to conventional microstructures are already in use. In surface science and surface engineering, nanotopographies offer substantially different properties related to adhesion, tribology, optics and electronic behaviour. Supramolecular chemistry and catalysis have led to novel surface and size dependent chemistry, such as enantioselective catalysis at surfaces. In biological sciences, fundamental understanding of molecular motors and molecular functional entities on the nanometer scale has been responsible for advances in drug design and targeting. Nanoscale functionalised entities and devices are in development for analytical and instrumental applications in biology and medicine, including tissue engineering and imaging. The application areas in which these advances in nanoscience are making their biggest impact include electronic, electro-optic and optical devices. The transition from semiconductor (conventional and organic) technology to nanoscale devices has anticipated improved properties and resolution, e.g. fluorescence labelling, scanning probe microscopy and confocal microscopy. Data storage devices based on nanostructures provide smaller, faster, and lower consumption systems. In medicine, greater understanding of the origin of diseases on the nanometer scale is being derived, and drug delivery through functionalised nanostructures may result in improved pharmacokinetic and targeting properties.

A wide variety of functional nanoscale materials and functional nanoscale surfaces are in use in consumer products, including cosmetics and sunscreens, fibres and textiles, dyes, fillers, paints, emulsions and colloids.

Among different semiconductor metal nanoparticles, silver nanoparticles exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface
plasmon resonance (SPR), well-developed surfaces, catalytic activity, high electrical double layer capacitance, etc. This is why they serve as a material in the development of new-generation electronic, optical and sensor devices.\(^\text{19}\)

When compared with gold particles, silver nanoparticle have excellent optical properties and display a unique ability for amplifying signals in fluorescence and Raman spectroscopies.\(^\text{19}\) Gold nanoparticles find wider scientific and practical application due to their chemical inertness and simple synthesis. Silver nanoparticle non-stabilised in a proper way undergo fast oxidation and easily aggregate in solutions, which complicates their use in the development of sensors and optical instruments. So, methods for the synthesis and effective stabilization of silver nanoparticles is of primary importance.

The main theme of the present work is the synthesis of silver nanoparticles and their encapsulation in the scaffolds of hyperbranched polyglycerol and in the well defined cavities of -cyclodextrin for developing antimicrobial agents with potential applications in chemistry, biology and medicine. Though silver nanoparticles have excellent anti microbial and optical properties and display a unique ability for amplifying signals in fluorescence and Raman spectroscopies, the fast oxidation and easy aggregation in solution complicates their use in sensors and optical instruments and antimicrobial agents.\(^\text{20}\)

Polymer coated functionalized noble metal nanoparticles have recently emerged as an active field of research due to many novel properties of these materials. A growing interest has been developed in the antimicrobial modification of surfaces to prevent the growth of harmful organisms. Coatings based on a slow- release of toxic agents are very effective and have been employed successfully for a long time. However, due to environmental concerns many such systems are to be abolished. Therefore, systems which show antimicrobial effects toward germs on contact without releasing toxic biocides are of strong current interest. Silver nanoparticles possess antimicrobial property and are also non toxic and environment friendly. However due to the lack of binding properties to the surface, they are unsuited as an antimicrobial coating. Hyperbranched polyglycerol is an excellent coating material and it can effectively stabilize silver nanoparticles in its cavities.

Cyclodextrins are a class of non toxic oligosaccharides with a hydrophobic exterior and a hydrophilic interior. They have been extensively investigated in host guest chemistry for construction of versatile supramolecular aggregations.\(^\text{21}\) The antibacterial and antifungal property of silver nanoparticle stabilized in -cyclodextrin aggregates has also been investigated in this work.
Objectives and Scope of the Work

The purpose of the current research work is to explore the synthesis of photochemically modified linear and hyperbranched polymers with photoresponsive and light fastening properties. It also aimed to explore the synthesis of silver nanoparticle dispersed β hyperbranched polyglycerols and – cyclodextrin and assess their utility for bio applications. In view of these challenges, the main objectives of the present work are fixed as:

- To design novel dendritic macromolecules with specific structural architecture and functionally modified with photoresponsive groups.
- To generate silver nanoparticles protected by long aliphatic amines such as dodecyl amine.
- To develop nanoparticle – dispersed dendritic macromolecules modified with photoresponsive groups by substitution – type reactions.
- To study the light induced trans - cis and reverse cis trans isomerisation of the azo chromophoric system bound to linear and hyperbranched polymers.
- To study the light absorption (UV visible radiant power), light stabilization and fluorescence emission properties of dendritic polymers functionalized with photoactive groups.
- To study the light fastening ability of the polymer bound chromophoric systems.
- To study the electron modulation and molecular switching properties and photoresponsive behaviour of the nanoparticle-dispersed dendritic macromolecules.
- To study the antibacterial and antifungal activities of the silver nanoparticle dispersed hyperbranched polyglycerols against various bacterial and fungal strains.
- To study the antibacterial and antifungal activities of the silver nanoparticle dispersed β - cyclodextrin against various bacterial and fungal strains.

Description of the Research Work Conducted

Experimental Methods

Functional modification of linear polyglycerol polyol, hyperbranched polyglycerols and its naphthoylated analogues with chromophoric systems could be used to develop photoresponsive materials which have applications in the areas such as coating materials. In the present work we
developed a chromophoric system with push pull electron modulation, and a series of azo chromophores with potential cis trans isomerisation behavior. Photosensitive linear and hyperbranched polyglycerols were developed by esterifying the hydroxyl functionalities of these polymers with the chromophoric systems through DCC coupling using DMAP as the catalyst.

![Schematic view of the synthesis of chromophore functionalized linear and dendritic macromolecules](image)

In linear polyglycerol, the end hydroxyl groups were preferably esterified with the photoresponsive groups. Thus entanglements like twisting, coiling etc. are possible in this system. In the dendritic system, large numbers of peripheral hydroxyl groups were esterified with the chromophores yielding a non entangled structure.

All the products were purified by column chromatography or membrane dialysis using semipermeable cellulose membrane and the products were characterized by FTIR, UV-visible and FTNMR spectroscopies and fluorescence spectral studies. The light absorption, fluorescence emission and photoisomerisation behaviour of the functionally modified products were systematically investigated.

Another important objective of the present work was to conduct a detailed investigation on the synthesis and stabilization of silver nanoparticles. Silver nanoparticles were synthesized in a versatile and inexpensive method. The synthesis of nanoparticle was achieved by reduction. The reduction process has led to the decomposition of silver acetate that resulted into the formation of spherical silver nanoparticles. The so formed silver nanoparticles were characterized by UV visible spectroscopy, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The synthesized silver nanoparticles were stabilized by encapsulating it in the polyether scaffolds of hyperbranched polyglycerolβ matrix and in the cavities of - cyclodextrin
aggregates. The silver nanoparticles were incorporated into the cavities of the β aggregates of hyperbranched polyglycerol matrix and - cyclodextrin under an inert atmosphere. It was achieved by reacting chloroform solution of silver nanoparticle and an aqueous solution of the host system at room temperature.

The silver nanoparticles were encapsulated in the polyether scaffold of hyperbranched β polyglycerol and in the cavities of - cyclodextrin aggregates to develop materials with antimicrobial properties. The antibacterial and antifungal activity of the nanoparticle β encapsulated hyperbranched polyglycerol and - cyclodextrin were also demonstrated against different bacterial and fungal strains. For in vitro screening, bacteria such as Staphylococcus aureus, Serratia marcescens, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae were used. The antibacterial property was investigated by disc diffusion method. Agar well diffusion method was adopted for analyzing the antifungal activity of the nanoparticle dispersed systems. The antifungal activity was tested against Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Curvularia geniculata, Mucor circinelloides, Mucor ramosissimus, Syncephalastrum racemosum, Chrysosporium sps. and Pencillium species.

Nanoparticle dispersed and chromophore functionalized hyperbranched polyglycerols were also synthesized. Light responses such as photoisomerisation, light absorption, fluorescence emission etc were investigated in these systems.

**Important Results**

Hyperbranched polymers constitute a special class of branched macromolecules characterized by their randomly branched topology. Hyperbranched polymers are attractive because they resemble dendrimers, but they can be prepared on a large scale and at a reasonable cost in a single step synthesis. They possess irregularly branched non-perfect structures with one focal unit and at least two branching points. Hyperbranched polyglycerol represents the first hyperbranched polymer that can be prepared in a controlled manner. Hyperbranched polyglycerols possess an inert polyether scaffold. Each branch ends in a hydroxyl function and it is these densely populated peripheral hydroxyls that render hyperbranched polyglycerols a highly functional material.

