

MINISTRY OF EDUCATION
AND TRAINING

VIETNAM ACADEMY OF SCIENCE
AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY



NGUYEN DINH TIEN DUNG

**SYNTHESIS AND SURFACE MODIFICATION OF
LIPOSOME HYBRID MESOPOROUS SILICA
NANOPARTICLES FOR ANTI-CANCER DRUG
DOXORUBICIN DELIVERY**

DOCTORAL DISSERTATION IN MATERIALS SCIENCE

Major: Polymeric and composite materials

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DECLARATION

I declare that the dissertation: "**Synthesis and surface modification of liposome hybrid mesoporous silica nanoparticles for anti-cancer drug doxorubicin delivery**" is my own research under the scientific guidance of the supervising team. The dissertation uses information cited from various reference sources, and all cited information is clearly acknowledged. The research results of mine that have been published jointly with other authors have been agreed upon by the co-authors to be included in the dissertation. The data and results presented in the dissertation are completely truthful and have not been published in any other work besides the author's published works. The dissertation was completed during the time I was a PhD student at the Graduate University of Science and Technology, Vietnam Academy of Science and Technology.

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LIST OF ABBREVIATIONS

Abbreviation	Full words
APTES	(3-aminopropyl)triethoxysilane
Brij (B100)	Polyoxyethylene (100) stearyl ether
B20	Polyoxyethylene (20) cetyl ether
B10	Polyoxyethylene (10) stearyl ether
BET	Brunauer–Emmett–Teller
BJH	Braett-Joyner-Halenda
CTAB	Cetyltrimethylammonium bromide
CTS	Chitosan
CPF	Chitosan-Polyethylene glycol-Folic acid
DiO	3,3'-Diocetadecyloxacarbocyanine perchlorate
DMEM	Dulbecco's Modified Eagle Medium
DOX	Doxorubicin
DLE	Drug loading efficiency
DLC	Drug loading capacity
DLS	Dynamic Light Scattering
EDA	Ethylenediamine
EDX	Energy dispersive X-ray spectroscopy
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
FA	Folic acid
FT-IR	Fourier Transform Infrared Spectroscopy
FBS	Fetal bovine serum
GLT	Gelatin
GPF	Gelatin-Polyethylene glycol-Folic acid
LB	Liposome-Brij
LBF	Liposome-Brij-Folic acid
LP	Liposome
MSN	Mesoporous silica nanoparticles
ML	Mesoporous silica nanoparticles-Liposome

MLB	Mesoporous silica nanoparticles-Liposome-Brij
MLBF	Mesoporous silica nanoparticles-Liposome-Brij-Folic acid
MLCPF	Mesoporous silica nanoparticles-Liposome-Chitosan-Polyethylene glycol-Folic acid
MLGPF	Mesoporous silica nanoparticles-Liposome-Gelatin-Polyethylene glycol-Folic acid
MTT	Thiazolyl Blue Tetrazolium Bromide
NMR	Nuclear magnetic resonance spectroscopy
NPC	p-Nitrophenyl chloroformate
NHS	N-hydroxysuccinimide
PBS	Phosphate buffer saline
PEG	Polyethylene glycol
QCT	Quercetin
SA	Succinic anhydride
SEM	Scanning electron microscope
TEOS	Tetraethoxysilane
TGA	Thermal gravimetric analysis
TEM	Transmission electron microscope
UV-Vis	Ultraviolet-Visible spectroscopy
XRD	X-ray diffraction

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INTRODUCTION

Recent advances in nanotechnology have enabled the development of materials with exceptional physicochemical properties, driving rapid progress across a wide range of scientific and technological disciplines. Within biomedical materials science, nanomaterials have emerged as a next-generation platform with broad potential, finding applications in biomolecule recognition, biosensing, live-cell and tissue imaging, nanotherapeutics, and nanoscale drug delivery [1]. Among these, drug delivery systems have attracted particular attention, especially in oncology, where nanocarriers capable of high drug loading, controlled release, and targeted delivery are actively pursued to improve therapeutic efficacy and reduce off-target toxicity [2].

Following decades of intensive research, nanocarrier platforms now encompass a diverse array of chemistries, morphologies, sizes, and surface functionalities. These systems can be broadly classified into organic nanocarriers, such as polymeric and lipid nanoparticles, and inorganic nanocarriers, including metal, metal-oxide, carbon-based, and silica nanoparticles. Each class offers distinct advantages depending on the biomedical context. However, despite significant laboratory-scale advances, the clinical translation of nanocarrier systems for cancer therapy remains limited. This gap is largely attributed to intrinsic limitations associated with single-component materials. Inorganic carriers, while exhibiting high physicochemical stability, often suffer from limited biocompatibility, low drug loading efficiency, and rapid clearance by the mononuclear phagocyte system. In contrast, organic carriers generally demonstrate favorable biocompatibility and prolonged circulation times but may lack structural stability under physiological conditions [2, 3].

To overcome these limitations, hybrid nanomaterials have been developed to integrate complementary features of multiple material classes, thereby enhancing therapeutic performance while minimizing adverse effects. Hybrid nanomaterials can be broadly categorized into inorganic-inorganic, organic-organic, and inorganic-organic systems. Among these, inorganic-organic hybrids have gained particular interest due to their ability to synergistically combine the structural robustness of inorganic materials with the biological advantages of organic components [2, 3].

A representative and highly promising inorganic-organic hybrid system for biomedical applications consists of mesoporous silica nanoparticles (MSN)

integrated with nanoliposomes (LP). Individually, both MSN and LP are well-established drug carriers. MSN are characterized by high structural stability, large surface area and pore volume for loading diverse therapeutic agents, and tunable particle size and morphology to optimize biodistribution. Incorporation of a liposomal component further enhances membrane mimicry and biocompatibility, thereby expanding the functional versatility of the nanocarrier [4].

Nevertheless, the standalone use of MSN is associated with several drawbacks, including premature drug leakage from mesopores, hemolytic activity, and a relatively short circulation half-life *in vivo*. Encapsulation or coating of MSN with a liposomal bilayer effectively addresses these limitations. The liposomal shell not only improves biocompatibility and mediates favorable interactions with cellular membranes but also allows the incorporation of hydrophobic drugs within the lipid bilayer. Moreover, the liposomal coating modulates drug release kinetics by suppressing burst release and enabling sustained drug liberation, thereby maintaining therapeutically relevant concentrations at the target site and ultimately enhancing anticancer efficacy [4]. Motivated by these considerations, this dissertation “***Synthesis and surface modification of liposome hybrid mesoporous silica nanoparticles for anti-cancer drug doxorubicin delivery***” was undertaken to enhance drug-delivery performance using MSN-LP hybrid platforms.

Objective of the dissertation

This study aims to develop a multifunctional hybrid nanoplatform based on mesoporous silica nanoparticle-liposome (MSN-LP) structures, surface-functionalized with folate-conjugated biocompatible polymers, to enhance colloidal stability, achieve stimuli-responsive drug release, and improve anti-tumor efficacy.

Main contents of the dissertation

1. Synthesize MSN-LP hybrid nanocarriers.
2. Surface-functionalize MSN-LP with folic acid-conjugated biocompatible polymers (Brij, gelatin-PEG, and chitosan-PEG).
3. Characterize the physicochemical profile of MSN-LP-based nanoplatforms.
4. Evaluate drug loading efficiency and *in vitro* release profiles.
5. Assess *in vitro* biocompatibility, cellular uptake, and anticancer efficacy.

Scientific and Practical Significance

The research findings enrich the database on inorganic-organic hybrid nanomaterials while providing scientific rationale regarding the influence of surface organic coatings on the physicochemical properties and drug release kinetics of hybrid materials. This provides a foundation for designing smarter and more effective carrier systems in the future.

The MSN-Liposome-Copolymer hybrid material synthesized in this thesis exhibits high loading capacity for the anticancer drug doxorubicin and targeted, controlled drug release, maintaining high local drug concentrations at cancer cells, thereby enhancing anticancer efficacy and minimizing side effects. Conducted entirely in Vietnam, this study contributes to mastering the technology for fabricating smart nanomaterials domestically.

New contributions of the dissertation

1. Developed the synthesis and characterization protocols for three novel inorganic-organic hybrid nanomaterials for anti-cancer drug delivery applications: surface-modified liposome-mesoporous silica hybrid nanoparticles (ML) functionalized with Brij-FA (MLBF), Gelatin-PEG-FA (MLGPF), and Chitosan-PEG-FA (MLCPF). Demonstrated enhancements in stability, pH-responsive controlled drug release, cytotoxicity against cancer cells, and cellular internalization efficiency of the synthesized nanomaterials.
2. Provided comprehensive data on the influence of Brij ratio and PEO chain length on the properties, stability of the materials. Analyzed the effects of the ratio, concentration, and PEG content of Gelatin-PEG-FA and Chitosan-PEG-FA on the physicochemical properties and stability of the resulting nanoplateforms.

CHAPTER 1. LITERATURE REVIEW

1.1. Smart drug delivery nanomaterials in cancer therapy

Cancer remains a leading cause of mortality worldwide and poses a substantial socioeconomic burden. According to estimates from the International Agency for Research on Cancer, approximately 19.98 million new cancer cases and 9.74 million cancer-related deaths were reported globally in 2022 (updated in 2026) [5]. Although various novel therapeutic strategies such as gene therapy and immunotherapy have recently been developed, chemotherapy, radiotherapy, and surgical resection remain the most prevalent clinical modalities. Radiotherapy and surgery exhibit high efficacy in treating early-stage malignancies with localized primary tumors. For advanced-stage cancers involving metastatic dissemination, or in cases where tumors reside in anatomical sites that pose prohibitive surgical risks, chemotherapy becomes the more appropriate therapeutic option. Nevertheless, conventional anticancer drugs lack selectivity and frequently damage healthy tissues, leading to severe adverse effects such as bone marrow suppression and renal dysfunction [6]. In parallel, the emergence of multidrug resistance in many tumor cell lines markedly diminishes therapeutic efficacy and often necessitates dose escalation, further exacerbating systemic toxicity. These limitations have motivated the development of smart nanocarriers.

Smart nanocarriers are engineered nanoscale delivery systems capable of encapsulating and selectively transporting therapeutic agents to targeted disease sites, thereby enhancing therapeutic efficacy while minimizing off-target interactions and systemic toxicity. Reflecting their clinical impact, the global market for nanotechnology in cancer treatment has expanded rapidly, growing from USD 159.07 billion in 2025 to USD 183.23 billion in 2026 at a compound annual growth rate (CAGR) of 15.2%. This trajectory is expected to persist, with the market projected to reach USD 319.04 billion by 2030 at a CAGR of 14.9% [7]. Notably, several nanocarrier-based formulations have already received regulatory approval for clinical use, including Doxil (doxorubicin-loaded liposomes for ovarian and breast cancers), Onivyde (irinotecan-loaded liposomes for pancreatic cancer), and Genexol-PM (paclitaxel-loaded polymeric micelles for breast and lung cancers). Despite these commercial successes, continuous research is actively underway to further optimize nanomedicine platforms. Current efforts focus on addressing key challenges, such as: (1) enhancing drug loading capacity; (2) engineering target specificity; (3) achieving

controlled release kinetics; (4) improving penetration across biological barriers; and (5) optimizing overall biocompatibility and safety profiles. The principal strategies to achieve these goals involve synthesizing novel material frameworks, functionalizing established platforms, or fabricating hybrid nanostructures to yield superior synergistic properties.

1.2. Mesoporous silica nanoparticle

1.2.1. Silica nanoparticle

Nanosilica is a representative class of nanoceramic materials that has attracted significant research interest over recent decades. From a chemical perspective, silica nanoparticles (SiO_2) consist of an internal siloxane network (Si-O-Si) and surface-exposed silanol groups (Si-OH), which play a critical role in their interfacial chemistry. Owing to their amorphous nature, nanosilica particles exhibit favorable characteristics for biomedical applications, including excellent biocompatibility and negligible intrinsic toxicity [8]. In addition, their synthesis can be readily scaled using cost-effective processes, making them attractive for large-scale production [9].

Since the pioneering work of Stöber in 1968 on the synthesis of monodisperse silica spheres, extensive efforts have been devoted to tailoring the size, morphology, and internal structure of silica nanoparticles. By systematically adjusting reaction parameters, such as precursor concentration, catalyst type, solvent composition, and surfactant use, a broad spectrum of silica nanostructures with well-defined physicochemical properties has been realized. In general, nanosilica materials can be classified according to two principal criteria: structural architecture and particle morphology.

From a structural standpoint, silica nanoparticles may be categorized as nonporous, mesoporous, hollow, or cage-like systems. Mesoporous silica nanoparticles (MSN) are defined by the presence of ordered pore networks with diameters typically ranging from 2 to 50 nm. The earliest example of mesoporous silica, the M41S family, was reported in the 1990s by researchers at Mobil Oil Corporation. This family comprises three representative phases, such as MCM-41, MCM-48, and MCM-50, which differ primarily in pore geometry and symmetry. MCM-41 possesses a hexagonally ordered array of one-dimensional channels, MCM-48 exhibits a three-dimensionally interconnected gyroid structure with cubic symmetry, and MCM-50 features lamellar pore arrangements separated by surfactant layers. Among these materials, MCM-41 remains the most extensively investigated

due to its relatively straightforward synthesis and high thermal stability [10]. Building upon these frameworks, more complex silica nanostructures have been developed, including hollow mesoporous silica nanoparticles (HMSNs), which consist of a porous outer shell surrounding a central void, and cage-like architectures featuring an external shell and an internal cavity that may enclose either identical or dissimilar materials.

With respect to particle morphology, nanosilica can be engineered into a variety of shapes, such as spherical, cubic, ellipsoidal, or rod-like forms. Each morphological class may exhibit either solid or porous internal structures, depending on the synthesis strategy employed. Variations in particle shape are predominantly governed by the relative proportions of reactants and structure-directing agents, as well as by kinetic and thermodynamic factors during nucleation and growth. These structural and morphological tunabilities collectively underpin the versatility of nanosilica as a platform material for advanced biomedical nanotechnology.

1.2.2. Mesoporous silica nanoparticles

Porous silica-based nanomaterials have emerged as particularly promising platforms among nanosilica systems designed for drug delivery. Within this category, mesoporous and hollow mesoporous silica nanoparticles are most frequently investigated due to their distinctive structural advantages. Specifically, their exceptionally high specific surface areas, coupled with interconnected pore networks and spacious internal cavities, provide favorable conditions for accommodating substantial quantities of therapeutic agents [11, 12]. These features collectively underpin the superior loading efficiency exhibited by such materials when compared with nonporous silica systems.

The development of MSN can be traced to early breakthroughs achieved independently by the research teams of Qiang Cai [13] and Fowler [14]. In these studies, the conventional Stöber sol-gel process was strategically modified through the incorporation of structure-directing components, including surfactants such as cetyltrimethylammonium bromide (CTAB), micelle-forming additives, or polymeric templating agents [15]. This methodological innovation enabled the formation of ordered mesoporous architectures that were previously unattainable using classical synthesis routes.

Building upon these foundational studies, subsequent research has demonstrated that precise modulation of synthesis parameters is essential for tailoring

MSN structural characteristics. Reaction variables such as solution alkalinity, the chemical nature of surfactants and co-surfactants, and precursor concentration were shown to exert significant influence over particle morphology, size distribution, pore diameter, and overall pore organization. Such control is particularly critical, as the physicochemical properties of MSNs are directly linked to their performance as drug transport vehicles.

From a biological perspective, the morphology and surface charge of MSN play a pivotal role in determining their biodistribution and preferential accumulation within diseased tissues [16]. In parallel, particle size has been identified as a key determinant of cellular internalization efficiency. While optimal size ranges vary depending on cell type, most studies indicate that effective uptake generally occurs for particles between 50 and 500 nm in diameter. Notably, MSN with sizes in the narrower range of approximately 50–300 nm exhibit a favorable balance of deep tissue penetration, prolonged circulation, and enhanced accumulation at target sites. In contrast, excessively small particles (<50 nm) often display limited porosity and, consequently, reduced drug loading capacity. Conversely, particles larger than 300 nm tend to localize within the interstitial spaces of tumor tissue, which can compromise therapeutic outcomes [17].

In addition to external dimensions, the internal mesoporous framework critically influences drug encapsulation behavior. By adjusting pore size and pore volume, MSN can be engineered to selectively host therapeutic molecules spanning a wide range of molecular dimensions. Larger pore volumes generally translate into increased encapsulation capacity. Furthermore, an increase in pore volume is frequently associated with thinner silica walls, a characteristic that has been linked to improved biodegradability under physiological conditions [18]. This relationship is particularly advantageous for biomedical applications, where controlled degradation is necessary to minimize long-term tissue accumulation.

Finally, beyond structural optimization, colloidal stability represents a fundamental requirement for the successful implementation of MSN-based drug delivery systems. In physiological and biomimetic environments, nanoparticles must remain well dispersed to avoid aggregation. Undesirable aggregation within the bloodstream may lead to vessel blockage, altered biodistribution, or severe adverse effects. Therefore, ensuring stable dispersion behavior under biologically relevant conditions is essential for achieving both safety and therapeutic efficacy.

1.2.3. Synthesis method of MSN

1.2.3.1. Synthesis method of nanosilica

The Stöber process provides the canonical framework for the preparation of silica nanoparticles and, by extension, underlies numerous variants that produce diverse particle morphologies and architectures. In essence, this sol-gel route generates monodisperse silica spheres from alkoxy silane precursors, commonly tetraethyl orthosilicate (TEOS), in mixed alcohol-water media under basic conditions. Operationally, an aqueous-alcoholic solution is first rendered alkaline, typically with ammonia, to furnish a catalytic environment for hydrolytic and condensative transformations. The alcohol co-solvent improves TEOS miscibility and moderates the hydrolysis kinetics, thereby contributing to uniform nucleation and growth. Upon introduction of TEOS into this basic medium, the ethoxy groups are hydrolyzed to form silanol (Si-OH) functionalities. Subsequent condensation reactions among silanols (or between silanols and residual ethoxy groups) generate siloxane (Si-O-Si) linkages, progressively building an inorganic network [19].

