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Materials and characterization methods

2.1: Materials

2.1.1: Pluronic Polymers

Pluronic is a brand name for a class of triblock copolymer surfactants produced by BASF (commonly known as *Poloxamer*). These polymers are composed of blocks of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) arranged in a PEO-PPO-PEO structure [1].

The unique structure of Pluronic polymers gives them surfactant properties, as they have both hydrophilic (water-attracting) and hydrophobic (water-repelling) blocks above >15°C temperature within the same molecule. Pluronic surfactants are frequently employed for synthesizing and stabilizing metal nanoparticles because of they do adsorb at interfaces and forming the micelles. These properties allow them to serve as reducing agent, capping agent, regulating the size distribution, and stability of the resulting nanoparticles [2].

The structure of Pluronic polymers utilized in this study is depicted in Figure 1, while their features are outlined in Table 2.1.

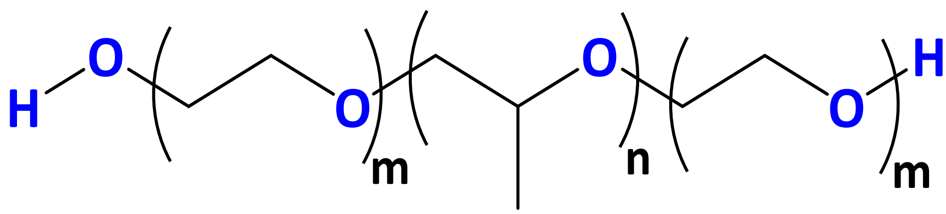

Figure 2.1: Molecular structure of Pluronic polymer (m= no. of EO, n= no. of PO)

Table 2.1: Characteristics of the studied Pluronics.

Pluronics®	Mol. Wt. (g mol^{-1})	Composition	% PEO	CP 1% (°C)	HLB
F68	8400	$EO_{104}PO_{48}EO_{104}$	80	>100°	28
F127	12600	$EO_{100}PO_{65}EO_{100}$	70	>100°	22
L64	2900	$EO_{13}PO_{30}EO_{13}$	40	58°	14
P123	5750	$EO_{20}PO_{70}EO_{20}$	30	90°	8
L121	4400	$EO_{5}PO_{70}EO_{5}$	10	14°	1

Pluronic polymers were collected from Sigma-Aldrich (St. Luice, MO, USA) and directly used in the research.

Figure 2.2: Real images of samples of Pluronic polymers used in the study.

2.1.2: Chitosan (Ch)

Ch, a biopolymer that originates from chitin, offers numerous benefits. It is non-toxic, has a high charge density, and contains reactive hydroxyl and amino groups. Moreover, it exhibits good hydrogen bonding capacity. These characteristics of chitosan make it an attractive material for a wide range of uses [3].

Ch is well-known for its non-toxicity, biocompatibility, antimicrobial characteristics, and biodegradability. These attributes make it valuable in diverse fields, including pharmaceuticals, agriculture, wastewater treatment, biomedical industries, food, and cosmetics [4-6]. Amino (-NH_2) and hydroxyl (-OH) functional groups are present in Ch, and these groups have the ability to interact with metal ions. Such functional groups can act as reducing agents, taking part in reduction reactions by providing electrons to metal ions and also stabilize nanoparticles from agglomerating or precipitating out of solution [7].

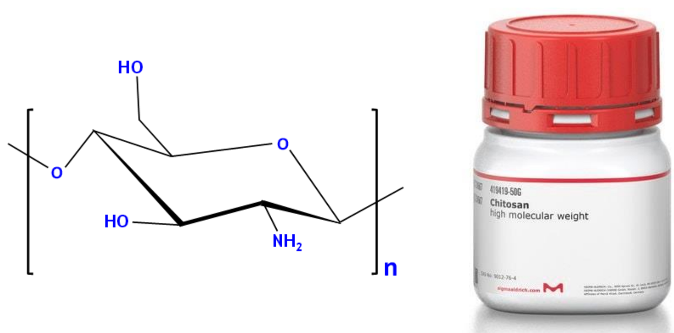

Figure 2.3: Molecular structure and real image of sample of chitosan used.