Functional modification of linear polyglycerols polyol, hyperbranched polyglycerols and its naphthoylated analogues with chromophoric systems could be used to develop photoresponsive materials which have applications in the areas such as photoresponsive materials. Photochemically active chromophoric systems were developed by condensation or by diazotization –coupling strategy.
Photosensitive linear and hyperbranched polyglycerols were developed by esterifying the hydroxyl functionalities of these polymers with the chromophoric systems through DCC coupling. All the products were purified by column chromatography or membrane dialysis using semipermeable cellulose membrane and the products were characterized by spectroscopic methods.

The light fastening ability of the chromophore functionalized polymers and the chromophores were systematically investigated by exposing to visible light for different time intervals and recording the UV visible spectra as a function of time. The light stabilisation property of the chromophores were tremendously increased by attaching to the polymer backbone. All the chromophores showed sudden fading on continuous exposure to visible light. But the chromophores attached to polymer backbone showed remarkable stability when exposed to visible light. Among the polymeric analogues, the linear polyglycerol functionalized chromophore showed least stability. The hyperbranched systems showed appreciable stability even on prolonged irradiation. The hyperbranched system anchored with naphthoyl group showed maximum resistance to degradation by visible light. The fading out of colours is a serious problem in dye industry. Thus the combination of photoresponsive chromophore and UV absorbing aromatic groups like naphthoyl group in hyperbranched polyglycerols will find extensive applications as coating materials in ink, paint and dye industries.

The main interest of azo chromophore anchored to polymers are their photoisomerisation. The photoisomerisation was investigated by UV visible spectroscopy. The azo dyes and the chromophore functionalized polymers exhibited effective light induced isomerisation from trans to cis form. In all the systems the photostationary state was achieved within 2-3 minutes. Thus, these newly developed systems could be used as molecular switches in sensor applications.

The ground and excited state properties of the chromophore functionalized polymers were also investigated. The light absorption behaviour of various chromophore functionalized polyglycerols has been investigated by UV-visible measurements. The chromophore functionalized hyperbranched polyglycerols showed a notable red shift while the linear polymer showed a blue shift in the absorption maximum relative to the monomeric dye. The red shift observed in hyperbranched polyglycerols arises from the “J aggregation” of chromophore which arises from the irregular branching in the polymer backbone. The entangled structure of the linear polymer is responsible for its blue shift. The differences in the light absorption behaviour of the functionalized polyglycerols in various solvents with differing polarity were investigated. As the solvent polarity is increased from
chloroform to dimethyl sulfoxide, the absorption maximum was shifted to higher wavelengths. This indicates greater degree of solute-solvent interaction on increasing the solvent polarity. Polar solvents stabilize the ground and excited states by solvation. Stabilization of excited state is more pronounced and this leads to absorption at a longer wavelength.

The photophysical properties of the functionalized products were also analyzed by steady state fluorescence measurements. The luminescence properties were significantly enhanced by attaching to hyperbranched polymer backbone. The enhanced emission is due to the formation of intramolecular excimers. In linear polymer and in the monomeric dye, there is the possibility of intermolecular energy transfer between excited and unexcited molecules and thus less intense emissions were observed. The effect of solvent polarity on the luminescence properties of the photosensitive materials was also studied. The emission maximum showed a red shift in polar solvents. This high sensitivity of fluorescence toward solvent polarity is due to the interactions that occur in the local environment during excited state lifetime. Relaxation effects and associated spectral shifts occur as a function of solvent polarity. Increasing the solvent polarity lowers the solvent relaxed energy level and increases the solvent reorientation with respect to fluorophore dipole in the excited state and this result in red shift. When the absorption and emission spectra were compared, a large spectral shift was observed in the emission spectra as compared to the absorption spectra. The fewer shifts in absorption spectra indicate that the ground state energy distribution is not affected to a greater extent. The larger shift in fluorescence is due to the higher dipole moment in the excited state.