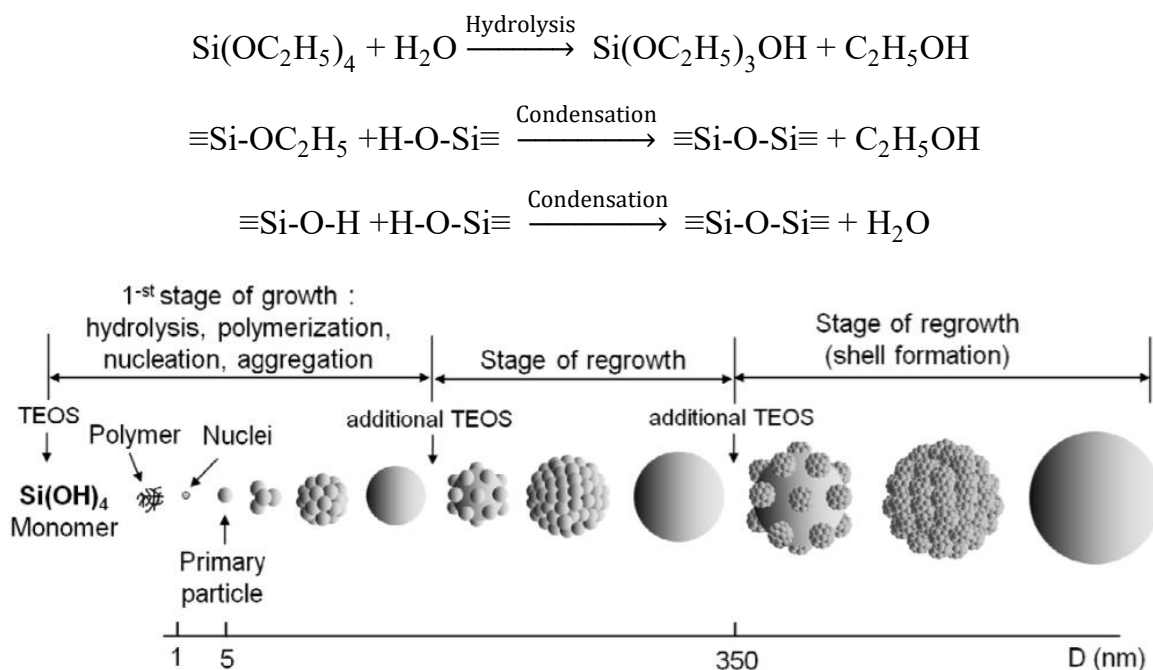


Figure 1.1. The description of multilayer SBF model in the silica particle growth during TEOS hydrolysis in alcohol-water-ammonia medium. Reproduced with permission from ref [20]. Copyright 2026 IOP Publishing Limited.

Critically, the interplay between base-catalyzed hydrolysis (which governs monomer supply) and condensation (which drives particle growth and densification) establishes the nucleation-growth balance that dictates final particle size distribution

and structural uniformity. Thus, control over parameters such as ammonia concentration, alcohol-to-water ratio, and precursor dosing not only ensures reproducibility but also provides tunability for downstream adaptations that yield tailored shapes and porosity.

The growth of silica nanoparticles is commonly rationalized by the multilayer Stöber-Fink-Bohn (SFB) framework, which views particle formation as a stagewise process in which previously generated nanoparticles serve as seeds for subsequent deposition cycles [20]. Within this framework, nucleation is initiated once the concentration of silicic acid species surpasses the supersaturation threshold, promoting preferential condensation of monomers onto existing particle surfaces rather than the generation of new nuclei. This selective surface growth yields primary silica granules that subsequently assemble through controlled aggregation involving elementary units with diameters typically in the range of 5-10 nm. Progressive condensation and aggregation lead to the development of larger silica colloids until growth becomes kinetically limited at an effective terminal particle diameter of approximately 350 nm, beyond which further agglomeration or layer formation is suppressed due to monomer depletion and surface stabilization effects (**Figure 1.1**).

1.2.3.2. Synthesizing methodologies of MSN

Surfactants play a central role in the formation of MSN, as they govern both pore generation and the regulation of pore geometry and dimensions. Their primary function is to act as structure-directing agents, organizing the inorganic precursor around self-assembled micellar templates that ultimately define the internal mesoporous architecture.

A major methodological advance was reported by Grün and co-workers in 2001, who introduced surfactant-assisted modifications to the classical Stöber synthesis, giving rise to a sol-gel-based strategy for producing porous silica materials [21]. Through this approach, spherical silica nanoparticles containing well-defined internal pore networks were successfully obtained, marking a significant step toward controlled mesostructure engineering in MSN systems.

In this synthetic framework, pore-forming templates are typically surfactant molecules that assemble into micellar or liquid-crystalline phases. Cationic surfactants, particularly cetyltrimethylammonium bromide (CTAB) and cetyltrimethylammonium chloride (CTAC), are most frequently employed due to their strong electrostatic interactions with negatively charged silica species during

condensation. In addition to ionic surfactants, nonionic amphiphiles such as pluronic block copolymers and polyethylene glycol (PEG) have also been widely utilized as soft templates. These neutral surfactants enable alternative pore structures and sizes by modulating micelle organization and silica-template interactions, thereby expanding the versatility of MSN design [19].

1.2.4. Surface modification of MSN

Despite extensive investigations exploring diverse strategies to harness MSN for therapeutic delivery, particularly in oncological applications, their translation into clinical practice remains nontrivial and continues to pose significant scientific challenges. One primary limitation arises from the intrinsic architecture of MSN: while open and interconnected pore networks facilitate efficient inward diffusion of drug molecules during loading, this same structural openness predisposes the system to premature drug release. Specifically, reductions in concentration gradients during purification steps or systemic circulation can promote undesired drug leakage, thereby diminishing the effective payload and increasing off-target exposure and treatment-associated side effects [22].

Beyond these structural concerns, chemical characteristics of MSN further constrain their biomedical applicability. The abundance of surface silanol groups can induce unfavorable interactions with phospholipid components of red blood cell membranes, leading to hemolytic activity and compromised blood compatibility [23]. Addressing such limitations has therefore become a central focus of current MSN engineering efforts.

To mitigate both premature drug release and hemocompatibility issues, surface and pore modifications have been widely employed. Functionalization of MSNs with organic or inorganic capping agents can effectively block pore openings, suppress uncontrolled drug diffusion, enhance loading efficiency, and shield reactive silanol groups from direct biological contact [10]. In parallel, chemical modification of the MSN surface can impart additional advantages, including improved colloidal stability under physiological conditions, enhanced biocompatibility, and reduced recognition by the mononuclear phagocyte system.

Recent advances have further expanded the functional scope of MSN surface engineering by enabling the conjugation of targeting ligands, such as peptides, aptamers, and folic acid derivatives. These targeting motifs facilitate selective binding and uptake by cancer cells, thereby improving drug delivery efficiency and

therapeutic specificity [24]. A broad range of organic molecules and polymers can be anchored onto MSN surfaces through either covalent bonding or noncovalent interactions. Common strategies for establishing stable chemical attachment include co-condensation approaches (frequently applied to pore interior functionalization), hydrolysis-condensation reactions, post-synthetic modification, and post-grafting techniques [17]. Collectively, these surface engineering methodologies provide a versatile toolkit for overcoming the inherent limitations of pristine MSNs and advancing their prospects for clinical drug delivery.

1.2.4.1. MSN surface modification by functional groups

Surface functionalization of MSN is pivotal not only for mitigating the intrinsic drawbacks of unmodified particles but also for imparting desirable attributes such as enhanced biocompatibility, strengthened drug-carrier interactions, and chemical handles for subsequent conjugation with organic moieties. These capabilities derive from the rich interfacial chemistry of MSN, in which abundant silanol (Si-OH) groups provide reactive sites for covalent modification and interfacial assembly, thereby enabling controlled interface engineering and diversified hybrid constructs.

Two principal strategies are typically employed to introduce functional groups onto MSN: co-condensation and post-grafting [25]. In co-condensation, tetraalkoxysilane precursors are sol-gel co-hydrolyzed and co-condensed with one or more organoalkoxysilane monomers, optionally in the presence of structure-directing agents, so that functional moieties are incorporated throughout the inorganic framework in a single step. This route often yields a more uniform spatial distribution of functional groups across the mesostructure. However, it can also perturb the self-assembly process and reduce the degree of mesoscopic order relative to pristine materials. By contrast, post-grafting proceeds sequentially: first, the silica surface is activated or exposed (often after template removal) to reveal reactive silanols; second, organosilanes or other functional reagents are covalently attached to accessible sites. This two-stage approach offers site selectivity, facilitating targeted modification at external surfaces, pore entrances, or internal pore walls. A recognized limitation, however, is heterogeneous distribution within confined mesopores, as steric constraints frequently bias attachment toward pore mouths and outer surfaces [26]. In practice, a repertoire of chemical motifs, most commonly amines, thiols, and carboxylates, is employed to tailor interfacial charge, hydrogen bonding, coordination, and subsequent bioconjugation chemistry [27].

1.2.4.2. MSN surface modification by polymers

Despite the favorable pharmacological attributes of MSN, clinical translation remains difficult. A principal obstacle is rapid recognition by the reticuloendothelial system (RES), which curtails circulatory half-life and redirects particles to the liver, spleen, and bone marrow. This off-target sequestration not only undermines delivery to diseased tissues but also raises concerns regarding organ accumulation and toxicity. To mitigate RES uptake and prolong systemic persistence, design strategies increasingly emphasize surface engineering with biocompatible polymers. In particular, grafting or coating MSN with PEG, pluronic block copolymers, or chitosan has shown promise in reducing opsonization and improving pharmacokinetics. Beyond stealth effects, polymer layers can serve as dynamic gatekeepers at pore openings, thereby suppressing premature release during circulation. Notably, block copolymers (e.g., pluronic) and multifunctional polysaccharides (e.g., chitosan, heparin) can be configured as capping shells that limit drug leakage while preserving loading capacity, collectively enhancing delivery efficiency and safety [10, 28, 29].

1.2.4.3. MSN surface modification by targeting agents

Targeted drug delivery seeks to lower dose requirements, strengthen therapeutic response, and limit adverse effects by discriminating between pathological and healthy cells. In practice, however, substantial barriers persist: nanoparticles frequently undergo off-target sequestration in normal tissues, most notably the lungs, spleen, and liver, before reaching tumors, and the extent of vascular leakiness can vary markedly across tumor types, undermining passive targeting. Consequently, active targeting has gained prominence as a complementary strategy, leveraging specific interactions between ligands displayed on the carrier and receptors overexpressed on malignant cells. MSN have been extensively functionalized with diverse recognition motifs to enhance selectivity and cellular uptake, including small molecules (e.g., vitamin derivatives), proteins such as antigens and transferrin, nucleic acid aptamers, short targeting peptides, and polysaccharides. These ligand-receptor pairings can improve binding avidity, promote receptor-mediated endocytosis, and thereby increase intratumoral accumulation while mitigating nonspecific deposition in healthy organs [1].

1.2.4.4. MSN surface modification by responsive-stimuli agents

Stimuli-responsive control is particularly attractive for MSN, whose open pore channels facilitate facile loading yet render payloads prone to premature diffusion. To prevent early release, pore entrances are commonly “gated” with macromolecular caps that remain closed during circulation but open upon a specific trigger, enabling on-demand drug discharge. Conceptually, cap engineering can be grouped into three modes: (i) reusable caps, where bulky gatekeepers are attached through reversible interactions and can be reclosed; (ii) fully reversible affinity systems, which rely on trigger-dependent switching of macromolecular binding strengths; and (iii) irreversible gates, in which cap–surface linkages are cleaved by a stimulus, permanently detaching the gate. The latter often uses a detachable shell covering the MSN exterior that disassembles under appropriate excitation, thereby initiating release [1].

Release triggers are broadly classified as endogenous or exogenous. Endogenous cues exploit physicochemical disparities between healthy and pathological milieus, such as those found in tumors or inflamed tissues, including pH gradients, redox conditions (elevated glutathione or reactive oxygen species), disease-associated enzymes, local hypoxia, and the accumulation of selected small molecules. In this paradigm, capped MSN remain stable in physiological fluids but open within the tumor microenvironment or intracellular compartments, enhancing therapeutic index while limiting systemic toxicity. Because no external apparatus is required, endogenous triggering is noninvasive and can be advantageous for disseminated or poorly localized disease [17].

By contrast, exogenous triggers, including light, heat, magnetic fields, and ultrasound, are applied by clinicians, offering spatiotemporal precision and on/off control. These operator-directed stimuli enable localized activation at the disease site, reducing off-target exposure while maintaining high payload integrity during systemic transit [2].

1.2.4.5. Hybridization with other materials

Hybridizing MSN with complementary nanomaterials confers multifunctionality that is difficult to achieve with single-component systems. Beyond sealing pore openings to suppress premature drug leakage, suitable hybrid shells can attenuate hemolysis associated with surface silanols while introducing new therapeutic or diagnostic capabilities. Representative partners under active investigation include dendrimers (for high-density ligand display and enhanced

loading), superparamagnetic/ferromagnetic nanoparticles (for magnetic guidance and imaging), noble metal nanostructures (for photothermal or plasmon-enhanced effects), and liposomes (LP) (for biomimetic membranes and improved pharmacokinetics). Among these, MSN-liposome (ML) hybrids are particularly compelling: a lipid bilayer can function as a dynamic gate over mesopores, reduce protein adsorption and RES recognition, and provide a versatile platform for incorporating targeting ligands or stimuli-responsive lipids. This architecture combines the high payload capacity and structural robustness of MSN with the stealth and membrane fluidity of LP, yielding high drug-loading efficiency, controlled release, and promising translational potential across diverse therapeutic indications.

1.3. Liposome

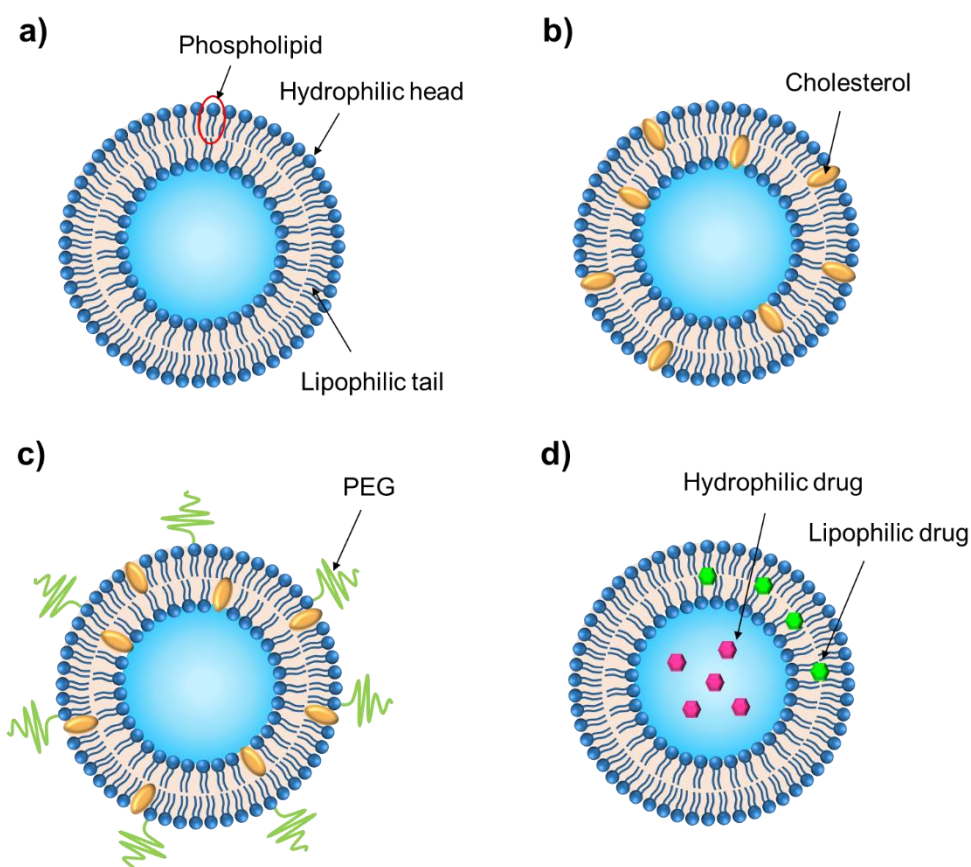


Figure 1.2. Structure of conventional liposome (a), liposome with cholesterol (b), and PEGylated liposome (c). Drug encapsulating mechanism of liposome (d)

Liposome (LP), represent the earliest class of vesicular drug carriers, originally proposed by Gregoriadis as vehicles for site-directed therapies [23]. Architecturally, LP are self-assembled into uni- or multilamellar vesicles in which amphiphilic lipids, predominantly phospholipids, organize into one or more bilayers that enclose

aqueous core. This compartmentalization enables dual-mode loading: hydrophilic therapeutics are sequestered within the aqueous core, whereas hydrophobic or amphiphilic agents partition into the bilayer domain (**Figure 1.2c**). Owing to their close mimicry of cellular membranes, LP exhibit high biocompatibility and promote effective interactions with biological barriers and cells [30]. Notably, LP constitute one of the few nanocarrier platforms with regulatory approval by the U.S. Food and Drug Administration, and multiple formulations have reached clinical use. Exemplars include liposomal chemotherapeutics, such as doxorubicin, daunorubicin, and irinotecan, for oncology indications, as well as liposomal amphotericin B for systemic fungal infections [30]. Together, these attributes underscore the translational maturity of LP and their continued relevance as modular, clinically validated drug delivery systems.