The chitosan was collected from Sigma-Aldrich (St. Luice, MO, USA) and directly used in the research.

2.1.3: Ascorbic acid (AA)

AA, commonly known as Vitamin C, is a vital water-soluble vitamin that plays numerous essential roles in the human body. It acts as a potent antioxidant, shielding cells from harm caused by free radicals. The body cannot produce AA on its own, so it must be obtained through diet or supplements [8].

AA is highly soluble in water and forms a clear, colorless solution. This characteristic makes it readily available for absorption and utilization in the human body [9]. AA is an organic acid, exhibiting mild acidity with a pKa value around 4.2. This acidity is essential for its physiological functions and plays a role in its antioxidant properties.

AA acts as a reducing agent due to its ability to donate electrons. This property allows it to participate in redox reactions [10, 11].

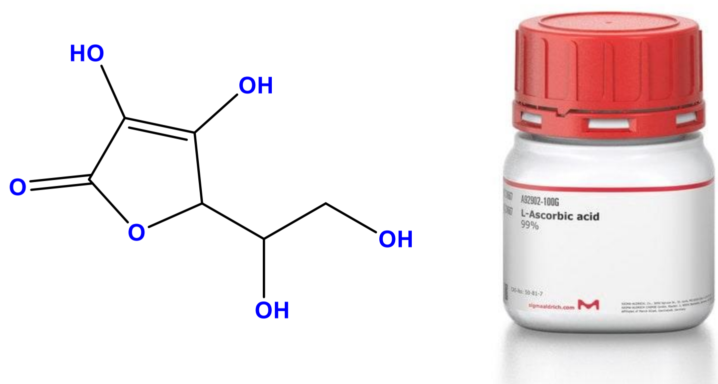

Figure 2.4: Molecular structure and real image of sample of AA used.

L-ascorbic acid was acquired from Merck (India) has the purity of >99% anddirectly used in the research.

2.1.4: Peanut shells (PS)

PS exist as agricultural waste after production of peanuts and its oil. PS made with the composition of lignin, cellulose, proteins, hemicellulose biopolymers, along with other compounds [12].

As agricultural source, PS contains various compounds, including a good amount of silica, which can be extracted and utilized in the synthesis of silica nanoparticles (SiO_2NPs). The extraction process involves silica from the peanut shells ash (PSA) and then converting it into SiO_2NPs [13]. This type of nanoparticle synthesis offers a sustainable and eco-friendly approach by reusing agricultural waste. Here, mainly PS used for synthesis of SiO_2NPs and applications in the development of biosorbent for catalytic activity.

Figure 2.5: Peanut shells

The peanut shell was collected from a farm in Somnath, Gujarat, India.

2.1.5: Organic dyes

2.1.5.1: Congo red (CR)

CR is known to have mutagenic and carcinogenic properties. It has primarily been utilized in industries such as textile, paper, printing, and dyeing rubber and plastic. It is resistant to disappearing due to its synthetic production, which makes it difficult to degrade naturally. It is classified as a potential carcinogen, and its release into the environment through wastewater can lead to contamination, adversely affecting aquatic life [14].

