R&D on Healthcare Materials

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Preamble:

Chemistry Group (CG), Bhabha Atomic Research Centre (BARC) has a robust programme on developing colloidal and nanostructured materials for healthcare applications.This article highlights the evolution of the healthcare materials programme at Chemistry Group, commencing from the colloid research activity originally envisaged in the Physical Chemistry Section of Chemistry Division. While the initial research activity was primarily focused on fundamental understanding of colloidal and supramolecular interactions, over the period of time, a large variety of materials with potential applications in diverse areas have evolved. Naturally, this has led to the expansion of this activity with special emphasis on materials for healthcare.

Introduction and historical background:

As colloids with tailored size distribution, internal structure and surface charge offer great potential in a variety of technologies, a good amount of work has been carried out at Chemistry Group (CG) to understand the preparation, properties and applications of colloids. The colloid chemistry research in CG was pioneered by Dr. C. Manohar during early 1980s. Initial studies were focussed on understanding the microstructure and phase transitions in thermotropic liquid crystalline materials. With the development of first LCD based pocket calculator by Sharp Corporation in 1973, the interest in liquid crystal research gathered momentum worldwide. Phase transitions in thermotropic liquid crystals were of great interest for the development of new generation display devices and Chemistry Division (ChD), CG has made extensive contributions to the theoretical understanding of such systems. In parallel, electro-flotation of colloids and the role of surfactants in electro-floatation was also investigated. With the setting up of small angle neutron scattering facility (SANS) at CIRUS reactor, the group utilised this facility to understand the structure and interactions in surfactant based colloidal aggregates. Using SANS technique, the mechanism of clouding phenomenon in non-ionic micelles were investigated and appropriate theoretical models were proposed to analyse the SANS data.

Structure of surfactant assemblies:

Micelles are aggregates, formed by spontaneous organization of amphiphilic molecules in selected solvents. This organization is largely governed by the hydrophobic effect that drives the lyophobic regions of each molecule away from the solvent and towards one another. Surfactants, block copolymers and several biological molecules are known to produce structures such as micelles, vesicles, tubules and ribbons with at least one of its dimensions in a few nanometers. The microstructure of these organized assemblies can be tuned by judicious selection of molecular/solution parameters and have impact on its applications. The group carried out extensive investigation of physico-chemical properties and microstructure of assemblies formed by a large variety of amphiphiles by utilizing complementary techniques such as dynamic light scattering (DLS) and SANS.

Microemulsions (MEs) are another class of nanostructured fluids that have been investigated using scattering techniques. Microemulsions are isotropic liquids composed of water, oil, and amphiphile with typical dimensions in the sub-100 nm range and hence SANS/small angle X-ray scattering (SAXS) have been extensively used to study its structure.

Some of the pioneering studies in the area of surfactant assemblies were related to the structural transitions in micelles induced by external stimuli. Structural transitions in ionic micelles can be induced by the screening of the electrostatic repulsions between the ionic species in the micelles. Studies carried out in Chemistry Group demonstrated that both inorganic and organic salts can be used to induce such transition. Unlike inorganic counterions, organic counterions are more efficient in inducing micellar growth and subsequent formation of long micelles that exhibits its solution characteristics similar to that of polymer solutions. Typical organic counterions for cationic micelles that have been investigated in detail include salicylate, p-toluene sulfonate, hydroxy-naphthalene carboxylate etc. These counterions come under the class of hydrotropes, compounds that can increase the solubility of organic substances in aqueous solution and are weakly surface active in nature. Long micelles formed by such oppositely charged surfactant-hydrotrope mixtures possess length of the order of microns and radii of typically 2-3 nm. Due to its polymer like structure, these micelles impart strong viscoelasticity to the solution, and the rheological properties of such solutions have been examined in detail.The soft matter research in Chemistry Group gained momentum with the setting up of rheological measurement facility in 2004 for characterisation of such non-Newtonian fluids. The linear and nonlinear viscoelastic properties of wormlike micellar solutions have been explained on the basis of models developed for polymer solutions. It was demonstrated that the self-assembly can be employed to modulate the flow behaviour of fluids with high sensitivity to temperature as the polymer-like structure is formed through non-covalent interaction.