1.3.1. Classification of LP

LP are commonly classified according to the organization of their lipid bilayers [31].

- Small unilamellar vesicles (SUVs): Single lipid bilayer enclosing an aqueous core; small diameter, typically below 100 nm.
- Large unilamellar vesicles (LUVs): Single lipid bilayer enclosing an aqueous core; diameter generally greater than 100 nm.
- Multilamellar vesicles (MLVs): Multiple concentric lipid bilayers alternating with aqueous layers, yielding an onion-like architecture; sizes typically exceed 500 nm.
- Multivesicular vesicles (MVVs): Contain numerous smaller internal vesicles; sizes often greater than 1000 nm.

Particle size is a critical parameter for drug delivery applications, as larger LP are more rapidly opsonized and cleared by the reticuloendothelial system (RES). An optimal size range of approximately 50-200 nm is often targeted to exploit the enhanced permeability and retention (EPR) effect [32].

1.3.2. LP components

Phospholipids constitute the core building blocks of LP, with frequently employed species including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylglycerol (PG), along with their derivatives (**Table 1.1**). The physicochemical attributes of these lipids, alkyl chain

length, degree of unsaturation, headgroup charge, and the gel–fluid phase transition temperature (T_c), collectively dictate key vesicular properties such as membrane permeability, colloidal/chemical stability, and bilayer elasticity [30].

To further refine performance, auxiliary constituents (e.g., sterols, polysaccharides, surfactants) are incorporated at controlled ratios to bolster mechanical integrity, stabilize physicochemical and colloidal behavior, modulate particle size and zeta potential, enhance loading, and tune both release kinetics and blood residence time [33]. Among these, cholesterol is pivotal: its hydrophilic hydroxyl group aligns toward the aqueous phase while the hydrophobic sterol moiety intercalates among acyl chains, thereby stiffening the bilayer. However, cholesterol also decreases bilayer permeability to hydrophilic solutes and lowering permeability to hydrophilic solutes. Consequently, an appropriate phospholipid-to-cholesterol ratio must be selected to balance encapsulation efficiency and release profiles for optimal performance [34].

To mitigate opsonization and mononuclear phagocyte system (MPS) uptake, hydrophilic polymers (most notably PEG) are grafted onto LP surfaces to form a hydrated steric corona that prolongs circulation. The PEG layer's conformation depends on grafting density and chain length: at low densities, chains adopt a mushroom regime that effectively reduces immune recognition, whereas high densities induce a brush regime that increases lateral steric pressure, potentially stressing the bilayer and compromising stability [35].

Table 1.1. Main components of LP

Phospholipid				
Name	Length of alkyl chain	Saturation level	Charge	Origin
Phosphatidylcholine (PC)	Mixture	Mixture	Zwitterionic	Natural
Phosphatidylethanolamine (PE)	Mixture	Mixture	Positive	Natural
Phosphatidylserine (PS)	Mixture	Mixture	Negative	Natural
phosphatidylglycerol (PG)	Mixture	Mixture	Negative	Natural
Sphingolipid	Mixture	Mixture		Natural

Phospholipid				
Name	Length of alkyl chain	Saturation level	Charge	Origin
16LPC	16 carbons	One saturated alkyl chain	Zwitterionic	Synthetic
18LPC	18 carbons	One saturated alkyl chain	Zwitterionic	Synthetic
DMPC	14 carbons	Two saturated alkyl chain	Zwitterionic	Synthetic
DPPC	16 carbons	Two saturated alkyl chain	Zwitterionic	Synthetic
POPC	One 16C-unit chain One 18C-unit chain	One saturated alkyl chain One unsaturated alkyl chain	Zwitterionic	Synthetic
DOPC	18 carbon	Two unsaturated alkyl chain	Zwitterionic	Synthetic
DPPG	16 carbon	Two saturated alkyl chain	Negative	Synthetic
POPG	One 16C-unit chain One 18C-unit chain	One saturated alkyl chain One unsaturated alkyl chain	Negative	Synthetic
POPS	One 16C-unit chain One 18C-unit chain	One saturated alkyl chain One unsaturated alkyl chain	Negative	Synthetic
DOPG	18 carbons	Two unsaturated alkyl chain	Negative	Synthetic
DOTAP	18 carbons	Two unsaturated alkyl chain	Positive	Synthetic
Phospholipid grafted PEG				
DSPE-PEG	18 carbons	Two saturated alkyl chain		Synthetic
DPPE-PEG	16 carbons	Two saturated alkyl chain		Synthetic
Sterol				
Cholesterol, hydroxycholesterol, acid cholestenoic...				

1.3.3. Methodologies in synthesis of LP

At present, a variety of techniques are available for the fabrication of LP delivery systems, which can be broadly classified into passive and active (remote) loading strategies. These two approaches differ primarily in the stage at which therapeutic agents are incorporated and in the physicochemical driving forces involved in encapsulation.

Passive loading introduces drug molecules either prior to or during vesicle formation and relies mainly on drug partitioning between lipid and aqueous phases. This category encompasses several methodical families, including mechanical dispersion, solvent dispersion, and surfactant-based dispersion, each employing distinct physical or interfacial principles to promote vesicle assembly. In contrast, active loading exploits transmembrane gradients, such as ionic, pH, or lipid gradients, to drive drug accumulation into preformed LPs, typically resulting in higher encapsulation efficiency and improved loading control.

Despite methodological diversity, LP fabrication generally follows a common workflow consisting of three fundamental steps: (i) dissolution of lipids in organic solvents followed by solvent removal to yield a dry lipid film; (ii) hydration or dispersion of the lipids in an aqueous medium to induce vesicle formation; and (iii) purification and physicochemical characterization of the resulting LP suspension [36]. Representative preparation techniques include the thin-film hydration (Bangham) method, reverse-phase evaporation, organic solvent spraying, and surfactant dialysis, each offering distinct advantages with respect to scalability, particle size control, and encapsulation performance.

1.3.4. Advantages and limitations of LP

LP offers several notable benefits for drug delivery. Owing to their phospholipid bilayer, which closely mimics cellular membranes, LP exhibits high biocompatibility and favorable interactions with biological barriers. Its compartmentalized architecture supports dual-mode loading: hydrophilic or amphiphilic agents can be entrapped within the internal aqueous lumen, whereas hydrophobic cargos partition into the lipid bilayer. This versatility enables formulation of diverse therapeutics, improves dispersion in physiological media, enhances bioavailability, and reduces off-target toxicity. In addition, LP can co-encapsulate synergistic drug combinations to amplify therapeutic response, while encapsulation shields payloads from enzymatic and chemical degradation. When engineered at ~50-150 nm, LP may

leverage the EPR effect for intratumoral accumulation and afford controlled release, thereby improving efficacy with fewer adverse effects [37].

Nonetheless, important limitations remain: LP can be prone to physical (aggregation, fusion) and chemical (oxidation, hydrolysis) instability, may face biological challenges (rapid clearance), often require costly precursors, and can display modest loading capacity with difficulties encapsulating large macromolecular drugs.

1.4. Hybrid LP-MSN nanoparticles

The MSN-LP (ML) hybrid comprises a MSN core completely cloaked by a surface lipid bilayer. This design adapts prior methodologies in which lipid vesicles are deposited onto solid supports to form continuous bilayers that emulate cellular membranes and enable diverse biotechnological applications [38, 39]. Such supported lipid architectures have been widely exploited as membrane mimetics to interrogate bilayer dynamics, protein-lipid interactions, surface-lipid phenomena, and to provide substrates for cell growth and analysis [40].

Functionally, the lipid envelope serves as a dynamic gate over mesopores, sealing pore entrances to suppress premature drug efflux during administration. Under defined stimuli, such as temperature or pH variations, or phospholipase A2 activity, the bilayer can destabilize or reorganize, thereby triggering controlled payload release and improving drug retention profiles [41, 42]. Beyond gating, the bilayer markedly reduces nonspecific adsorption (by roughly an order of magnitude or more compared with bare MSN). This is achieved by shielding surface silanol ($\equiv\text{Si}-\text{OH}$) and deprotonated silanolate ($\equiv\text{Si}-\text{O}^-$) groups that otherwise promote unintended cellular uptake via microgliosis or other nonspecific endocytic routes [43]. Consequently, the bilayer mitigates off-target adhesion and limits carrier infiltration into healthy cells.

Although efficient cytosolic delivery is essential for biomedical efficacy, traversing the plasma membrane remains intrinsically challenging due to bilayer mechanics and nanoparticle physicochemical constraints [44]. Notably, Tada and co-workers observed distinct intracellular distributions for lipid-coated versus uncoated silica particles: while ML were broadly dispersed throughout the cytoplasm, unmodified MSN localized predominantly within vacuolar compartments. A putative sequence involves: (i) initial adhesion without aggregation for ML (contrasted with clustering of bare MSN); (ii) vesicular internalization; and (iii) cytoplasmic

dissemination for ML versus vacuolar confinement for uncoated MSN [45]. These findings highlight the role of the lipid bilayer in modulating cell–particle interactions, uptake pathways, and ultimate intracellular fate.

Coating MSN with a continuous lipid bilayer has been shown to effectively abolish the hemolytic activity and potential cytotoxicity associated with unmodified MSN. This behavior provides strong evidence for the formation of an intact and impermeable lipid membrane that physically isolates both surface-exposed silanol ($\equiv\text{Si}-\text{OH}$) and deprotonated silanolate ($\equiv\text{Si}-\text{O}^-$) groups from direct contact with erythrocyte membranes during hemolysis assays [46]. By masking these reactive surface functionalities, the lipid coating markedly improves the biocompatibility of the resulting protocell systems relative to bare mMSNs. Furthermore, this protective shell attenuates nonspecific protein adsorption, minimizes recognition and sequestration by the reticuloendothelial system, and thereby prolongs systemic circulation time [43].

Beyond biocompatibility, MSN offer distinct capacity and stability advantages over lipid-based carriers. Owing to their exceptionally high specific surface areas (often exceeding $1000 \text{ m}^2 \text{ g}^{-1}$), MSN can accommodate markedly larger quantities of therapeutic and imaging agents than liposomal systems of comparable dimensions. For example, the drug-loading capacity for doxorubicin has been reported to be higher than that achieved with conventional LP. In parallel, strong interfacial adhesion between the lipid bilayer and the rigid silica surface constrains excessive membrane deformation, thereby suppressing bilayer fluctuations and structural instability. As a result, ML exhibit enhanced mechanical robustness and colloidal stability relative to LP alone, particularly under physiological conditions [47].

1.4.1. Mechanism of LP-MSN formation

The formation of a continuous lipid bilayer on MSN proceeds through a sequential, adsorption-driven mechanism (**Figure 1.3**). Initially, intact lipid vesicles establish contact with the MSN surface through interactions between the hydrophilic phospholipid headgroups and surface silanol functionalities, governed predominantly by electrostatic forces with additional contributions from van der Waals interactions [48]. This initial attachment promotes vesicle deformation, during which the lipid assemblies flatten and expand their interfacial contact area with the silica substrate, thereby reducing interfacial energy. As surface-lipid interactions intensify, the structural integrity of the adhered vesicles becomes compromised, leading to vesicle

rupture and the reorganization of lipids into bilayer patches on the MSN surface. These nascent bilayer domains progressively grow through the adsorption and collapse of additional vesicles, followed by lateral spreading and fusion of adjacent lipid plaques. Ultimately, this cooperative process culminates in the formation of a uniform and stable lipid bilayer coating that fully envelops the MSN core [44].

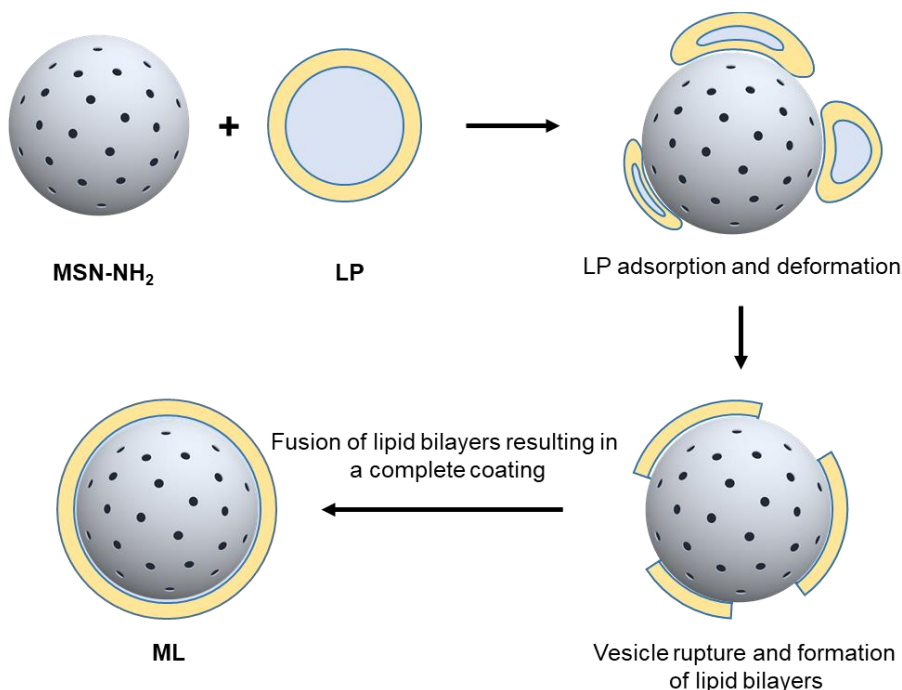


Figure 1.3. Mechanism of lipid bilayer formation on the surface of MSN

Bilayer formation on charged substrates (e.g., glass or silica) proceeds through a multistep adsorption-rupture-spreading sequence. Initially, LP vesicles adhere to the surface when interfacial attraction is sufficient; strong adhesion or vesicle stress (elevated osmotic pressure, membrane tension) can drive deformation and ultimately rupture, yielding supported lipid bilayer patches [49, 50]. When adhesion alone is inadequate, as reported for PC bilayers on silica, rupture requires lateral crowding by neighboring vesicles; this threshold is often described as the critical LP surface concentration on silica [51]. Once the first vesicle collapses, subsequent vesicles fuse at the high-curvature, energetically unfavorable bilayer edges through hydrophobic interactions between exposed lipid tails, promoting lateral propagation of the membrane. The process continues until a continuous bilayer envelops the substrate, after which excess lipid and water re-disperse into the solution [52]. Notably, vesicle rupture can expose either the inner or outer leaflet to the solid support, but these two pathways are experimentally difficult to distinguish. On MSN surfaces, the adsorption-collapse sequence can be summarized as: initial LP attachment; surface

accumulation to a critical density; tension-induced rupture and bilayer spreading, with leaflet orientation set by the specific rupture pathway; relaxation of high-energy defects and expulsion of surplus lipid/water; and coalescence of patches into a uniform bilayer. After bilayer completion, residual vesicles may adsorb weakly without further rupture or spreading [48].

Phase reversibility in liquid-liquid systems is governed by multiple parameters, including droplet size, phase concentration, and interfacial as well as bulk solution conditions. Within this framework, certain transitions are reversible, whereas others proceed irreversibly once initiated. Although supported lipid bilayers can exhibit considerable mechanical robustness, they are not permanently stable; under conditions of reduced LP concentration or elevated temperature, partial or complete delamination may occur. Importantly, the detachment pathway is not necessarily a simple reversal of assembly. Intermediate structures, such as tubular lipid protrusions that bud from the supported bilayer, can form prior to release into the surrounding medium, despite not being depicted in [53]. Dynamic responses differ markedly with the nature of the perturbation: temperature variations induce rapid structural rearrangements, whereas changes in LP concentration produce significantly slower, delayed responses due to the kinetics of adsorption and desorption processes [48].

Consistent with the mechanistic framework outlined above, Mornet et al. provided direct visual evidence of lipid vesicle adsorption and collapse on nanosilica surfaces using cryo-electron microscopy (cryo-EM) [54]. Their observations revealed that LP vesicles with diameters of approximately 30-50 nm undergo rapid adsorption followed by structural disintegration, forming double lipid patches with lateral dimensions ranging from 50 to 100 nm. Notably, the morphology of these lipid domains is not uniform but depends strongly on the surface roughness of the underlying nanosilica. High-resolution imaging further showed the presence of a thin interfacial water layer (~ 1 nm) separating the inner lipid leaflet from the silica surface, while the total spacing from the silica interface to the outer lipid leaflet measured approximately 4-5 nm. In contrast, nanosilica particles functionalized with lipid-protein complexes exhibited surface adsorption without vesicle rupture. A similar adsorption-only behavior was observed for particles bearing both pre-formed lipid bilayers and intact LP vesicles, indicating that bilayer integrity plays a critical role in governing collapse dynamics. Importantly, the transition from vesicle adsorption to bilayer formation occurred on a rapid timescale, typically within a few minutes. Quantitative analysis further suggested that complete surface coverage of an

individual nanosilica particle can be achieved with as few as ~10 LP vesicles of 30 nm diameter, highlighting the efficiency of this self-assembly process.

1.4.2. Factors affecting the formation of lipid bilayer

Building on the mechanistic insights discussed above, the formation of MSN-LP constructs is governed by several interrelated interfacial interactions. These include contacts between LP vesicles and the MSN surface, the adhesion strength of individual vesicles, and vesicle-vesicle interactions during surface accumulation [55]. Importantly, variations in these interaction forces, arising from changes in surface chemistry, vesicle properties, or solution conditions, can substantially influence adsorption behavior, vesicle deformation, and rupture dynamics, thereby directly affecting the initiation and progression of lipid bilayer formation on the MSN surface.