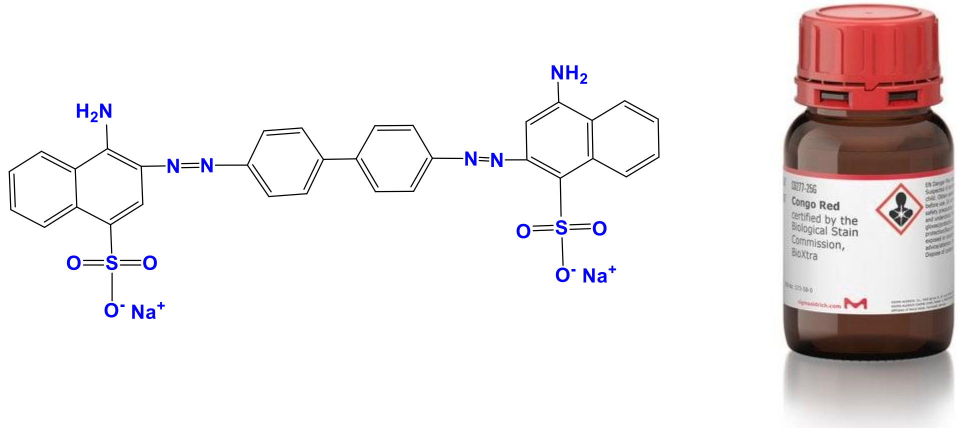

Figure 2.6: Molecular structure and real image of sample of CR used.

CR dye was acquired from Sigma-Aldrich (USA) and directly used in the research.

2.1.5.2: Methyl Orange (MO)

This organic compound is classified as an azo dye and is distinguished by its vibrant orange-red color. It is used in various industries such as textile, food, printing, and pharmaceutical. MO dye can be harmful to both the environment and human health. In water systems, it is resistant to natural degradation, which can lead to pollution and negatively affect aquatic ecosystems. It may also cause skin, eye, and respiratory irritation in humans upon direct exposure. Additionally, the dye's presence in industrial wastewater poses challenges for treatment, contributing to long-lasting environmental contamination if not properly managed [15, 16].

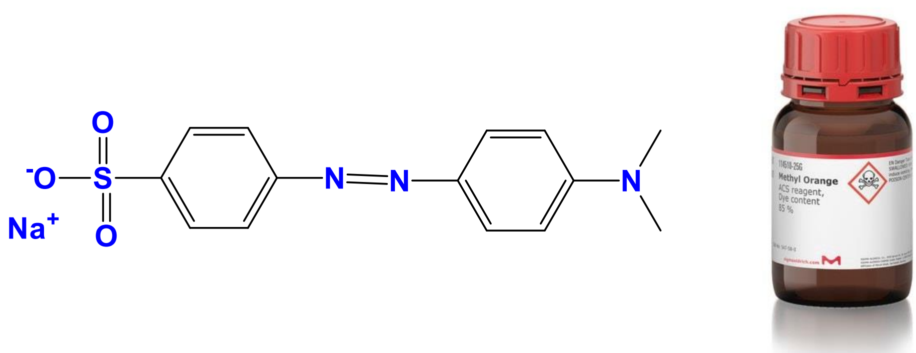

Figure 2.7: Molecular structure and real image of sample of MO used.

MO dye was acquired from Sigma-Aldrich (USA)and directly used in the research.

2.1.5.3: Methylene Blue (MB)

MB is used in medicine to treat methemoglobinemia and as a dye in laboratory settings to stain cells for better visibility. It is extremely harmful to the environment and can cause eye damage, respiratory problems, excessive sweating, cognitive impairment, and methemoglobinemia. its release into the environment through wastewater can harm aquatic life, making proper disposal important to prevent ecological damage [17, 18].

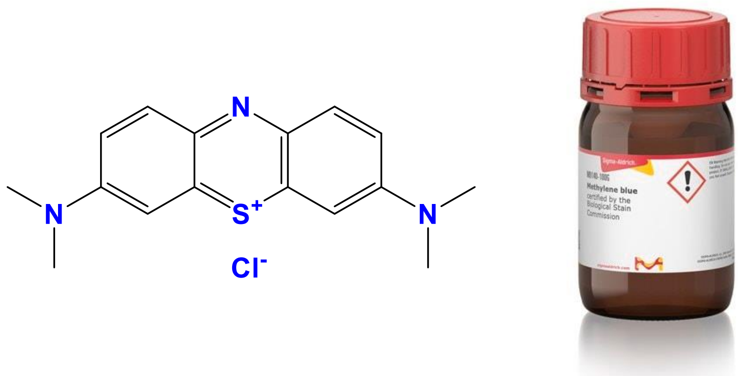

Figure 2.8: Molecular structure and real image of sample of MB used.