R&D on block copolymer assemblies:

Another interesting class of molecules that has been investigated and can self-assemble to form diverse structures in solution is block copolymers. The maximum extended length of a hydrocarbon chain in a conventional surfactant molecule is typically 10-30 Å. This limits the maximum radius that can be attained for a rod-like assembly of surfactants. On the other hand, block copolymers can form assemblies with much larger dimensions. These are

typically polymers having different solubility characteristics (say poly-ethylene and poly-propylene) linked together by covalent bond. The difference in the solubility of the two segments drives them into self-assembled structures similar to those of surfactants but with a larger size compared to conventional micelles. Thus, these block copolymers can be viewed as surfactants for "macro" micelles. Changes in the molecular weight of the polymer in the two blocks offer a way to control the dimensions of the micelles. Several groups have investigated the synthesis, properties and applications of a wide variety of block copolymer assemblies. Structural transitions in such block copolymer assemblies can be induced by changes in the solubility of the two polymer blocks.

Material synthesis using surfactants:

The hydrophobic/hydrophilic compartments that are present in the self assembled structures can be used as solubilization sites for various reactants and hence as a microreactor for different classes of materials such as metals, semiconductors, polymers and ceramics. Technological developments in various fields require the production of materials with controllable size, shape or pore dimensions. Mesoporous materials with pore dimensions at the scale of a few nanometers find applications in separation science, catalysis etc. Surfactants find extensive applications in producing such morphology tuned materials. Mesoporous silicate is an excellent example of such materials prepared using a surfactant self-assembly template. Quaternary ammonium surfactants such as cetyltrimethylammonium bromide (CTAB) were used as template to produce hexagonally packed pores in mesoporous silicates.

In Chemistry Division, research has been pursued to explore the application of surfactants in the production of inorganic and polymeric materials. Conducting polymers such as polyaniline (PANI) with tailored electronic properties were developed using micelles as the reaction medium. New experimental tools such as electrochemical workstation, Langmuir-Blodgett apparatus and atomic force microscope (AFM) were acquired in the Division. The potentiostat instrument, procured in the year 1999, enabled synthesis of conducting polymers like polyaniline, polypyrrole and polythiophene by electropolymerisation of the corresponding monomers. The conductivity and redox behaviour of these polymers is influenced by several factors like extent of doping, dopant anions, method of synthesis etc., which were investigated by electrochemical techniques to get a fundamental understanding of this versatile polymer. The ability to manipulate the shapes of inorganic nanoparticles remains an important goal of modern materials science. Significant efforts were made to produce size/shape selective nanoparticles of silver, gold etc using surfactant medium. The role of crystal defects, surface energy, preferential adsorption of surfactants etc in inducing anisotropic growth of inorganic crystals was delineated.

Surface chemistry for bio-diagnostics:

The obvious application of colloidal particles with tailored surfaces led to the evolution of this field into development of healthcare materials. Surface functionalization of materials is of great importance in bio-diagnostics whether it is used as colloidal particles as in latex agglutination assays or as solid-phase supports as in immunoassays. Immobilization of

biomolecules on a suitable substrate is crucial in developing efficient biosensors. Polystyrene is a commonly used solid support for immobilization of antibodies in immunoassays. Surface modification through self-assembled monolayers, adsorption of polymers or nanoparticles with specific functionalities and grafting of functional materials etc. are also used for efficient binding of biomolecules on the surfaces during biosensor fabrication. Polyaniline modified surfaces have shown improved binding of triiodothyronine(T_3) antibodies on polystyrene surface that can be used as a substrate for the detection of human thyroid hormone in nanograms/ml level using radioimmunoassay (RIA). Polyaniline thin film was also covalently modified by thiol-ene click chemistry to obtain carboxylic acid tethered polyaniline.The functionalized polymer was used for construction of enzymatic biosensor by covalent immobilization of glucose oxidase, uricase and horse radish peroxidase (HRP) for detection of the corresponding substrate glucose, uric acid and H_2O_2, respectively. The biosensor displayed excellent sensitivity with improved detection limit of these analytes in blood serum. A sandwich electrochemical biosensor was constructed using carboxylic acid functionalized polyaniline as antibody immobilization matrix and used for detection of liver cancer biomarker, α-fetoprotein (AFP). The strategy involved dual amplification, where the signal was amplified not only by the immobilization matrix but also by the detection probe consisting of silver nanoparticle loaded HRP tagged secondary antibody. The sensor was validated by detecting AFP in human blood serum samples where the AFP concentration obtained are consistent with the values estimated using ELISA.