1.4.2.1. Surface charge

Extending the preceding discussion on vesicle adsorption and rupture, electrostatic forces emerge as a primary determinant of lipid bilayer formation on nanosilica. Because both the LP vesicles and the silica surface contribute surface charges, their zeta potentials critically govern the likelihood of adhesion, deformation, and subsequent bilayer assembly. In systematic experiments, Mornet et al. compared LP bearing distinct net charges: positively charged (cationic phospholipids), neutral (zwitterionic amphiphiles), and negatively charged vesicles with tunable charge density, on silica substrates characterized by negatively charged silanol groups. Consistent with electrostatic expectations, positively charged and neutral LP not only adsorbed efficiently but also proceeded to rupture and spread, generating supported bilayer patches. By contrast, negatively charged LP displayed a charge-density-dependent response: at low negative charge, coating efficiency was largely preserved, whereas at high negative charge density, vesicles predominantly adsorbed without rupture, yielding sparse nanosilica-LP constructs and extensive populations of intact, non-spreading vesicles [54]. Taken together, these observations indicate that charge complementarity or neutrality between LP and nanosilica surfaces is generally required to drive the full adsorption-deformation-rupture sequence. Consequently, rational selection of LP composition and nanosilica surface chemistry, to ensure attractive or minimally repulsive electrostatics, constitutes a practical prerequisite for the reliable synthesis of continuous lipid coatings on silica nanoparticles.

Building on the role of electrostatics in bilayer formation, Shi et al. demonstrated a charge-complementary strategy to construct MSN-LP assemblies: negatively charged LP derived from soybean lecithin were deposited onto amine-functionalized (positively charged) MSN, whereas cationic LP containing DOTAP were used to coat carboxylated (negatively charged) MSN [56]. Consistent with this design principle, charged MSNs proved highly effective for transporting oppositely charged cargos, including proteins and dyes. In parallel, Liu et al. employed positively charged LPs to envelop silica nanoparticles bearing net negative surface charge [57], while another study achieved robust coatings by adsorbing anionic LP onto MSNs modified with amine and imine-polyethylene groups to yield a positively charged substrate [58].

Notably, effective coating is not limited to oppositely charged pairs. Several reports have also realized MSN-LP formation using like-charged combinations: for example, Dengler produced stable constructs from MSN-NH₂ and positively charged LP [59], and Yang achieved coatings using MSN and LP that were both negatively charged [58]. These observations indicate that, although charge complementarity accelerates adsorption, deformation, and rupture, additional factors including ionic strength, buffer composition, lipid formulation, vesicle size, and surface roughness, can compensate for electrostatic repulsion and ultimately govern bilayer nucleation and continuity. Consequently, rational selection of both surface chemistry and solution conditions is essential for reproducible synthesis of uniform MSN-LP coatings.

1.4.2.2. Particle size

a) Particle size of LP

In addition to surface charge effects, LP particle size plays a decisive role in governing bilayer formation on silica substrates. As demonstrated by Jing et al., larger LP diameters progressively diminish the propensity for vesicle deformation and rupture at a fixed temperature. Specifically, LP with a mean diameter of approximately 90 nm readily adhere to silica and undergo collapse to form a supported lipid bilayer once a critical surface coverage is achieved; below this threshold, adsorption is transient and vesicles detach without spreading. In contrast, larger LP (110-130 nm) exhibit markedly reduced flattening and bilayer formation despite comparable adsorption, indicating insufficient membrane tension to drive rupture. Vesicles of ~160 nm diameter show little to no structural collapse.

Mechanistically, this size dependence arises from the scaling of adhesion forces with vesicle curvature: as size increases, the local curvature at the contact point decreases, weakening the stresses required to induce membrane rupture and lateral spreading. These findings underscore particle size as a key design parameter, alongside electrostatics and concentration, in determining the efficiency and continuity of lipid bilayer assembly on nanoscale silica surfaces. [40].

b) Particle size of nanosilica

Beyond LP size, the diameter of nanosilica critically influences bilayer assembly. When nanosilica smaller than ~ 20 nm is mixed with LP, particles tend to cluster into larger colloids, and the measured ζ -potential of the silica shifts toward that of the LP, indicating adsorption of silica onto vesicle surfaces rather than bilayer formation. As LP concentration increases, vesicles can be cross-linked by ultrafine silica acting as nanoscale bridges, yielding gel-like aggregates instead of supported bilayers (**Figure 1.4**) [57]. This behavior is consistent with a curvature threshold: below ~ 20 nm, the surface curvature imposes prohibitively high bending and stretching energies on the membrane, exceeding the adhesion energy available at the interface; consequently, stable bilayer wrapping is unfavorable [60, 61]. Taken together, these considerations indicate that sub-20 nm silica is generally unsuitable for constructing nanosilica-LP hybrids.

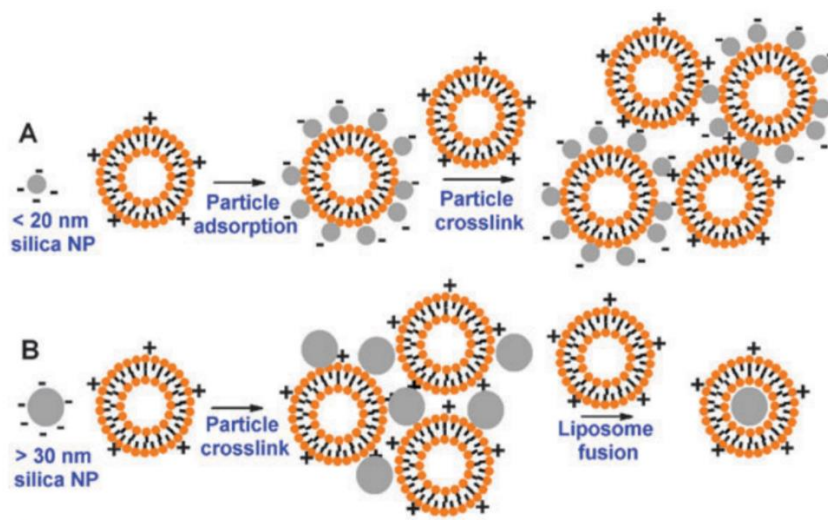


Figure 1.4. The formation of agglomeration with LP by small-size nanosilica (A), LP-hybridized material formation by large-size nanosilica (B). Reproduced with permission from ref [57]. Copyright 2026 Royal Society of Chemistry.

Conversely, for nanosilica with diameters ≥ 30 nm, the interfacial energetics shift in favor of bilayer formation. At low LP doses, particles may still cross-link and

form large, gel-like agglomerates; however, upon increasing the LP amount, surface coverage rises, vesicles flatten and rupture, and continuous lipid bilayers emerge on the silica surface. This transition is accompanied by zeta-potential convergence of the hybrid toward that of the LP, corroborating complete lipid coverage and effective fusion of silica with the lipid phase [57]. In practical terms, selecting nanosilica above the curvature threshold and tuning LP concentration provide a controllable pathway from initial adsorption and bridging to bilayer spreading and plaque coalescence, culminating in robust nanosilica-LP hybrid materials.

Beyond adsorption–rupture mechanics, the lateral organization of phospholipids within supported bilayers is strongly modulated by the curvature of the nanosilica substrate. As particle diameter decreases, the outer leaflet experiences higher curvature, which effectively enlarges intermolecular spacing among headgroups and creates voids within the lipid lattice. To compensate, acyl chains must interdigitate more extensively or adopt conformational adjustments at terminal carbons to maintain hydrophobic continuity. When curvature becomes extreme (very small silica cores), these constraints can drive the formation of lipid bridges spanning adjacent particles, promoting interparticle aggregation and the emergence of large plaque-like assemblies rather than uniform coatings [60].

At constant curvature (i.e., for nanosilica of the same size), bilayer composition further determines packing outcomes. Notably, bilayers formed from DSPC (C18 chains) exhibit a greater tendency for chain interweaving than those from DPPC (C16 chains), consistent with the expectation that longer alkyl chains more effectively compensate for curvature-induced voids by enhanced interdigitation. This compositional effect aligns with the broader hypothesis that increasing chain length amplifies the capacity to bridge curvature-generated gaps, thereby stabilizing outer-leaflet packing on curved supports [60].

Extending the curvature-dependence discussed above, Savarala et al. showed that DMPC LP form supported lipid bilayers on nanosilica only when the silica cores fall within a ~20-100 nm diameter window; smaller particles do not yield stable bilayer coatings. Under favorable conditions, the resulting interfacial membrane exhibits an apparent thickness of ~4-6 nm, consistent with a phospholipid bilayer architecture [62].

From a thermodynamic perspective, the influence of substrate curvature on bilayer fluidity becomes significant only when the particle radius R is much smaller

than a characteristic length scale set by the ratio of the bending modulus (κ) to an interfacial energy term (ϵ). Using representative values reported for DOPC/DPPC, the criterion implies that curvature effects are pronounced primarily for $R \ll 100$ nm, a conclusion supported experimentally by measurements showing that bilayer fluidity can be retained on nanowires <50 nm in diameter [47, 63].

1.4.2.3. Surface properties

The architectural characteristics of MSN, including pore diameter, pore geometry, and internal pore volume, are fundamental determinants of their performance as drug delivery vehicles. These parameters directly influence payload accommodation, encapsulation efficiency, and therapeutic stability. Because medicinal compounds span a wide range of molecular sizes and physicochemical properties, effective carriers must offer tunable and well-distributed pore features. Consequently, evaluating the compatibility of lipid bilayer formation across MSN with distinct pore structures is essential for designing carriers that balance size uniformity, structural integrity, and physiological compatibility [43].

From an interfacial perspective, surface porosity reduces the accessible silica area while increasing nanoscale roughness, thereby limiting the effective contact region between LP and the MSN surface. This reduction in contact weakens van der Waals and electrostatic interactions, diminishing the forces required to induce vesicle deformation and rupture. Consistent with this mechanism, Durfee et al. showed that lipid bilayers can form on MSN with diverse morphologies (spherical, hexagonal, rod-like) and pore diameters of 2.8-9 nm, accompanied by an average hydrodynamic size increase of ~ 30 nm post-coating. However, when pore diameters increased to ~ 18 nm, LP failed to rupture and instead adsorbed without spreading, leading to the formation of large aggregates rather than continuous bilayers [43]. Complementarily, Epler et al. demonstrated that MSN with sizes of 100-300 nm and pore diameters between 10 and 30 nm can be reliably enveloped by lipid bilayers to produce stable MSN-LP hybrids [64].

High-resolution cryo-electron microscopy further corroborates these findings, revealing lipid bilayers that conform closely to the underlying MSN topology. Importantly, surface irregularities do not impede vesicle rupture or bilayer continuity, countering earlier assumptions regarding the incompatibility of rough substrates [54]. In fact, MSN with roughened surfaces can enhance bilayer edge mobility, thereby facilitating lipid spreading relative to smooth, nonporous silica cores [47].

Notably, interfacial roughness also alters lipid thermodynamics. MSNs induce a marked reduction in lipid phase transition temperature ($\approx 6^\circ\text{C}$), leading to increased bilayer fluidity, an effect observed for both DPPC- and DOPC-based membranes. This behavior arises from nanoscale lateral tension gradients generated by three-dimensional surface features and interspersed silica-water domains at the interface, collectively promoting in-plane lipid mobility and stabilizing bilayer formation [47].

1.4.2.4. pH

Variations in solution pH exert a decisive influence on lipid bilayer assembly by modulating the surface charge state of MSN. When pH is lowered from neutral to moderately acidic conditions (e.g., pH 6.0 $\rightarrow$ 4.0), progressive protonation of surface silanol groups shifts the silica interface toward a less negatively charged state ($\equiv\text{Si-OH}$ rather than $\equiv\text{Si-O}^-$). This charge attenuation weakens electrostatic repulsion and simultaneously enhances electrostatic-dipole attractions, allowing van der Waals interactions, hydration forces, and steric effects to dominate the interfacial energetics. As a consequence, adhesion energy increases and bilayer formation kinetics transition from a two-step adsorption-rupture pathway to an apparent single-step process. While such one-step kinetics do not unequivocally prove single-vesicle rupture, they indicate that vesicle-vesicle interactions become nonessential, and that surface interactions together with bilayer edge effects are sufficient to complete membrane spreading once LP adsorption reaches saturation [65].

In contrast, alkaline conditions markedly inhibit bilayer formation. At pH ≈ 10.0 , deprotonation of silanol groups intensifies negative surface charge on both the silica substrate and LPs, substantially increasing electrostatic repulsion. Concurrently, enhanced ordering of the silica-solution interface raises the energetic barrier to vesicle rupture, favoring nonproductive LP adsorption over bilayer assembly [66]. Near pH 9, close to the second pKa of silica, the system exhibits mixed behavior, where bilayer formation and unfavorable adsorption may coexist, reflecting the delicate balance between electrostatics and interfacial organization [65].

1.4.2.5. Temperature

Temperature critically modulates lipid bilayer mobility and, by extension, the propensity of LP vesicles to deform and collapse into supported membranes on silica. In the study by Jing et al., smaller vesicles ($\sim 90\text{-}130\text{ nm}$) readily formed bilayers at temperatures near the lipid phase transition (T_m), whereas larger vesicles ($\sim 160\text{ nm}$)

showed negligible bilayer formation even at 50 °C ($>T_m$). Conversely, when systems were heated stepwise from 22 °C to 50 °C, a clear kinetic progression emerged: initial adsorption at 22 °C was followed by vesicle flattening as the temperature approached ~41 °C, which increased LP-LP lateral interactions and reduced edge curvature of nascent patches, culminating in complete bilayer coverage at 50 °C. These observations indicate that elevating temperature toward or above T_m lowers membrane bending rigidity and line tension, thereby facilitating the adsorption-to-rupture transition (**Figure 1.5**). Notably, vesicle size imposes an additional constraint: larger LP, despite adsorption, may not achieve sufficient membrane tension to rupture under comparable thermal conditions, highlighting the coupled roles of thermal state and geometric curvature in dictating bilayer assembly kinetics [40].

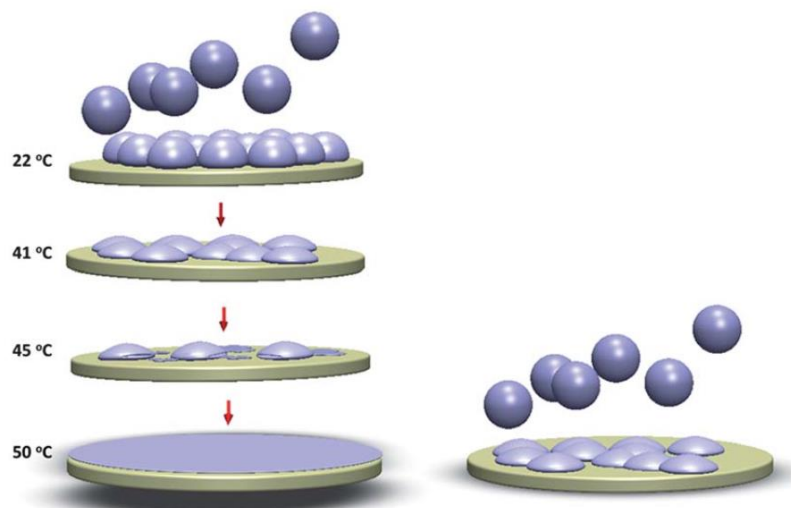


Figure 1.5. Adsorption and bilayer formation of 160 nm LP on silica surfaces.

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1.4.2.6. Ionic strength

Beyond pH and temperature, ionic strength is a key regulator of lipid bilayer assembly on nanosilica, as it modulates electrostatic screening, vesicle-surface adhesion, and vesicle-vesicle interactions [54, 62, 67-69]. In the study of Savarala et al., 100 nm DMPC LP failed to coat nanosilica (4-100 nm) in deionized water at $\text{pH} \approx 9$; at low NaCl, only larger silica cores formed coatings, whereas progressively higher salt concentrations enabled hybrid nanosilica-LP formation on smaller particles down to ~20 nm. Kinetically, NaCl < 0.75 mM yielded slow,

time-dependent bilayer formation, with smaller cores showing the slowest rates; above this threshold, rapid bilayer formation was observed for larger cores, while small cores aggregated. These outcomes likely reflect ion-headgroup interactions: small, weakly polarizable ions with compact solvation shells can associate with phospholipid phosphates, effectively shifting their electrostatic character and enhancing adhesion to the silica interface. Added ions may also increase silica surface charge density by promoting silanol protonation, collectively favoring adsorption and rupture [62].

Complementary results by Durfee et al. showed that at low LP:nanosilica surface-area ratios, raising PBS from 0 to 160 mM induced particle aggregation, increasing hydrodynamic diameter and PDI. In contrast, at higher LP:nanosilica ratios, the presence of PBS produced smaller LP-coated silica relative to PBS-free conditions, and further increases in PBS had minimal additional impact on size [43]. These findings suggest that ionic strength exerts a dual effect: it can (i) screen repulsions to promote adsorption, vesicle flattening, and rupture when sufficient lipid is available to achieve near-continuous coverage, yet (ii) drive bridging and aggregation at low lipid coverage or for highly curved (small) silica cores. Thus, rational control of salt identity and concentration, in tandem with LP dose and particle curvature, is essential for reproducible, uniform bilayer formation.