Another objective of the present work was to conduct a detailed investigation on the synthesis and stabilization of silver nanoparticles by encapsulating it in the polyether scaffolds of hyperbranched polyglycerol. Silver nanoparticles were synthesized in a versatile and inexpensive method and stabilized by encapsulating in hyperbranched polyglycerol matrix. The synthesis of nanoparticle was achieved by reduction. The reduction process has led to the decomposition of silver acetate that resulted into the formation of spherical nanoparticles. From the UV visible absorption spectrum, a surface plasmon absorption peak for silver nanoparticles was observed at 402nm. No splitting in the absorption spectrum was observed, which suggested that spherical shaped nanoparticles were formed. SEM and TEM analysis supported the formation of spherical nanoparticles with a size range of 8 to 32nm. The antibacterial and antifungal activity of the
nanoparticle encapsulated hyperbranched polyglycerol were also demonstrated against different bacterial strains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serretia marcescens*, *Escherichia coli*, and *Klebsiella pneumoniae* and fungal strains such as *Aspergillus flavus*, *Aspergillus niger*, *Curvularia geniculata*, *Mucor geniculata*, and *Pencillium species*. The results suggested that nano-Ag encapsulated hyperbranched polyglycerol is a potential compound in the treatment of bacterial and fungal infectious diseases. The primary significance of this study is the observation that nano-Ag encapsulated hyperbranched polyglycerol could inhibit the growth of microbes, which cause superficial infections. Secondly, the fact that preparation method of nano-Ag described here is cost-effective is also of importance. Therefore, it could be expected that nano-Ag encapsulated hyperbranched polyglycerol may have potential as an anti-infective agent for disease caused by bacterial and fungal infections.

Cyclodextrins are cyclic oligosaccharides composed of hydrophobic cavities that can form complexes with various organic molecules and hydrophilic rims of hydroxyl groups. Thus cyclodextrins have also been used in the present work for the stabilization of silver nanoparticles. Silver nanoparticles were encapsulated in cyclodextrin aggregates and the antimicrobial activity of this supramolecular system was demonstrated against bacterial strains like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serretia marcescens*, *Escherichia coli*, and *Klebsiella pneumoniae* and fungal strains such as *Aspergillus fumigatus*, *Mucor ramosissimus* and *Chrysosporium species*. Nanoparticle dispersed and chromophore functionalized hyperbranched polyglycerols were also synthesized. Light responses such as photoisomerisation, light absorption, fluorescence emission etc. were investigated in these systems. All the systems showed effective photoisomerisation and light fastening abilities. The efficient absorption and emission properties of the nanoparticle dispersed and chromophore functionalized hyperbranched polyglycerols could be applied in the development of antimicrobial coating materials, paint, additives, adhesives and printing inks.

**Conclusions**

Chromophore functionalized linear and hyperbranched polyglycerols as well as its naphthoylated analogues were synthesized by esterification reactions. The experimental results obtained show that incorporation of chromophores in the polymer backbone enhances the chromophore stability. It was also proven that the light fastening ability of the chromophore can be improved by attaching to polymer backbone. When the polymer backbone was modified with UV
absorbing groups, the light fastening was further improved. The cis trans isomerisation of the azo chromophores was also retained even after incorporation into polymer framework, which could be used for photoswitching applications. The light absorption and fluorescence emission properties of the chromophores were found to be tremendously enhanced by incorporating onto polymer backbone.

The successful synthesis and stabilization of spherical silver nanoparticles was another important result that we obtained from the present work. Silver nanoparticles were stabilized by encapsulating in hyperbranched polyglycerol networks and -cyclodextrin aggregates. The antibacterial and antifungal activity of the nanoparticle dispersed hyperbranched polyglycerol and -cyclodextrin was also demonstrated against various bacterial and fungal strains.

Silver nanoparticles are effective biocompatible antimicrobial agents. Antibacterial and antifungal studies of silver nanoparticle encapsulated hyperbranched polyglycerols may be carried out on more bacterial and fungal stains. The mechanism of growth inhibition also presents an interesting study for our future works. It is possible to conduct a detailed investigation on the photophysical properties of nanoparticle dispersed and chromophore functionalized hyperbranched polyglycerols such as life time measurements etc. The effect of fluorophore concentration, pH and excitation wavelength on the fluorescence emission properties of chromophore functionalized polymers could also be studied. The excited state decay of the functionalized polymers could also be investigated.

References