As most of the cancers are associated with more than one marker, simultaneous detection of multiple biomarkers can improve the accuracy of diagnosis. From this point of view, an electrochemical sensor was developed for simultaneous determination of two cancer biomarkers, α-fetoprotein (AFP) and carcinoembryonic antigen (CEA). Bio-functionalized nanoparticles grafted with redox tags were used as the detection probes which produced amperometric signal for the sandwich immunoassay (Fig. 1). The sensor displayed wide linear dynamic ranges that are potentially suitable for point-of-care detection of these cancer biomarkers in blood serum samples.In addition to biomarker detection, covalently modified polyaniline derivatives were synthesized and evaluated in physiological fluids (serum, blood, urine and sweat) for potential application as electrode material in implantable biosensors and energy storage devices.

Owing to abnormal metabolism, cancer cells exhibit upregulation of glycolysis leading to difference in their extracellular microenvironment as compared to normal cells. A novel electrochemical approach has been developed for cancer detection by exploting the difference in glycolysis rate of cancer cells and normal cells. A software controlled portable device, namely, Extra Cellular Acidity Analyzer (ECAA) has been developed based on this approach for rapid screening of cancer in biopsy samples (Fig. 2). The instrument was validated using several cancer cell lines and mice tumor tissues. Clinical studies were carried out with human breast tissue samples obtained from Tata Memorial Hospital under a BARC-TMH MoU. This innovation has been put up as a technology on BARC website and the know-how is transfered for commercialization to five private companies till date.