1.4.2.7. Ratios of LP to nanosilica

At sub-saturation surface coverage, individual LP vesicles behave largely independently, experiencing primarily LP-surface interactions and temperature-dependent membrane effects. Under these conditions, and depending on ancillary variables, adhesion forces are often insufficient to induce vesicle rupture and bilayer spreading. Once the adsorbed LP density approaches saturation, however, emergent vesicle-vesicle interactions (lateral crowding, edge wetting, and cooperative flattening) can supply the additional driving force needed to trigger collapse and membrane coalescence [40]. Consistent with this cooperative requirement, Garcia-Manyes reported that a surface-area (SA) ratio of lipid : silica > 1 is typically necessary for robust bilayer formation on silica supports [70], and Mornet et al. successfully produced nanosilica-LP hybrids at $SA_{\text{lipid}} : SA_{\text{silica}} \approx 15$ [54]. Notably, formulation and medium effects can relax this threshold: Durfee et al. observed effective coating even at $SA_{\text{lipid}} : SA_{\text{silica}} \approx 0.5$ in deionized water, underscoring the context-dependence of the critical coverage condition [43].

When the LP dose is modest relative to nanosilica, cross-linking bridges can form between adsorbed vesicles and particles, yielding gel-like aggregates rather than continuous bilayers [57]. In the extreme lipid-deficient regime, where nanosilica outnumbers LP by ~38-150 times and the silica cores are much smaller than the vesicles (e.g., 10-20 nm silica with ~100 nm LP), the silica can decorate the LP surface, forming a silica corona around individual vesicles and thereby stabilizing the dispersion without bilayer rupture [71].

1.4.2.8. Permeability pressure of LP

LP exhibiting a high internal osmotic pressure, typically arising from elevated ionic concentrations within the aqueous core, are generally less deformable and display increased membrane rigidity. This reduced flexibility limits the ability of adsorbed vesicles to flatten and undergo tension-induced rupture on silica substrates. Consequently, even when such LP attach to the silica surface, their constrained mechanical response can suppress membrane collapse, thereby inhibiting the transition from surface adsorption to the formation of a continuous supported lipid bilayer. This observation underscores the importance of osmotic conditions and internal vesicle composition in regulating the mechanical deformability required for successful bilayer assembly on nanosilica surface [40].

1.4.2.9. Fatty acid composition of LP

LPs can be formulated from a wide spectrum of phospholipids spanning different net charges, and this compositional choice strongly influences bilayer formation on MSN surfaces. Representative classes include cationic lipids (e.g., DOTAP), anionic lipids (e.g., DOPS, POPS, DOPG, DPPG, POPG), and zwitterionic/neutral lipids (e.g., DOPC, DMPC, POPC, DPPC). Charge-dependent interactions at the LP-silica interface modulate adhesion, deformation, and rupture, thereby governing the efficiency and continuity of supported bilayer assembly.

Beyond headgroup charge, the acyl-chain chemistry of constituent phospholipids exerts a decisive effect on MSN-LP stability. Durfee and co-workers reported that bilayers constructed from DOPC (unsaturated) and DSPC (saturated) both formed stable dual layers initially; however, their time-dependent behaviors diverged in physiologic buffers. DOPC-based MSN-LP displayed progressive size growth over 24-72 h in PBS, whereas DSPC-based protocells remained stable for >72 h at 37 °C and for >6 months at ambient temperature. Notably, DOPC systems were stabilized in oxygen-reduced media but aggregated in standard PBS, while

DSPC systems showed no discernible sensitivity to oxygen [43]. Altogether, these observations demonstrate that unsaturated lipid chains are preferentially susceptible to oxidation, establishing lipid peroxidation as a key driver of bilayer destabilization and aggregation, whereas saturated lipids such as DSPC confer improved oxidative resilience and long-term colloidal stability.

1.4.3. The ability of LP-coated MSN to control drug release

1.4.3.1. pH

pH fluctuations can profoundly modulate the organization of supported membranes, leading to localized swelling of the lipid bilayer and the emergence of transient pores that facilitate drug permeation [41]. The extent of pore formation depends on bilayer composition, particularly the identities and ratios of constituent phospholipids and sterols, which dictates headgroup hydration, acyl-chain order, and overall membrane mechanics.

Mechanistically, acidification diminishes headgroup hydration and increases acyl-chain ordering, thereby altering membrane dipolar asymmetry and bending rigidity. According to membrane mechanics, bending is favored when asymmetric stresses develop across the two leaflets. For bilayers supported on nanosilica, this asymmetry arises because the substrate suppresses thermal undulations of the inner leaflet and perturbs its hydration layer. Low pH further modifies the hydration structure at the silica-water interface, so that the inner leaflet (in close contact with the substrate) experiences a distinct local pH compared with the outer leaflet. Moreover, upon an external pH shift, the outer leaflet responds rapidly, whereas pH equilibration to the inner leaflet is delayed, with the timescale governed by bilayer defect density. The resulting pH gradient across leaflets induces differences in spontaneous curvature, elevates interfacial tension, and drives the formation of blister-like protrusions and associated pores via lateral rearrangements of phospholipids. Such defects are favored when interleaflet coupling is sufficiently weak to permit out-of-plane undulations beyond a flat configuration [41].

An additional pathway involves proton penetration of the membrane under acidic conditions. Protons can reach the silica-water interface, strengthening bilayer-substrate interactions and decreasing the interleaflet-substrate spacing. Because confined interfacial water cannot readily dissipate laterally (high near-surface viscosity) or traverse the membrane vertically (greater bilayer density at low pH), the

bilayer deforms to create voids that accommodate this water, again producing blisters and pores [41].

Consistent with these mechanisms, accelerated drug release at low pH also correlates with reduced bilayer stability, evidenced by MSN-LP aggregation, which further compromises the barrier function of the coating [43].

1.4.3.2. Temperature

Temperature exerts a pronounced influence on the physicochemical characteristics of dual lipid bilayers, particularly in the vicinity of the lipid phase transition temperature (T_m), which marks the transformation from an ordered gel phase to a disordered liquid-crystalline state. At this transition point, membrane properties, including permeability, microviscosity, and mechanical rigidity, undergo substantial and highly cooperative changes as ordered and fluid lipid domains coexist within the bilayer architecture [72]. Notably, membrane permeability typically reaches a maximum near T_m , creating a transiently destabilized state that can be harnessed for controlled release applications.

This thermoresponsive behavior has been strategically exploited in the design of dual lipid bilayer coatings capable of temperature-triggered drug release. In particular, elevated temperatures characteristic of tumor microenvironments ($\approx 42^\circ\text{C}$) or locally induced hyperthermia generated by external stimuli can activate membrane permeabilization, thereby promoting site-specific payload release [73]. To this end, several studies have reported the formulation of thermosensitive LP composed of phospholipids with phase transition temperatures close to physiological hyperthermic ranges (approximately 41°C). Such systems have demonstrated the ability to release encapsulated therapeutic agents or imaging probes within the temperature window of $40\text{--}45^\circ\text{C}$, rendering them promising candidates for cancer diagnosis and treatment [74].

As the field advances toward more sophisticated temperature-responsive delivery platforms, a deeper understanding of bilayer interactions at T_m becomes essential. In particular, interfacial phenomena governing LP-LP interactions and LP-surface contacts during phase transition play a critical role in determining membrane stability, fusion behavior, and release kinetics. Model systems based on surface-adsorbed LP offer a controlled experimental framework for probing these interactions and elucidating the mechanistic pathways associated with thermally induced membrane restructuring [40].

1.4.4. Synthesis method of ML

1.4.4.1. *Mixing method*

The mixing method represents a straightforward and widely applied strategy for the fabrication of ML hybrid nanostructures. This approach is based on the spontaneous adsorption of LP onto the surface of mesoporous silica nanoparticles, followed by lipid reorganization and collapse, which collectively result in the formation of a continuous lipid bilayer enveloping the MSN core [47, 54]. Owing to its operational simplicity and mild experimental conditions, this method has been extensively employed for MSN-LP assembly.

From a procedural standpoint, the mixing method typically comprises three sequential steps. First, independent colloidal dispersions of MSN and LP are prepared under controlled conditions to ensure stability and uniformity. Subsequently, the two suspensions are combined and gently mixed, allowing electrostatic interactions, hydrophobic forces, and lipid fluidity to drive bilayer formation on the MSN surface. Finally, unbound or excess LP are eliminated through purification processes such as centrifugation or filtration to yield well-defined ML hybrids.

Importantly, this assembly strategy also offers flexibility with respect to drug encapsulation pathways. For the synthesis of drug-loaded ML systems, therapeutic agents may be pre-encapsulated within the MSN pores or incorporated into the LP prior to the mixing step, depending on the physicochemical properties of the drug and the desired release profile. As a result, the mixing method not only facilitates efficient lipid coating but also supports modular drug loading, making it a versatile platform for the construction of ML-based delivery systems.

a) Simple mixing

Several studies have demonstrated the feasibility of ML hybrid nanostructures using the mixing strategy, although differences in lipid composition, surface charge, and processing parameters strongly influence the resulting architectures. For instance, Ashley and co-workers prepared ML systems by combining MSN with preformed LP composed of amphiphilic phospholipids. Two LP formulations were investigated: DOPC (C18, unsaturated, $T_m \approx -20^\circ\text{C}$) and DPPC (C16, saturated, $T_m \approx 41^\circ\text{C}$), each blended with cholesterol and PE-PEG2000, yielding LP with an average diameter of approximately 120 nm. Negatively charged MSNs (100-150 nm), pre-encapsulating therapeutic cargos such as small-molecule drugs, siRNA, or proteins, were dispersed in $0.5\times$ D-PBS and subsequently mixed with excess LP (MSN:LP volume ratios of

1:2 to 1:4) for 30-90 min at room temperature. The resulting ML hybrids were maintained in LP-rich suspensions, and unrelated LP were removed immediately prior to use by centrifugation (10 000 rpm, 5 min), followed by washing and redispersion in $0.5\times$ D-PBS [47].

Extending this methodology, Epler et al. explored protein-loaded MSN with diameters of approximately 190 nm and pore sizes ranging from 10 to 30 nm, which were integrated with LP composed of DOPC, DOPE, cholesterol, and PEG2000-PE. After lipid coating, the ML nanostructures exhibited a modest increase in hydrodynamic diameter, reaching approximately 205 nm, indicative of successful LP deposition without substantial particle aggregation [64].

In contrast to charge-complementary systems, Dengler and colleagues demonstrated ML synthesis via direct mixing of positively charged MSNs and positively charged LP. Homogenization was achieved using manual pipetting in microcentrifuge tubes, followed by incubation at ambient temperature for 1 h with intermittent agitation. Excess LP was then removed by brief centrifugation (6000 rpm, 2 min), and the purified ML particles were washed and redispersed in PBS [59].

Similar mixing-based strategies were reported by Liu et al., who combined 100 nm MSNs with LPs prefiltered through a 100 nm membrane [57], and by Tu et al., who optimized ML formation using an MSN:LP mass ratio of 1:1.88 in phosphate buffer (1 mM, pH 7.4) under continuous shaking prior to centrifugation-based purification [75].

Collectively, these studies underscore the operational simplicity of the mixing method for ML fabrication; however, they also reveal inherent constraints, as the approach is typically limited to small-scale preparation and relies on environments with relatively high ionic strength to promote stable LP adsorption.

b) Combined mixing with ultrasound

During the fabrication of ML hybrids via direct mixing, transient particle aggregation is frequently observed as an intermediate phenomenon. If such aggregation is not adequately managed, it may evolve into irreversible clustering, ultimately compromising colloidal stability and material performance. To address this challenge, recent studies have increasingly incorporated ultrasonication as a post-mixing treatment. The application of ultrasound energy promotes disaggregation

and uniform lipid redistribution, leading to improved particle homogeneity and enhanced stability of the final ML constructs [43, 56, 76, 77].

Building on this approach, Shi and co-workers combined MSN and LP with opposite surface charges, either positively charged MSNs (~210 nm) with negatively charged LPs (~150 nm), or vice versa, followed by sonication and incubation at room temperature for 4 h. Excess LP were subsequently removed by centrifugation. Notably, the resulting ML particles exhibited a size increase of approximately 30-40 nm relative to bare MSN, despite the lipid bilayer itself having a thickness of only ~6 nm, underscoring the influence of lipid adsorption and solvation on hydrodynamic diameter [56].

Similarly, LaBauve et al. employed brief ultrasonic treatment (30 s) to assemble ML hybrids from negatively charged MSN (96 nm, -25 mV) and LP (125 nm, -0.78 mV) at an MSN:LP mass ratio of 1:5. After removal of excess LP by high-speed centrifugation, the resulting hybrids displayed increased particle sizes (149.5 nm without drug loading and 164 nm when loaded with ML336). The authors further demonstrated that colloidal integrity could be preserved during storage through cryoprotection using sucrose and freezing at -80 °C [77].

Extending these findings to non-spherical systems, Zhang and colleagues reported successful coating of rod-shaped MSN by LP following brief sonication (2 min). Transmission electron microscopy confirmed the formation of a well-defined lipid bilayer around the anisotropic silica cores [78].

Most recently, Parra-Nieto et al. introduced additional surface functionality by synthesizing ML systems incorporating PEG-COOH or PEG-NH₂. LP derived from DSPC, cholesterol, and PEGylated lipids were mixed with MSN (150 nm) and subjected to short sonication prior to purification, yielding stable hybrids with sizes in the range of 150-180 nm [76].

1.4.4.2. Thin film hydration method

In contrast to the direct mixing approach, the thin membrane hydration method enables LP to assemble onto MSN during phospholipid membrane rehydration, thereby forming ML hybrids through a fundamentally different pathway. This strategy generally involves four sequential stages: generation of a thin lipid film, hydration of the film with an MSN dispersion, homogenization and size regulation, and purification of the final product by centrifugation. Although widely employed, the underlying formation mechanism remains incompletely understood. While

electrostatic attraction contributes to lipid-particle association, non-covalent interactions such as van der Waals forces are thought to facilitate rapid and extensive bilayer formation. Moreover, thermodynamic factors, including changes in interfacial free energy, hydration layer thickness, lipid mobility, and dispersion stability, are likely to play critical roles yet require further investigation [79].

Compared with mixing-based assembly, membrane hydration offers distinct advantages for drug-loaded systems. Direct lipid deposition enables immediate pore sealing and complete surface coverage, thereby minimizing drug leakage during post-processing. In addition, the purification steps are less detrimental to drug retention, and the method is amenable to scale-up, with reported yields reaching approximately 200 mg per batch [79]. Nevertheless, an inherent limitation arises from insufficient lipid availability during hydration, which may lead to incomplete coverage, lipid bridging, and particle aggregation. To alleviate these issues, high-energy ultrasonication is commonly applied, although excessive acoustic energy may compromise nanoparticle integrity or introduce contamination risks.

Several representative studies illustrate both the strengths and challenges of this technique. Meng et al. hydrated paclitaxel-containing lipid films with gemcitabine-loaded MSN (60-70 nm) at a near-equimolar MSN:LP ratio, producing coated particles following sonication and centrifugation [79]. Similarly, Liu and colleagues reported a pronounced size increase for lactoferrin-modified MSN-LP systems (90 nm to 177 nm), reflecting effective lipid deposition [80]. In contrast, Choi et al. observed partial aggregation, with clusters of MSNs encapsulated within a shared bilayer, highlighting sensitivity to processing conditions [81].

In a related protocol, Li and colleagues employed a comparable coating strategy in which MSN co-loaded with paclitaxel and tanshinone IIA were dispersed in PBS and then brought into contact with a lipid membrane, followed by ultrasonic hydration for 20 min and centrifugation at 10,000 rpm. Consistent with successful lipid deposition, the hydrodynamic diameter increased stepwise: drug-loaded MSN measured ~266 nm compared with ~230 nm for bare MSN, and subsequent LP coating yielded particles of approximately 294 nm [82]. Alternative homogenization strategies, such as high-pressure extrusion employed by Zhang et al., have successfully reduced aggregation while yielding controlled size increases (90 nm to 105 nm) [83].

1.4.4.3. Pre-hydration mixing method

Extending these coating strategies, several groups have prepared ML constructs using a pre-hydration mixing approach. In this workflow, MSN are homogeneously combined with lipid constituents in an organic solvent; the solvent is then evaporated to yield a thin, dry lipid film embedding the MSN, which is subsequently hydrated, homogenized, and centrifuged to recover the coated product. This route integrates cargo loading and membrane assembly, facilitating uniform lipid deposition prior to aqueous processing.

Illustrative of this method, Yang et al. dispersed dry MSN in chloroform containing selected LP components such as SP, DPPG, cholesterol, and PEG-DSPE, followed by low-pressure solvent removal to form a thin lipid film with MSN embedded. The film was then rehydrated and dispersed by vortexing and brief sonication (5 min), subjected to probe sonication (200 W, 10 cycles, 10 s each), and finally extruded through a 0.22 μm membrane. The resulting ML assemblies exhibited a substantial size increase relative to bare MSN (~ 167 nm vs. 86-107 nm), consistent with successful lipid encapsulation and formation of a continuous membrane shell [58].

1.4.4.4. Solvent transfer method

Within the solvent-transfer approach, lipid constituents (e.g., DOTAP, DOPC, POPC) are first dissolved in chloroform, vacuum-dried overnight to form a thin film, and then re-solubilized in a 4:6 (v/v) ethanol-water mixture. In parallel, the MSN dispersion is clarified by centrifugation at 19,000 rpm to collect the MSN pellet. The ethanol-water lipid solution is subsequently introduced to the MSN pellet, followed by gradual addition of water to shift the solvent composition toward 100% aqueous phase, thereby promoting lipid deposition on the MSN surface. The coated product is finally recovered by centrifugation. Using this protocol, Cauda et al. obtained ~ 100 nm MSN-LP hybrids from ~ 50 nm MSNs, confirming efficient lipid transfer and shell formation [84]. Nevertheless, this route is generally constrained to small-scale preparations and carries a heightened risk of aggregation, likely due to solvent exchange kinetics and limited control over colloidal stability during the water-driven coating step.