MB dye was acquired from Sigma-Aldrich (USA) and *directly used in the research.*

2.1.5.4: Rhodamine B (RhB)

RhB is commonly used as a fluorescent dye in biological research for staining cells and tracking biomolecules, as well as in the textile and paper industries for coloring. This heterocyclic dye poses serious risks to both humans and animals due to its capacity to irritate the eyes, skin, and respiratory system. Its potential carcinogenic effects in high concentrations raise concerns about long-term exposure, and improper disposal into water bodies can lead to environmental pollution, affecting aquatic ecosystems [19].

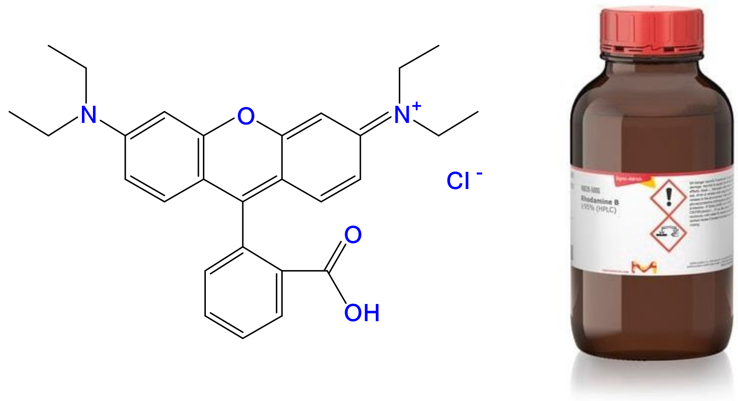

Figure 2.9: Molecular structure and real image of sample of RhB used.

*RhB dye was acquired from Sigma-Aldrich (USA)*and *directly used in the research.*

2.1.6: Other materials used in the synthesis of MNPs

- Sodium borohydride (>99%) was acquired from Merck (India)and used as reducing agent.
- Copper sulphate (99%) was purchased from Merck (India) and used as precursor for the synthesis of CuNPs.
- Nutrient agar (sterile) and agar culture media were obtained from HiMedia (India) for the use in the antimicrobial study.
- Acetic acid (>99%), sulphuric acid (97%), hydrochloric acid (37%), and sodium hydroxide (NaOH) were purchased from Merck (India).

2.2: Characterization Methods

2.2.1: UV-Visible spectroscopy (UV-VIS)

UV-VIS spectroscopy serves as an analytical method for examining how molecules within a sample absorb light in the ultraviolet and visible spectrums. It provides valuable information about a compound's electronic structure, concentration, and chemical environment, enabling qualitative and quantitative analysis [20].

The absorbencies (for determination of concentration and amount of MNPs and other studied organic compounds) of various samples were observed using the UV-VIS spectrophotometer (UV-1900, Shimadzu, Japan) over the range of 200 nm to 800 nm at necessary temperatures.

2.2.2: Fluorescence spectroscopy (FL)

This FL technique serves as a powerful analytical tool for examining the fluorescent properties of molecules. It involves the absorption of light at a specific wavelength, followed by the emission of light at longer wavelengths, a process known as fluorescence [21].

The fluorescence behavior of MNPs and Polymer mediated MNPs were analyzed through Spectro fluorophotometer (RF-6000, Shimadzu, UK) in the range of 200 to700 nm at RT (28°C).

2.2.3: Dynamic light scattering (DLS) and Zeta potential (Zeta)

DLS is a powerful technique used to analyze the particle size, polydispersity, and dynamics of particles or macromolecules in a liquid medium. The method is based on the analysis of fluctuations in scattered light intensity caused by the Brownian motion of particles or assemblies dispersed in a solvent [22, 23].

The DLS and Zeta potential analysis were used to determine the particle sizes in the form of average hydrodynamic dimeter (D_h), and charge on the surface of the MNPs and Pluronic mediated MNPs at RT using the Zetasizer Nano-ZSP system (Malvern, UK), which was outfitted with Nano ZS® software for data requisition and data analysis.