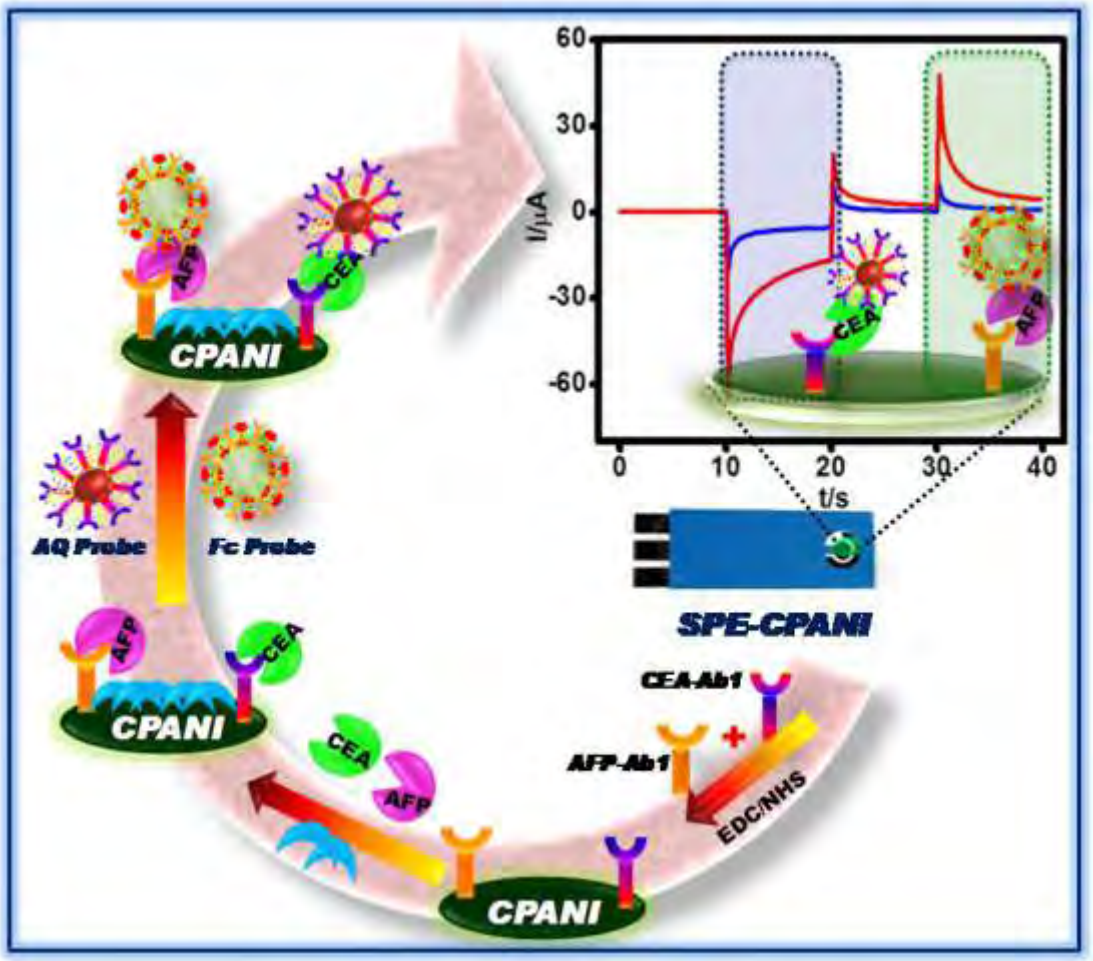

Fig. 1: Schematic representation of the surface modification of carboxylic acid tethered polyaniline over screen printed electrodes for construction of biosensor enabling simultaneous detection of multiple cancer biomarker.

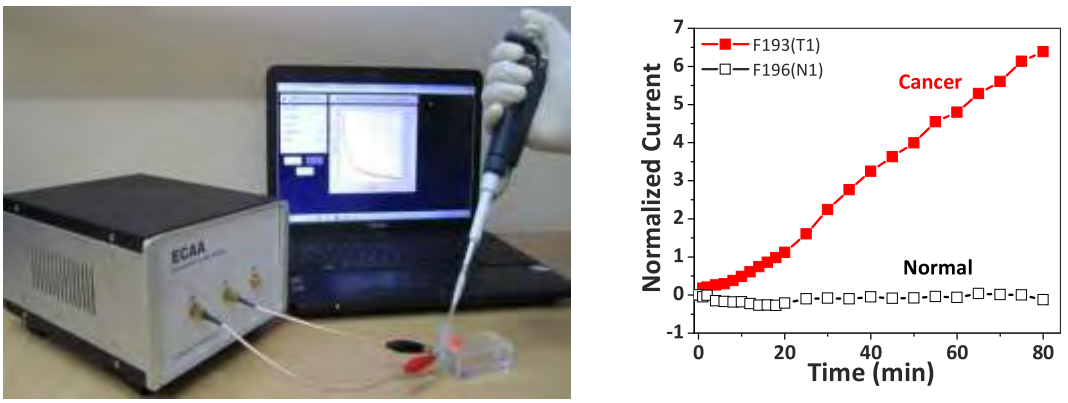

Fig. 2: ECAA setup and the sensor response to cancerous and normal human tissue samples

More recently, an electrochemical sensor array with portable interface has been developed for detection and identification of pathogenic bacteria from the point of view of medical diagnostics. The sensor has potential for use in pathology labs for detection of bacteria in urine/blood. Moreover, the concept can be extended to analysis of food and environmental samples.

A reliable distinction in the fluorescence and circular dichroism spectral features of a probe dye, thiazole orange, on binding to the genomic DNAs isolated from non-cancerous and cancerous tissues is developed as an effective, label-free optical method for the diagnosis of cancer tissues.

Materials for therapeutics:

The formation of colloidal or supramolecular systems with tailored properties is of eminent interest in drug delivery applications. Surfactants and block copolymers spontaneously assemble into structures such as micelles, vesicles, microemulsions and liquid crystalline phases with hydrophilic and hydrophobic microenvironments. One of the issues most frequently addressed in pharmaceutical products by these systems is poor solubility. A low solubility and poor dissolution characteristics of many drugs reduces their bioavailability. Other issues such as poor drug payload, undesirable drug metabolism by endogenous enzymes and excretion of active ingredient by the immune system etc are also addressed with the help of suitable delivery systems. It appears that the structural features of such assemblies can be used to address some of these challenges. Multilamellar vesicles obtained from a biodegradable surfactant PEG-8 Distearate (PEG-8-DS) can be employed for the delivery of sumatriptan succinate (SS), a potent antimigraine drug.