1.4.4.5. Inverse vapor-phase deposition method

As an alternative to solution-phase coating, the inverse vapor-phase deposition method proceeds by dissolving phospholipids and cholesterol in a chloroform:methanol (5:1, v/v) mixture, followed by the incremental incorporation

of an MSN dispersion (HEPES buffer containing NaCl, NaN_3 , and sucrose). After each addition, the mixture is sonicated to promote lipid–particle contact and then vacuum-evaporated to remove residual solvent and drive lipid organization at the MSN interface. This add-sonicate-evaporate cycle is repeated with a second aliquot of the MSN dispersion to reinforce bilayer assembly. Once the lipid bilayer has formed, the suspension is incubated at 45 °C for 30 min to enhance membrane annealing and uniformity, and subsequently extruded 30 times through a 0.4 μm polycarbonate membrane to narrow the size distribution and improve colloidal homogeneity [85]. This vapor-assisted workflow thus integrates controlled solvent removal with thermal and mechanical post-processing to yield reproducible LP coatings on MSN.

1.4.4.6. Hydrophobic MSN-based method

Beyond vesicle-mediated coating strategies, an alternative approach relies on direct alkyl-chain functionalization of the MSN surface to induce lipid assembly without the need for preformed LP. In this method, MSN are first modified with long-chain alkyl groups, after which phospholipids spontaneously assemble via hydrophobic chain–chain interactions, yielding a lipid bilayer directly on the silica surface. This strategy eliminates the requirement for LP synthesis and generates a bilayer that interacts strongly with the MSN through covalent or quasi-covalent anchoring, thereby enhancing interfacial stability. However, this strong coupling can also be a limitation: increased bilayer robustness may retard drug release, and the conversion of the MSN surface to a hydrophobic state can impair the loading of hydrophilic therapeutics.

Leveraging this strong interfacial bonding for stimulus-controlled release, Han et al. engineered MSN-LP-like systems incorporating redox-responsive linkages. Specifically, MSN were functionalized with thiol groups and connected to octadecanethiol via a disulfide bridge to form MSN-SS-C18. Subsequently, phospholipids and D- α -tocopherol polyethylene glycol 1000 succinate (TPGS) were deposited using a thin-film hydration technique, producing a lipid shell that can be destabilized under reductive conditions to trigger drug release [86].

In a closely related application, Kheirkhah and co-workers adopted alkylation to tailor MSN for poorly soluble drugs. MSN were rendered hydrophobic through palmitoyl chloride modification, facilitating efficient encapsulation of

dexamethasone, and were then coated with an external phospholipid-PEG layer via ultrasound assistance to restore colloidal stability and biocompatibility [87].

1.4.5. Application of MSN-LP in cancer treatment

As outlined above, conventional chemotherapy in cancer treatment faces major limitations, including severe side effects, low selectivity, and the risk of multidrug resistance. Employing smart nanocarriers for drug delivery offers a viable strategy to mitigate these drawbacks. A key design strategy to enhance nanocarrier performance involves integrating established materials to construct hybrid platforms with superior synergistic properties. In this context, ML systems, which integrate the high loading capacity of MSN with the biocompatibility and barrier function of LP, have attracted considerable attention as next-generation drug delivery platforms.

Building on this concept, Zhang et al. developed pluronic-functionalized ML to deliver irinotecan (CPT-11) for the treatment of multidrug-resistant MCF-7/BCRP breast cancer cells. In this system, the dual lipid layer preserves the drug within MSN pores during circulation and undergoes rapid disruption under acidic endosomal conditions, enabling triggered release. Moreover, surface-grafted pluronic P123 suppresses efflux pump activity, promoting intracellular drug retention. As a result, intracellular drug levels increased 12.9-fold, producing 7.1-fold higher cytotoxicity relative to free drug. In murine models, CPT-11-loaded ML achieved >81.2% greater tumor growth inhibition with reduced systemic toxicity [83].

Similarly, Amin et al. employed ML to encapsulate doxorubicin (DOX) for drug-resistant breast cancer therapy. The positively charged LP shell not only prevented premature drug leakage but also enhanced electrostatic interactions with cancer cell membranes, thereby improving endocytic uptake and antitumor efficacy while mitigating off-target effects [88].

Further emphasizing the versatility of ML, Liu et al. demonstrated a protonation-assisted strategy to improve irinotecan loading and release control. Amphiphilic irinotecan diffuses through the lipid layers and becomes protonated within MSN pores, transforming into a hydrophilic species that is efficiently retained. Compared with LP-only formulations, ML systems displayed enhanced stability, improved pharmacokinetics, prolonged drug release, and greater tumor accumulation, leading to superior tumor suppression and reduced toxicity to the liver and bone marrow [89].

One of the recent trends attracting attention is the research on carrier materials capable of delivering multiple drugs simultaneously. These drugs often exhibit synergistic effects, enhancing the efficacy of cancer cell killing.

Illustrating this concept, Meng et al. co-loaded gemcitabine (GEM) and paclitaxel (PTX) at a 10:1 ratio by confining GEM within the MSN core and embedding PTX in the dual lipid layer of ML. The LP coating retained GEM effectively (encapsulation ~40 wt%), while PTX was maintained at sub-toxic doses; nevertheless, the synergistic pairing yielded tumor growth inhibition ~12-fold greater than free GEM in a PANC-1 xenograft model. Mechanistically, ML encapsulation increased GEM phosphorylation and DNA interaction ~13-fold and reduced deamination ~4-fold, translating into enhanced antitumor activity without local or systemic toxicity [79].

Expanding to other drug pairs, Choi et al. designed ML carriers loading axitinib (AXT) in the MSN core and celastrol (CST) in the lipid shell for applications in brain cancer (SH-SY5Y), squamous cell carcinoma (SCC7), and breast cancer (BT474). The platform exhibited pH-responsive retention/release (minimal leakage at pH 7.4; sustained release at pH 5.0), enabling AXT-mediated anti-angiogenesis and CST-mediated mitochondrial disruption to co-activate apoptosis and suppress tumor growth. In vivo, AXT/CST-loaded ML achieved >64% greater tumor inhibition than comparator groups [81].

Targeting can further potentiate combination therapy. Li et al. reported folate-functionalized ML (ML-FA) co-carrying PTX and tanshinone IIA (TanIIA), a pair known to synergize by inducing apoptosis and blocking NB4 promyelocytic leukemia cell differentiation. The active-targeting surface enhanced tumor homing and cellular uptake, producing significantly stronger tumor growth suppression than free drugs, with no detectable injury to vital organs (heart, liver, spleen, lungs, kidneys) in mice [82].

Beyond small-molecule drugs, ML can deliver biomacromolecules and genetic cargo. Nouredine employed ML to transport CRISPR segments into HeLa and A549 cells, demonstrating the platform's capacity to enable gene-editing therapeutics [90]. Likewise, Epler et al. used peptide-modified ML to deliver ricin toxin A-chain (RTA) to Hep3B hepatocellular carcinoma: SP94 enhanced cancer-cell affinity, while H5WYG facilitated endosomal escape, yielding high, selective cytotoxicity to Hep3B with minimal toxicity to healthy hepatocytes [64].

Integrating therapy with imaging and stimulus-responsiveness further elevates ML utility. Sun et al. (2017) created DOX@GdMSN-ICG-TSL, combining Gd-doped MSN (MRI contrast), ICG (NIR fluorescence and photoacoustic imaging; photothermal/photodynamic agent), and a thermosensitive LP shell. Under NIR irradiation, ICG-generated heat disrupted the lipid barrier, triggering DOX release while simultaneously ablating tumors photothermally/photodynamically. After 20 days, tumors in treated mice were 5× smaller than those receiving DOX@GdMSN (no LP), 5× smaller than ICG-LP, and 10× smaller than untreated controls, with significantly prolonged survival, underscoring tri-modal imaging (MRI/NIRFI/PAI) and multi-mechanistic therapy within a single construct [91].

Finally, shape engineering can improve intratumoral transport. Zhang et al. (2021) used longitudinally aligned MSN coated with LP to co-deliver DOX and ICG for pancreatic ductal adenocarcinoma (**Figure 1.6**). The anisotropic MSN geometry enhanced mobility through dense tumor and mucosal matrices, while the lipid bilayer retained payloads until NIR-triggered release (via ICG-generated heat), achieving synergistic chemo-photothermal activity. Both *in vitro* and *in vivo* data showed superior tumor penetration and growth inhibition relative to free drugs or single-carrier controls (MSN-only or LP-only) [78].

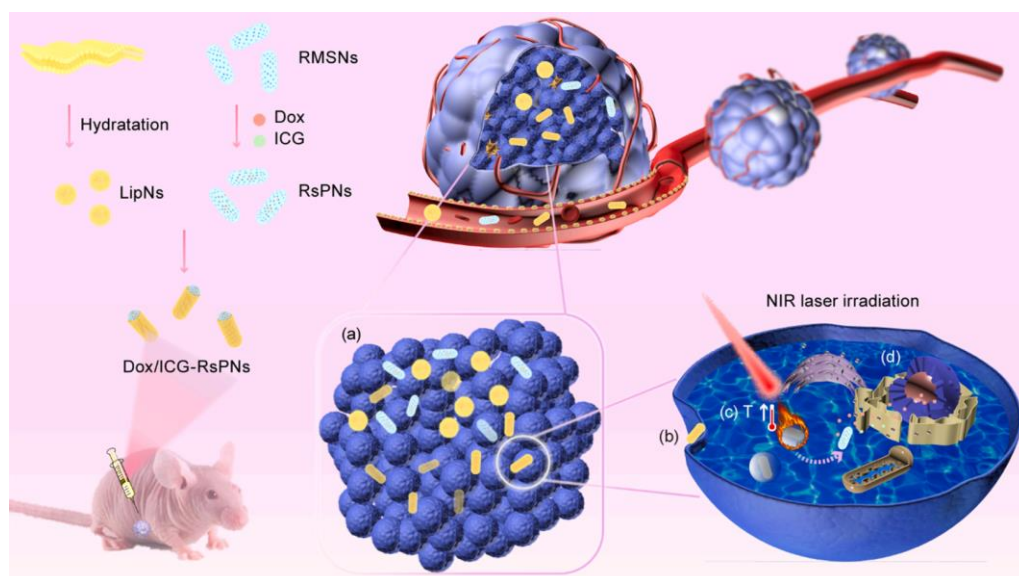


Figure 1.6. Illustration of the synthesis of Dox/ICG-RsPN to enhance: (a) tumor penetration capability, (b) cellular uptake, and (c) temperature elevation, (d) triggered smart drug release activated by NIR laser. Reproduced with permission from ref [78]. Copyright 2026 Elsevier.

1.5. Biocompatible polymers

1.5.1. Gelatin

Gelatin is a naturally derived, highly versatile biopolymer with a long record of safe use across pharmaceuticals, cosmetics, and foods. It combines essential attributes, such as non-toxicity, non-carcinogenicity, biodegradability, and non-irritability, and is classified by the U.S. Food and Drug Administration as Generally Recognized as Safe (GRAS) [92]. Its polymer backbone presents abundant reactive functional groups, enabling straightforward chemical modification and broad formulation flexibility. Compositionally, gelatin contains ~13% cationic amino acids (arginine, lysine), ~12% anionic amino acids (aspartate, glutamate), and ~11% hydrophobic residues (alanine, methionine, valine, leucine, isoleucine), with the remainder largely glycine, proline, and hydroxyproline. Importantly, enzymatic degradation yields constituent amino acids without harmful by-products, underscoring its biological compatibility [93].

From a materials perspective, gelatin is typically obtained by partial acid or alkaline hydrolysis of insoluble animal collagen. Collagen itself is a key structural protein, comprising ~25-35% of total body protein, abundant in skin, tendons, ligaments, and also present in cornea, bone, cartilage, vasculature, gut, and intervertebral discs [94]. Its hallmark triple-helix architecture arises from three α -chains coiled in a suprahelical arrangement dictated by the conserved sequence (Gly-X-Y)_n (with X often lysine, methionine, arginine, or valine). Glycine accounts for ~33% of residues, while another ~33% are proline/hydroxyproline (Y), conferring neutral and hydrophobic character; interchain hydrogen bonding stabilizes the fibrillar assembly [95]. Acidic or basic hydrolysis disrupts higher-order structure, erasing triple-helix order and producing gelatin.

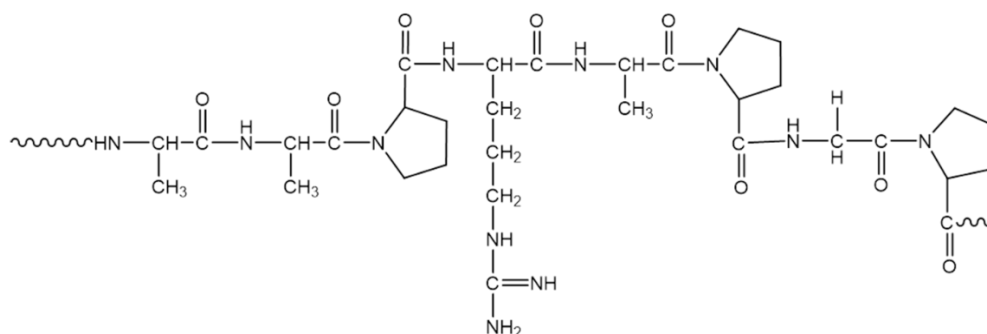


Figure 1.7. Chemical structure of gelatin

Commercially, the two principal grades, gelatin A and gelatin B, are distinguished by their isoelectric points (IEP) and processing routes. Type A (IEP 7-

9) arises from acid treatment, yielding a positively charged polymer near neutral pH; Type B (IEP 4.7-5.5) is produced by alkaline treatment, which hydrolyzes amide groups of glutamine/asparagine to carboxylates, imparting high carboxyl density and thus a negative charge at physiological pH [96]. The alkaline process targets glutamine and asparagine's amide groups, hydrolyzing them into carboxyl groups, which imparts a high density of carboxyl groups to the resulting gelatin, making it negatively charged [97]. This tunable charge landscape, together with rich chemical functionality, enables precise conjugation strategies (direct modification or linker-mediated coupling), a crucial advantage for designing targeted drug delivery systems with high payload loading and controlled release [98].

Clinically, gelatin is widely employed in systemic formulations as a plasma volume expander, and as a stabilizer in protein therapeutics, vaccines, and hemostatic sponges (e.g., gel foam). Beyond excipient roles, its bioactive sequences are notable: the Arg-Lys-Gly motif appears in numerous extracellular matrix proteins, mediating cell binding and signaling via integrin β -subunits; similarly, the Arg-Gly-Asp (RGD) sequence is central to cell adhesion [99]. These biochemical cues, combined with chemical modifiability and benign degradation, make gelatin an attractive platform not only for drug delivery but also for tissue engineering, where it serves as a matrix for mineralization and regenerative scaffolding [100].

1.5.2. Chitosan

First reported in a deacetylated form of chitin by Rouget in 1859, chitosan (CTS) has evolved from a historical curiosity to a modern biomedical workhorse [101]. Its pharmaceutical relevance expanded markedly in the early 1990s, when both academic and industrial groups began to exploit CTS as a platform for advanced therapeutic systems. Chemically, CTS presents a high density of primary amines that serve as versatile reaction handles, enabling the formation of diverse covalent linkages under mild conditions. In aqueous media, these amines are readily protonated, imparting a net positive charge that underlies CTS's designation as an amino-polysaccharide. The polymer backbone consists of repeating D-glucosamine and N-acetyl-D-glucosamine units, each monomer bearing one amino ($-\text{NH}_2$) and two hydroxyl ($-\text{OH}$) groups that collectively support broad chemical derivatization and intermolecular interactions [102].

The ionizable nature of CTS confers pH-dependent solubility and conformation. At neutral pH, CTS is poorly soluble; as the pH drops below its isoelectric region,

protonation of the amines increases solvation and promotes chain expansion. Conversely, at higher pH, the weakly basic amines interact less favorably with water, driving polymer contraction and reduced solubility. Moreover, targeted chemical modification, such as sulfonation, can invert the net charge, yielding anionic derivatives with enhanced water solubility, favorable rheology, high water retention, and antithrombotic behavior [103]. The material's properties span a wide range, with molecular weights commonly between ~ 300 and >1000 kDa and degrees of deacetylation from $\sim 30\%$ to 95% , reflecting source and processing history [104].

Owing to its origin from non-toxic, renewable feedstocks, CTS is generally regarded as biocompatible and biodegradable, with low immunogenicity and intrinsic antibacterial activity. These attributes initially motivated its adoption in wound dressings, tissue engineering, and even weight-management products. Over time, however, CTS has emerged as a leading candidate for drug delivery. Key functional advantages, mucosal adhesion, permeation enhancement, in situ gelation, controlled transport, colon targeting, and efflux pump modulation, are largely traceable to its protonatable amine groups and their capacity to engage in electrostatic interactions at biological interfaces [105, 106].