2.2.4: X-ray diffraction (XRD)

XRD stands as a robust analytical method for investigating the crystalline structure of materials. It operates on the principles of X-ray scattering by crystalline solids, delivering valuable details about a material's crystal structure, phase composition, crystallographic orientation, and other relevant structural properties [24].

XRD spectra were obtained by utilizing X-ray diffractometer (Phillips X-Pert MPD, USA). The analysis involved a large beta filter-Ni detector, 3DPixcel technology, and Cu-K radiation (λ = 1.5418). The examination aimed to investigate the phase composition and crystallinity of MNPs, polymers, and polymer mediated MNPs under the conditions of 45 kV and 40 mA. The XRD pattern was systematically recorded over the 20°–80° (2Ө range) at RT.

2.2.5: Field-emission scanning electron microscopy (FE-SEM) and Energy-dispersive X-ray spectroscopy (EDX)

FE-SEM is imaging technique used to visualize the surface morphology, topography, and composition of samples at high resolution. FE-SEM works by scanning a focused electron beam across the sample surface and collecting various signals generated by interactions between the electron beam and the specimen [25].

EDX is an analytical technique used in conjunction with electron microscopes (such as SEM and TEM) to perform qualitative and quantitative analysis of the elemental composition of materials. It provides information about the types and concentrations of elements present in a sample [26].

The surface morphology of various MNPs and other samples was conducted through the utilization of a FE/SEM (Carl Zeiss Supra-55, Germany). EDX was used along with this advanced imaging technology to give a full analysis of the purity and metallic composition of the MNPs.

2.2.6: Transmission electron microscopy (TEM)

TEM is an advanced optical microscopy used to examine the structure, morphology, and 2D structural details of materials at very high resolution. It operates on the principle of transmitting a focused electron beam through a specimen to produce an image with extremely high magnification and resolution [27].

The morphological features (shape, size and structural arrangements) of the synthesized MNPs and other modified MNPs were investigated using the TEM instrument (JEOL JSM-2100F, Tokyo, Japan). This sophisticated imaging technique allowed for a comprehensive investigation of the structural details at the nanoscale.

2.2.7: Fourier transform infrared spectroscopy (FTIR)

Infrared (IR) spectroscopy is a powerful analytical technique used to identify and study chemical substances by measuring their absorption of infrared light. It works by collecting a spectrum that represents the molecular vibrations of various functional groups within a sample [28].

FTIR spectra of the samples were obtained using a Perkin-Elmer 1000 spectrometer (Perkin Elmer, Waltham, MA, USA) with spectral range in between the 4000 to 400 cm^{-1}. The FTIR measurements were conducted in the transmittance mode, employing the KBr pellet approach.

2.2.8: Thermogravimetric analysis (TGA)

TGA is a technique used to measure changes in the weight of a material as a specified temperature range. This method provides valuable information about the thermal stability, composition, and decomposition patterns of substances. TGA is commonly employed in materials science, polymers, pharmaceuticals, and environmental studies to assess factors such as moisture content, thermal degradation, and residual weight [29].

The thermal properties of the MNPs, polymer-mediated MNPs, and other organic samples were examined by using a TGA instrument (Mettler Toledo TGA/SDTA851, Switzerland). The samples placed in an alumina pan, were subjected to heating in a nitrogen atmosphere. The temperature was increased from 25° to 600 °C at a rate of 10 °C/min, while a nitrogen flow of 30 mL/min was maintained.

2.2.9: Specific surface area and pore distribution

Surface area and pore distribution are crucial parameters that characterize the physical structure and properties of porous materials. Techniques such as gas adsorption, commonly using nitrogen adsorption-desorption isotherms, are employed to determine these properties [30].

Nitrogen adsorption/desorption isotherms were measured using a volumetric gas sorption analyzer (ASAP-2020, Micromeritics, USA). The pore volume versus pore diameter distribution was determined by analyzing the isotherms of adsorption/desorption using the Barrett-Joyner-Halenda (BJH) method. This technique was well executed to find out the mesoporous structural parameters of SiO_2NPs and chitosan-modified SiO_2NPs which further studied for their catalytic applications.

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