Magnetic nanoparticles with specific surface functional groups were prepared to modulate drug binding, controlled release and targeting to specific sites of interest. Surface functionalised Fe_3O_4 nanoparticles with good colloidal stability, protein resistance characteristics and room temperature superparamagnetic behaviour were prepared by soft-chemical approach. These particles show excellent heating efficacy under AC magnetic field, high loading affinity for positively charged anticancer drug, doxorubicin (DOX) and sustained drug release in acidic environments. Cellular uptake studies of DOX-folic acid conjugated biologically active Fe_3O_4 nanoparticles were also prepared and investigated for cellular internalisation using confocal microscopy. A significant uptake of DOX-folic acid conjugated nanoparticles into WEHI-164 cells was clearly observed. Glycine functionalized hydroxyapatite (Gly-HAp) nanoparticles were also prepared by co-precipitation of calcium nitrate and di-sodium hydrogen phosphate in the presence of glycine with an aim to be used for drug delivery applications. PXRD studies on as prepared sample revealed the formation of single phase hexagonal hydroxyapatite without any secondary phase formation.

Liposomal drug delivery systems have shown promising results due to their ability to penetrate through leaky vasculature and alter pharmacokinetics. A new SAXS facility was installed at Chemistry Group for soft matter characterisation. With a view to prepare liposomal drug delivery systems with PEG coating, the effect of block copolymers on particle size, stability and encapsulation efficiency of phospholipid vesicleswere investigated. The

phospholipid vesicles were prepared by reverse phase evaporation technique and characterised for particle size distribution, surface charge and stability. The interaction of block polymer Pluronic P123 with phospholipid was investigated. Addition of block copolymer offers control over the particle size of liposome and enhances the stability. The encapsulation of the drugs such as DOX, cisplatin, paclitaxel etc.in liposomes composed of mixtures with different polymer: lipid ratio was investigated.

^{177}Lu-labeled $NaGdF_4$:Ho-Yb@m-SiO_2 up conversion nanophosphors (^{177}Lu-UCNPs) were developed for theranostic application. *In vitro* stability assessment showed that ^{177}Lu-UCNPs retained radiochemical integrity in saline and rat serum. From a whole-body SPECT/CT imaging (4 h after injection of ~ 18 MBq of ^{177}Lu-UCNPs in healthy Wistar rat), it is evident that they are preferentially accumulated in liver. Particularly, the absence of any activity in skeleton confirmed no leaching of $^{177}Lu^{3+}$, which is essential for theranostic applications.

Research on the stabilization of G-quadruplex DNAs has received much attention in recent years as prospective targets for chemical intervention of cellular functions, especially inhibition of cancer progression. In this context, Thioflavin T (ThT) has been demonstrated in the exclusive role of inducing and stabilizing quadruplex folding in the human telomeric DNA, which can inhibit the activity of telomerase enzyme, expressed in 85% of tumor cells, and is recommended as a therapeutic agent to arrest cancer progression.

One of the mandates of CG is to develop candidate drugs molecules/formulations for radioprotection against high energy ionizing radiations and/or cancer therapy. In this context, the organometallic chemistry of platinum group metals and main group elements has been accomplished with the objective to evaluate their cytotoxic properties. Similarly, a number of aliphatic and aromatic selenium compounds have been synthesized and investigated for electron-transfer reactions, in-vitro/in-vivo evaluation for radioprotection, redox modulation and anti-cancer activities. The lead compound 3,3'-diselenodipropionic acid (DSePA) has shown potent efficacy against radiation-induced pneumonitis, a major side effect of thoracic-radiotherapy. Additionally, elaborate in-vivo studies have also confirmed anticancer activity of DSePA against leukemia and lung cancers. Recently, CG has also developed deuterated analogue of DSePA (D-DSePA) and biological studies have confirmed the higher therapeutic index of D-DSePA as compared to DSePA in cellular models. Currently, clinical studies aimed at collecting data on safety (Phase I) and efficacy (Phase II) of DSePA as a radiotherapy adjuvant drug is under progress at ACTREC/TMC with financial support from Biotechnology Industry Research Assistance Council (BIRAC), under its "Early Translation Accelerators Funding Scheme".