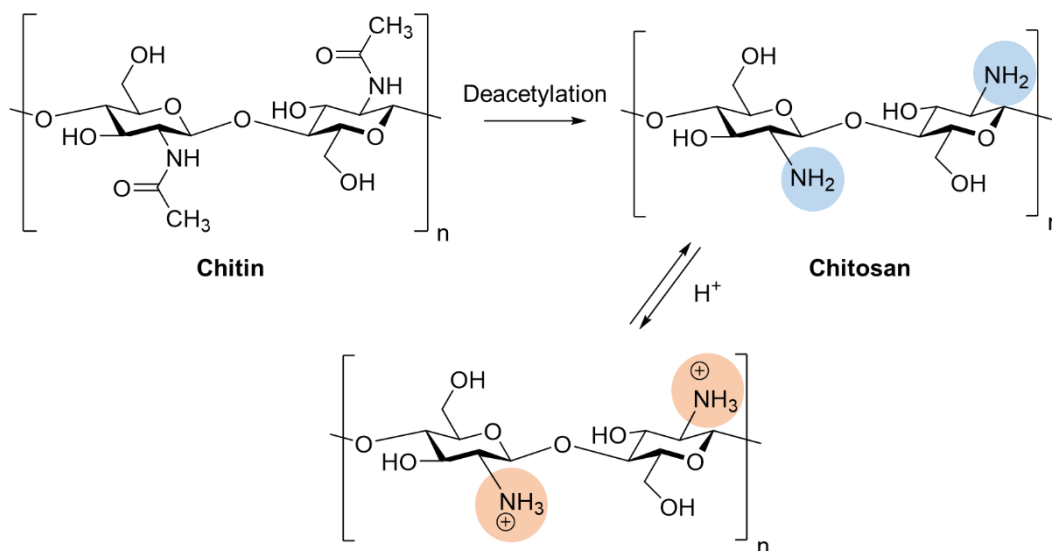


Figure 1.8. Preparation and protonation of chitosan

The protonation-deprotonation of CTS's amines across physiological pH ranges naturally lends itself to stimuli-responsive drug delivery. Under acidic conditions (e.g., below the isoelectric point), enhanced protonation improves hydration and swells the polymer matrix, facilitating diffusional transport of entrapped drugs and accelerating release. In contrast, at neutral or basic pH, polymer compaction slows

diffusion and attenuates release rates. By tuning environmental pH or engineering CTS derivatives with tailored pKa, one can modulate release profiles with precision. This principle has been widely applied *in vitro* to mimic *in vivo* contexts such as tumor acidity by varying medium pH, and to deploy CTS as a gatekeeper over porous carriers to curb burst release yet enable on-demand discharge in acidic microenvironments. From a translational standpoint, the ready availability and low cost of CTS are advantageous for clinical scale-up and commercialization, aligning material performance with manufacturing feasibility.

CTS is susceptible to enzymatic depolymerization by chitosanase, glucosaminidase, chitobiase, and N-acetyl-glucosaminidase. In mammalian systems, enzymes such as lysozyme and pepsin can also contribute to degradation under appropriate pH, temperature, and ionic conditions. The degradation rate is a pivotal design parameter: it must be harmonized with tissue remodeling dynamics for regenerative medicine, or tuned to achieve desired drug release schedules in therapeutic applications. A persistent limitation, however, is CTS's poor solubility at neutral pH, which has spurred extensive derivatization to create water-soluble analogues with improved performance and broader biomedical utility.

As a cationic polysaccharide, CTS forms strong electrostatic interactions with anionic substrates, including cell membranes and mucin, thereby enhancing residence time at mucosal surfaces and transiently modulating tight junctions to promote paracellular transport. These characteristics render CTS particularly attractive for intranasal delivery, a non-invasive, painless route that does not require sterility and offers direct access to the central nervous system [107]. Although mucociliary clearance and limited epithelial permeability can curtail drug absorption, mucoadhesive polymers like CTS mitigate these barriers by prolonging contact with the nasal epithelium and increasing the concentration gradient across the mucosa. Furthermore, CTS's positive charge enhances interaction with negatively charged cellular membranes, improving uptake and facilitating nose-to-brain transport.

1.5.3. Polyethylene glycol

Polyethylene glycol (PEG) is extensively employed across scientific and industrial domains owing to its excellent solubility in both water and common organic solvents, high biocompatibility, and favorable elimination kinetics. Its low intrinsic toxicity, protein-repellent (anti-fouling) characteristics, and minimal immune stimulation have underpinned widespread biomedical use, and PEG has received

authorization from the U.S. Food and Drug Administration (FDA) for multiple medical applications [108].

Chemically, PEG is produced by polymerization of ethylene oxide and can be prepared in linear, branched, star-shaped, or comb-like architectures. End-group hydroxyls confer strong aqueous solubility and provide convenient handles for further functionalization. Commercial materials span a broad molecular-weight continuum, from a few hundred daltons (~ 6 -7 repeat units) to multi-megadalton chains (≥ 10 MDa; $\sim 2 \times 10^5$ repeat units), and can be readily modified to introduce reactive termini for bioconjugation and surface grafting [109].

Grafting PEG onto nanoparticle surfaces (PEGylation) reduces serum protein adsorption (opsonization), thereby limiting hepatic uptake and accelerating escape from the mononuclear phagocyte system, which collectively prolongs systemic circulation [110]. While recent reports suggest that anti-PEG antibodies may arise in some mammalian systems, the magnitude, prevalence, and clinical significance of this response remain under active investigation [111]. Despite these concerns, PEG remains the dominant stealth coating in both academic research and commercial nanomedicines, exemplified by PEGylated liposomal doxorubicin (Doxil®/Caelyx®), which has been used clinically for more than two decades to treat select breast and ovarian cancers, multiple myeloma, and AIDS-related Kaposi's sarcoma [109].

Modern liposomal formulations frequently incorporate PEG-modified lipid bilayers to extend blood residence time and dampen immunogenicity, thereby promoting preferential accumulation at tumor sites through the enhanced permeability and retention (EPR) effect [112]. A key inflection point for long-circulating vesicles was the integration of PEG into LP compositions, which produced "stealth" LP with reduced recognition by the reticuloendothelial system (RES) and lower uptake by the mononuclear phagocyte system (MPS) [113, 114]. Beyond stealth behavior, steric stabilization by surface-grafted PEG suppresses vesicle-vesicle aggregation, improving colloidal stability during storage and use [115].

ML inherits the lipid bilayer interface of LP and thus can still adsorb nonspecific proteins or interact with cell membranes and blood components. Applying a PEG corona to the ML surface mitigates nonspecific binding and reduces off-target interactions [47]. Similar to conventional LP, unmodified ML can be opsonized and

sequestered by RES phagocytes, diminishing the fraction of carriers that reach tumor tissue. PEGylation of ML addresses this challenge by lowering immune recognition, prolonging circulatory half-life, and thereby increasing tumor accumulation via EPR-mediated transport [43].

1.6. Targeting agents

Targeting agents are functional moieties conjugated onto carriers to enhance their recognition capability or binding affinity toward therapeutic targets via specific receptor–ligand interactions. This targeted approach significantly improves selectivity, cell adhesion, and cellular internalization while mitigating off-target side effects. In cancer therapy, commonly employed targeting agents include antibodies (trastuzumab, rituximab,...), proteins (transferrin, epidermal growth factor,...), peptides (RGD, TAT,...), polysaccharide (hyaluronic acid, lactose,...), small molecules (vitamins, hormones,...). Among these, folic acid is widely utilized owing to its safety profile, cost-effectiveness, lack of immunogenicity, and exceptional thermal and chemical stability during nanocarrier synthesis [116].

Folic acid (FA; ~441 Da) is indispensable for nucleotide biosynthesis and cell proliferation, and it also offers a practical chemical handle for nanomedicine. Beyond being inexpensive, readily available, and serum-stable, FA is non-immunogenic, biodegradable, and easily conjugated to nanoparticle surfaces, making it a scalable ligand for targeted delivery [117]. Its value in oncology stems from high-affinity interactions with folate receptors (FR- α and FR- β), which are glycosylphosphatidylinositol-anchored membrane proteins frequently overexpressed across multiple human tumors (e.g., breast, ovarian, hepatic, renal, and pulmonary cancers). Reported dissociation constants indicate strong binding ($K_D \sim 0.1$ for FR- α and ~ 1 for FR- β) [118], enabling receptor-mediated endocytosis and efficient internalization of FA-decorated nanocarriers.

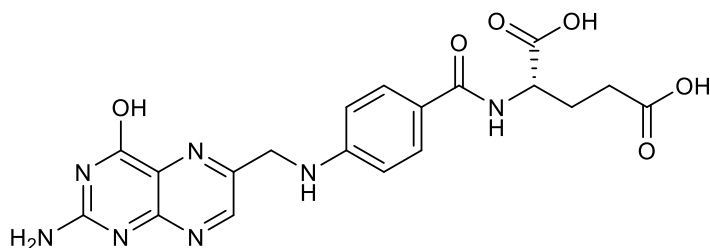


Figure 1.9. Molecular structure of folic acid

Leveraging this biology, several groups have integrated FA into ML hybrids to achieve active targeting. For example, Li and colleagues employed DSPE-PEG2000-FA to assemble a thin FA-bearing lipid membrane, which yielded MSN-LP-PEG-FA constructs with markedly enhanced cellular uptake and superior inhibition of NB4 cells relative to non-FA controls [82]. *In vivo*, FA functionalization shifted biodistribution toward tumors while reducing off-target accumulation, improving antitumor efficacy and lowering systemic toxicity. Consistent findings were reported by Sun and co-workers, who observed increased cellular internalization when FA was conjugated to ML [91].

1.7. MSN-LP hybrid nanoparticles functionalized with FA-conjugated polymers

The hybrid ML material is a synergetic combination of an inorganic core (MSN) and an organic shell (LP), engineered to overcome the intrinsic limitations of its individual components while leveraging their superior characteristics. The MSN core serves as an ideal carrier due to its exceptional physicochemical stability, high surface area, and large pore volume, enabling high drug loading capacity. However, pristine MSN often suffers from premature drug leakage in physiological environments and potential hemolytic toxicity caused by surface silanol groups. The integration of LP provides a supported lipid bilayer that seals the mesopores for controlled release and offers high biocompatibility, facilitating seamless cellular membrane fusion and internalization. Conversely, the rigid MSN core provides a structural scaffold that enhances the mechanical durability of the LP, preventing the vesicle rupture typical of conventional LP.

Despite these advantages, basic ML hybrids still face critical hurdles. A primary challenge is protein corona formation; in complex physiological media like blood, serum proteins (e.g., albumin, globulin) rapidly adsorb onto the ML surface. This leads to particle aggregation or rapid recognition and clearance by the reticuloendothelial system (RES). Furthermore, basic ML systems lack active targeting capabilities, resulting in non-selective drug distribution and potential off-target effects on healthy tissues.

To address these limitations, this study develops a multifunctional multilayered nanoplatform by surface-functionalizing ML with FA-conjugated polymers via three distinct strategies:

- Anchoring polymers into the lipid bilayer via hydrophobic tails (non-ionic surfactant Brij).
- Electrostatic coating using amphoteric PEGylated proteins (Gelatin-PEG).
- Electrostatic coating using cationic PEGylated polysaccharides (Chitosan-PEG).

These designs are hypothesized to enhance the performance of conventional ML materials through several mechanisms:

- Enhanced serum stability: All three polymer types incorporate PEG chains, which act as a steric barrier against serum protein adsorption. This improves colloidal stability in physiological environments and reduces RES uptake, thereby increasing the circulation time and the probability of tumor accumulation.

- Each polymer offers unique functional benefits:

- + Brij: Increases phospholipid membrane fluidity, improving cellular membrane permeability.

- + Gelatin: Contains RGD peptide sequences that enhance cell adhesion via strong affinity for integrin receptors overexpressed on cancer cells.

- + Chitosan: Provides a positive surface charge, strengthening interactions with negatively charged cell membranes. Furthermore, Chitosan confers pH-responsiveness, where the polymer shell swells or dissociates in the acidic tumor microenvironment (pH ~5.5), triggering accelerated drug release.

- Active targeting and internalization: The conjugation of folic acid (FA) exploits the high affinity between FA and folate receptors overexpressed on cancer cell membranes. Beyond mere recognition, FA conjugation significantly boosts receptor-mediated endocytosis, enhancing the overall cellular uptake.

In summary, the multifunctional multilayered nanoparticles synthesized in this work are expected to exhibit superior performance, characterized by: (i) high drug encapsulation and physicochemical stability (MSN core); (ii) excellent biocompatibility and controlled release (LP shell); (iii) enhanced cell interaction (Brij, gelatin, or chitosan); (iv) improved colloidal stability (PEG layer); and (v) improved cellular internalization (FA).

CHAPTER 2. MATERIALS AND EXPERIMENTAL METHODS

2.1. Materials

2.1.1. Chemicals

Ammonia solution, tetraethoxysilane (TEOS), cetyltrimethylammonium bromide (CTAB), (3-aminopropyl)triethoxysilane (APTES), cholesterol, polyoxyethylene (100) stearyl ether (Brij S100), polyoxyethylene (20) cetyl ether (Brij 58), polyethylene glycol 6000 Da (PEG 6000), p-nitrophenyl chloroformate (NPC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), N-hydroxysuccinimide (NHS), succinic anhydride (SA), porcine skin gelatin (GLT) (type A, bloom 300, M average = 75 kDa), low molecular weight chitosan (CTS) (M average = 120 kDa, deacetylation $\geq$ 75%), Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, thiazolyl blue tetrazolium Bromide (MTT), doxorubicin (DOX), 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) were purchased from Sigma. Folic acid (FA), soybean lecithin (SL) were supplied by TCI. Polyoxyethylene (10) stearyl ether (Brij S10) was purchased from Mackun. Chloroform, tetrahydrofuran (THF), acetic acid were supplied by Fisher. Ethanol, diethyl ether were purchased from Chemsol.

2.1.2. Equipments

Rotary evaporator (Buchi Rotavapor R-114, Switzerland), ultrasonic bath (Elmasonic S 80H, Germany), lyophilizer (EYELA FDU-1200, Japan), centrifuge (Hermle Z32HK, Germany), analytical balance (OHAUS pioneer PA114, USA), magnetic hotplate stirrer (VELP Scientifica, Italy).

2.2. Synthesis methods

2.2.1. Synthesis of MSN-NH₂

The process of synthesizing MSN-NH₂ includes two stages: synthesis of MSN and MSN surface modification with amine [119].

In the first stage, MSN was synthesized by hydrolysis and condensation of TEOS on CTAB micelle template in an alkaline environment (**Figure 2.1**). Firstly, 2.6 g CTAB was dissolved in deionized water at 60 °C for 30 minutes, then 11.25 mL absolute ethanol and 0.55 mL NH₃ 2.8% solution were added and stirred for 5 minutes. Sequentially, 8 mL TEOS was slowly dropped into the above mixture, stirred for 2 hours. The mixture was sonicated for 15 minutes, then placed into

MWCO = 12-14 kDa membrane, dialyzed in a mixture of ethanol and 2M acetic acid (1:1 v/v) for 3 days. The product was centrifuged at 10000 rpm for 30 minutes, washed with ethanol for 3 times, redispersed and stored in ethanol at 25 °C.

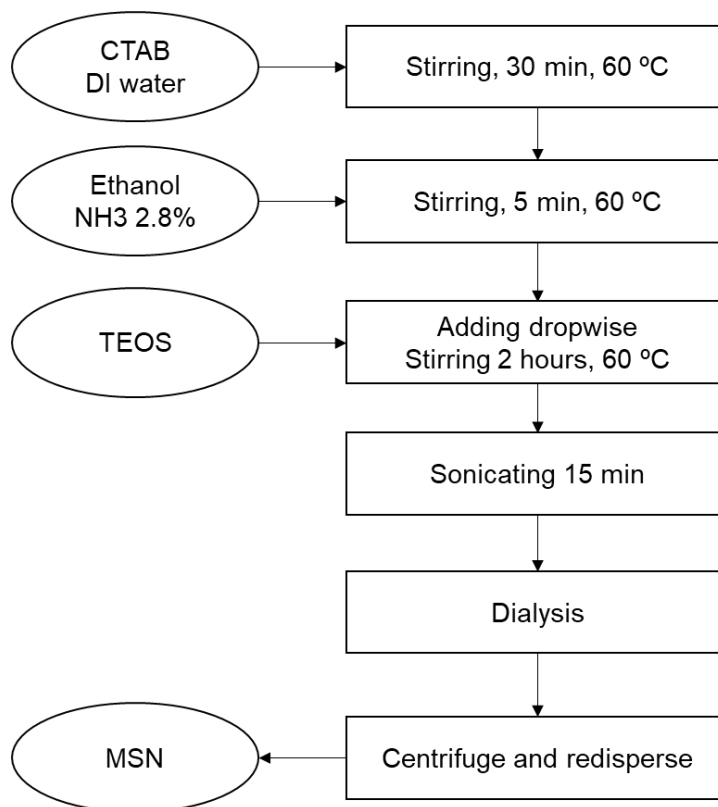


Figure 2.1. Synthesis process of MSN

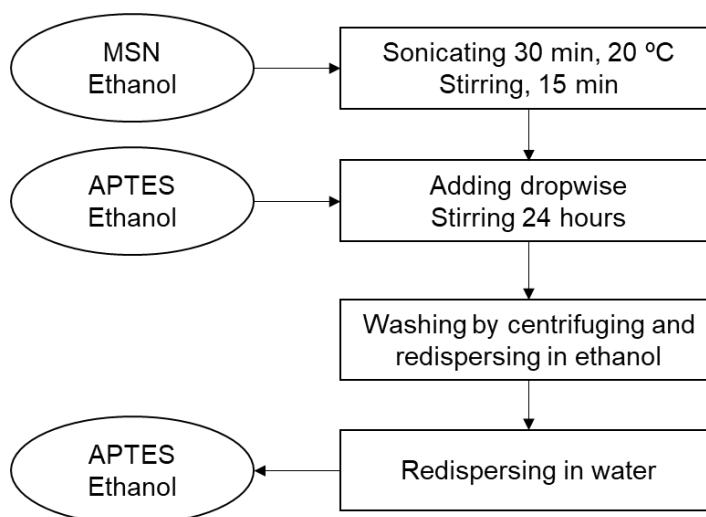


Figure 2.2. Synthesis process of MSN-NH₂

MSN-NH₂ was synthesized by APTES condensation on the MSN surface (Figure 2.2). Firstly, 100 mg of MSN was dispersed in 20 mL of absolute ethanol,

sonicated for 20 minutes, and stirred at 300 rpm for 15 minutes. A solution of 0.8 mL APTES in 0.2 mL absolute ethanol was added into the MSN solution, stirred for 24 hours at room temperature. The product MSN-NH₂ was centrifuged at 10000 rpm for 30 minutes, washed with ethanol for 7-10 times, redispersed and stored in deion water at 25 °C.