Various nanoformulations were developed to improve the bioavailability of nutraceuticals such as curcumin, the major active ingredient in turmeric. Curcumin has potential pharmacological benefits such as anti-inflammatory, antioxidant, antiviral, anti-microbial anticancer activities. However, its therapeutic efficacy is limited due to its poor aqueous solubility, low stability and thus low bioavailability. To overcome these problems, different formulations based on natural proteins, amphiphiles and carbohydrates were prepared. A nasal drop concentrate formulation of curcumin was also developed using biocompatible amphiphiles that self-assemble to form micelles. This nanoformulation has capability of encapsulating as high as 50 mg/mL of curcumin. Curcumin containing hydrogel

patches were also developed for wound healing applications. An optimised hydrogel patch with tensile strength 0.6MPa and elongation at break 42% have been prepared. They are made of biocompatible polysaccharide and contain nanocurcumin solubilized in Pluronic F127 micelles. Further, an easy, one step fabrication method has been developed for preparing a curcumin loadedgel. The gel facilitates the release of curcumin entrapped within vesicles which are formed in-situ through self-assembly. Subsequently, the synthesis method has also been extended to prepare a bio-mimic catalytic nitric oxide (NO) generating material by conjugating organ odiselenide with gelatin and demonstrated in animal model for wound care.

Amyloid fibrils, formed by the aberrant aggregation of proteins, are believed to be the root cause of several neurodegenerative diseases such as Alzheimer'sdisease (AD). In this regard, custom synthesized phenylalanine derivatized perylenediimide (L-Phe-PDI) dye has been established as an optical probe for the detection/imaging of amyloid fibrils both *in vitro* and *in vivo* which also established the blood brain barrier/blood retina barrier cross-over features of L-Phe-PDI dye with no toxic side effects. On the other hand, macrocyclic receptors such as *p*-sulfonatocalixarenes etc., modify the inter-protein interactions through molecular recognition leading to the inhibition of amyloid fibrillation. These negatively charged macrocyclic hosts are also employed for the disintegration of mature fibrils through surface charge interactions, a valuable therapeutic for amyloidosis.

Antimicrobial resistance (AMR) is a global health problem as microbes are acquiring new drug-resistance mechanisms day by day. One of the promising strategies to improve the antibacterial efficacy of the drugs is based on supramolecular host-guest chemistry without affecting the chemical constituents of the drug. Interaction of fluoroquinolone drugs (danofloxacin, norfloxacin, ofloxacin etc.) with cucurbit[7]uril (CB7) increases the antibacterial efficacy of the drugs against pathogenic bacteria and extended the shelf-life, which are encouraging for long-acting antibiotic formulations. Establishing the synthesis of *p*-sulfonatocalix[6]arene-functionalized silver nanoparticles composite (ScxAgNP), enhancement in the antibacterial activity of sanguinarine (SGR) drugs against multi drug resistant bacteria has been achieved by activating the biologically active iminium form of SGR on the ScxAgNP composite in presence of ciprofloxacin as a supplement.

Way forward:

The colloids research activity in Chemistry Group, initially focused on fundamental studies, has taken a different route altogether by aligning with the national mission of providing affordable healthcare to the public. The production of surface-functionalized materials has long been motivated as a result of their application in technologies that require the interaction of biomolecules with a given substrate either as film or as colloidal support. With regard to radioprotection and chemotherapy research, Chemistry Group has contributed significantly towards understanding the antioxidant, radioprotective and anticancer activities of organoselenium compounds and one of the lead compounds DSePA is being evaluated as a candidate drug molecule for lung radioprotection and lung chemotherapy. During the last decades, tremendous progress has been made towards translating the fundamental knowledge to tackle some of the intriguing problems in healthcare industry. However, for diffusion of these technologies into the society, there is lot more to be done in terms of regulatory

requirements. For immediate and long term progress into the future, it is required to build strong capability in interdisciplinary and translational research by involving clinicians, technologists and entrepreneurs. Some of the upcoming areas that should be focused for advanced therapeutics include photodynamic therapy, gene editing, immunotherapeutics, targeted delivery, stimuli responsive controls and liquid biopsies for biosensing. It is important that self-reliance in our current and emerging healthcare needs is achieved by inventing new molecules, diagnostic tools and new therapeutic platforms. All this needs to be achieved at an accelerated pace by concerted efforts of all stake holders.