2.2.2. Synthesis of LP

LP is synthesized by thin lipid film hydration and homogenization by ultrasound [120]. Soybean lecithin, cholesterol, and CTAB at a ratio of 90:10:1.5 (w/w) were completely dissolved in 20 mL of chloroform in a ground-neck flask. The mixture was dried by rotary evaporation to create a thin lipid film for 2 hours, then kept under vacuum conditions for 5 hours to completely eliminate chloroform. The thin film was then hydrated with 20 mL deion water for 30 min at 60 °C, then homogenized by sonication for 30 min to yield LP. The LP solution was stored at 15 °C.

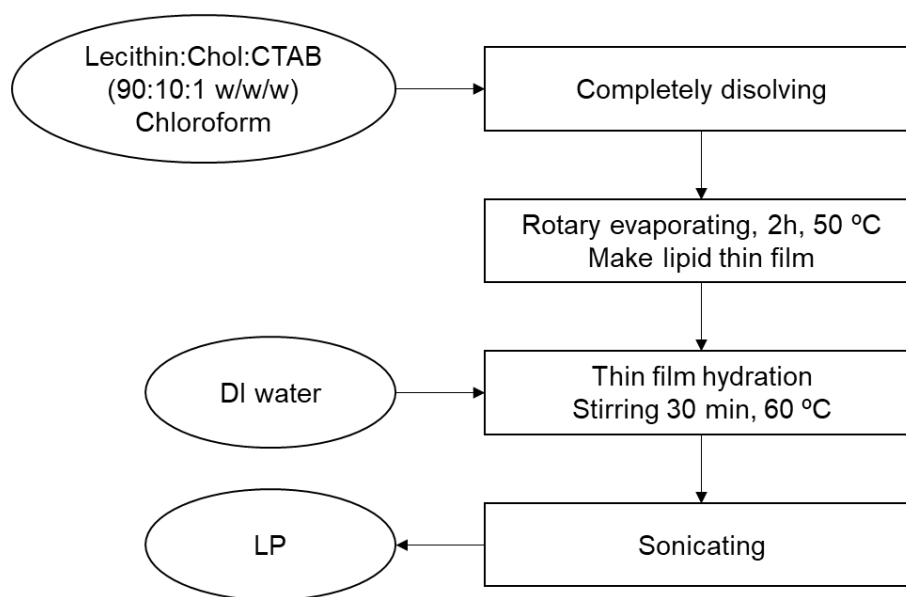


Figure 2.3. Preparation process of LP

2.2.3. Synthesis of ML

The hybrid nanomaterial ML is synthesized by two methods: thin film hydration method and mixing method combined with ultrasound.

2.2.3.1. Hydration of thin lipid film by MSN solution

The thin lipid film was prepared similarly to the LP synthesis process above, with lipid components including lecithin, cholesterol, and CTAB at a ratio of 90:10:1.5 w/w. After forming the lipid film, MSN-NH₂ solution was added to hydrate

the lipid film for 30 min at 60 °C, followed by homogenization by ultrasound for 30-120 min.

2.2.3.2. *Mixing and sonicating LP and MSN solutions*

The mechanism of this method including 3 stages: (1) LP adsorb onto the surface of MSN particles; (2) the LP collapse, forming a lipid bilayer covering the surface of the particles, gradually increasing the contact area; (3) the lipid bilayer completely covers the MSN surface, forming ML hybrid nanoparticles.

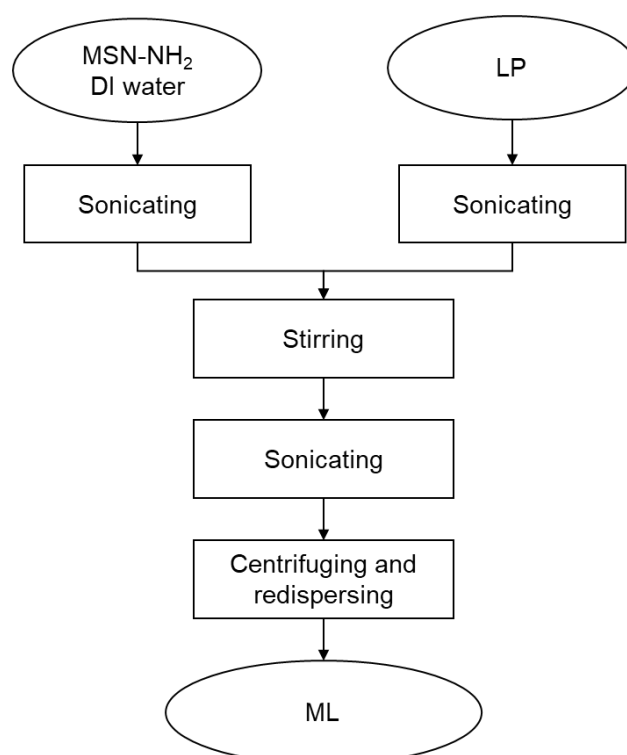


Figure 2.4. Synthesis process of ML

10 mg of MSN-NH₂ was dispersed in 2 mL of deionized water by sonicating for 15 minutes, then slowly added to LP solution (10 mg/mL) heated to 60 °C, stirred for 30 minutes. The mixture was sonicated for 30 minutes at 60 °C, then centrifuged at 10000 rpm for 10 minutes, removed the supernatant. The pellet was washed with deionized water 3 times, then dispersed in deionized water by ultrasound. The synthesis procedure is shown in **Figure 2.4**.

2.2.4. **Modification of polymers**

2.2.4.1. *Synthesis of Brij-FA*

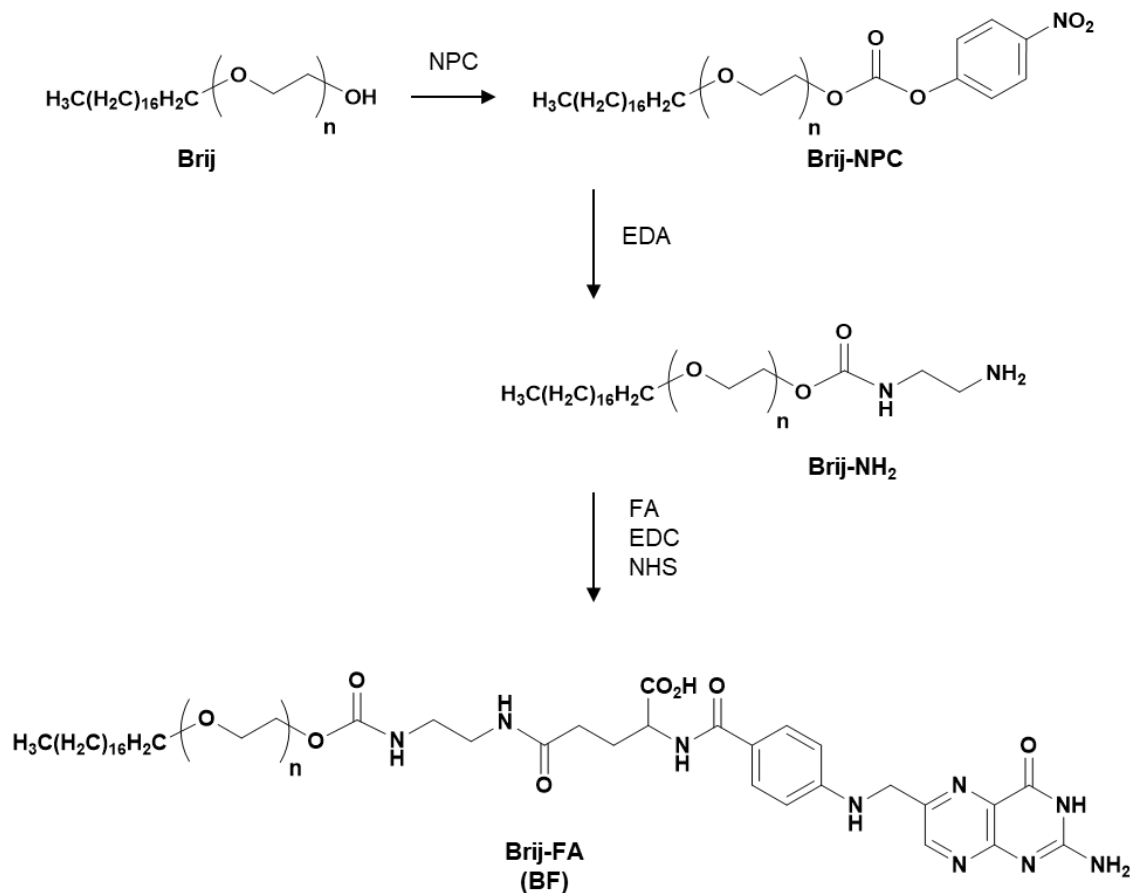


Figure 2.5. Synthesis scheme of Brij-FA

Brij-FA was synthesized following a previously reported procedure [120], as illustrated in **Figure 2.5**.

Preparation of Brij-NH₂: Preparing a three-neck flask, with one neck connected to a nitrogen gas cylinder and another to a vacuum pump, Brij S100 was added to the flask and heated to 65°C until completely melted. The mixture was stirred at 50 rpm while purging with nitrogen and vacuuming for 30 minutes. NPC (1.2 eq mol/mol relative to Brij) was then quickly added to the flask and the reaction was carried out for 5 hours. The reaction mixture was cooled, 10 mL of THF was added, and the reaction was continued overnight (solution (A)). EDA (10 eq mol/mol relative to Brij) was dissolved in THF to obtain solution (B). Solution (A) was slowly added dropwise into solution (B) with stirring at 300 rpm, and the reaction was carried out for 24 hours at room temperature. The product was precipitated in cold diethyl ether, washed 2-3 times with cold diethyl ether, and then dried by rotary evaporation.

Synthesis of Brij-FA: FA (1.2 eq mol/mol relative to Brij-NH₂) was dissolved in DMSO, and gentle heating may be applied to ensure complete dissolution. EDC (2 eq mol/mol relative to FA) was added and stirred for 15 minutes to obtain solution

(A). Brij-NH₂ was dissolved in DMSO with stirring until completely dissolved to obtain solution (B). Solution (B) was added to solution (A) with stirring, and the reaction was carried out for 24 hours at room temperature. The mixture was filtered using a membrane with a MWCO = 3.5 kDa in distilled water (3 days). Excess FA was removed by centrifugation. The product was then freeze-dried.

2.2.4.2. Synthesis of Gelatin-PEG-FA

Gelatin-PEG-FA was synthesized via a four-step reaction according to a modified method described in the previous study [121].

Synthesis of NH₂-PEG-NH₂: A 3-neck flask was prepared to connect to the N₂ gas tank and a vacuum outlet. The flask was added PEG to spontaneously immersed in an oil bath heated to 65°C to fully melt the PEG, which was 50-rpm magnetically stirred under a nitrogen atmosphere and under vacuum for 30 minutes to remove oxygen and moisture. Then, 2.2 equivalents of NPC relative to PEG was weighed and quickly added. The reaction proceeded for 5 hours prior to the addition of THF and reaction coolness to allow for nighttime continuation of the synthetic process (Solution A). Separately, 20 mol/mol equivalent of EDA relative to PEG was dissolved in THF to yield Solution B. Then, Solution A was slowly dropped into Solution B with stirring at 300 rpm and the reaction continued at room temperature for 24 hours. The crude product was precipitated in cold diethyl ether and washed with additional cold diethyl ether 2-3 times. Finally, the purified NH₂-PEG-NH₂ was obtained after the utilization of rotary evaporation to achieve solvent elimination.

Synthesis of H₂N-PEG-FA: 1.2 equivalents of FA relative to NH₂-PEG-NH₂ was completely dissolved in DMSO at a slightly hot temperature. The addition of EDC was performed in a condition of magnetic stirring to obtain solution A. NH₂-PEG-NH₂ was dissolved in DMSO under magnetic stirring to obtain solution B. Then, solution A was slowly dropped into solution B under magnetic stirring to conduct 24-hour reaction at room temperature prior the utilization of dialysis (3.5kDa membrane) in distilled water for 3 days. Finally, the unreacted FA was removed by leveraging the centrifugation technique before employing lyophilization to achieve H₂N-PEG-FA.

Preparation of HOOC-PEG-FA: NH₂-PEG-NH₂ was completely dissolved in THF prior the addition of TEA under magnetic stirring and ultrasound to prepare solution A. The solubilization of two equivalents of SA relative to NH₂-PEG-FA was performed in THF to acquire solution B. The 24-hour magnetic stirring of solution

was conducted after the addition of solution A to solution B at room temperature, which was continuously employed dialysis (3.5kDa membrane) in distilled water for 3 days and lyophilization to generate HOOC-PEG-FA.

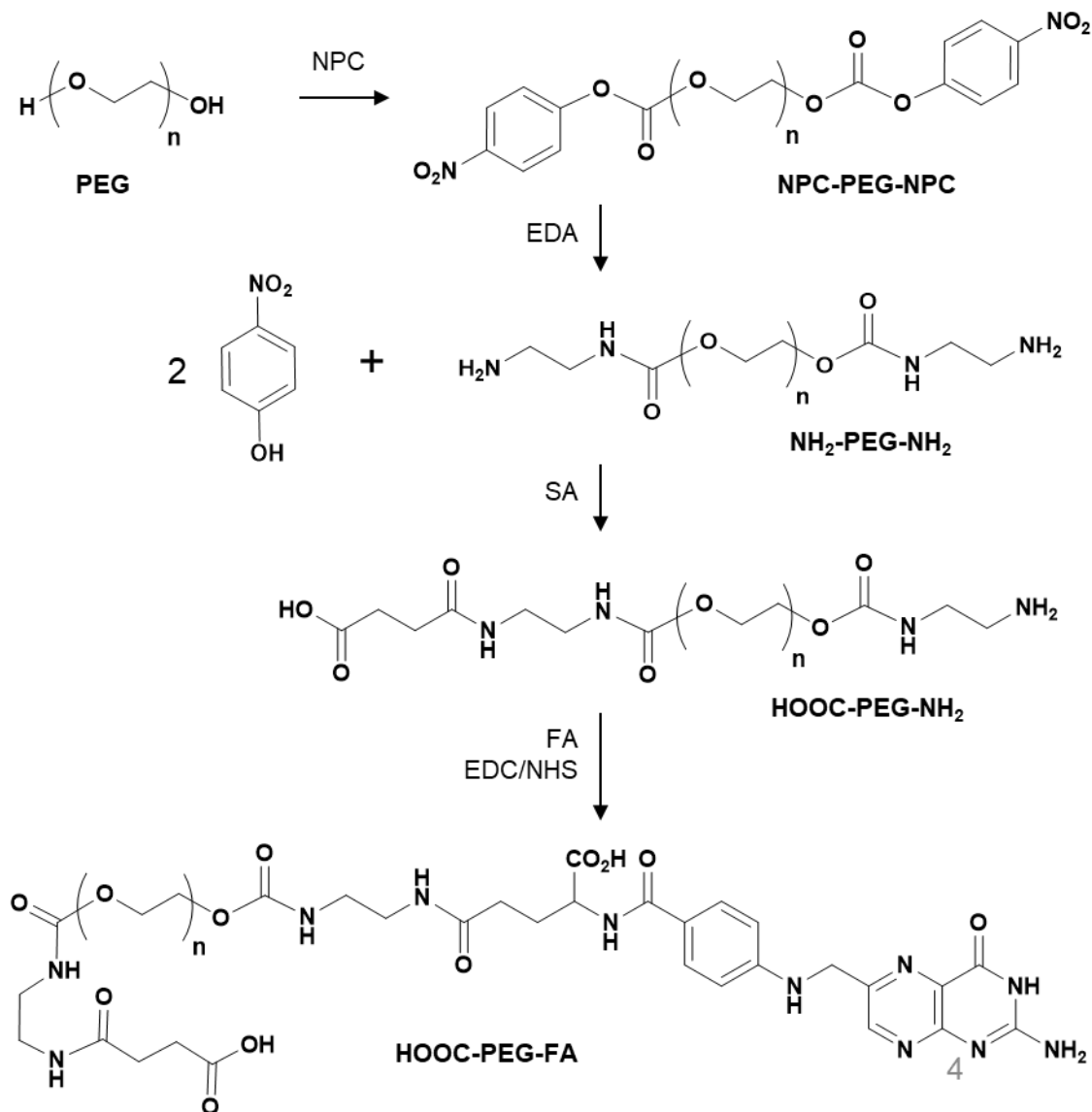


Figure 2.6. Synthesis process of HOOC-PEG-FA

Synthesis of GLT-PEG-FA (GPF): The gelatin (GLT) dissolution in distilled water was magnetically stirred at 50 °C until completely dissolved to obtain solution A. Solubilization of FA-PEG-COOH was employed ($n_{\text{FA-PEG-COOH}} / n_{\text{GLT}} = 5:1, 10:1, 20:1, 30:1, 40:1$) in buffer solution (pH = 4.5), followed by the addition of EDC (2 equivalents relative to FA-PEG-COOH) to acquire solution B. Then, solution B was slowly dropped into solution A under magnetic stirring to conduct 24-hour reaction at room temperature prior the utilization of dialysis (12-14kDa membrane) in distilled water for 3 days. Finally, the lyophilization was leveraged to achieve GPF.

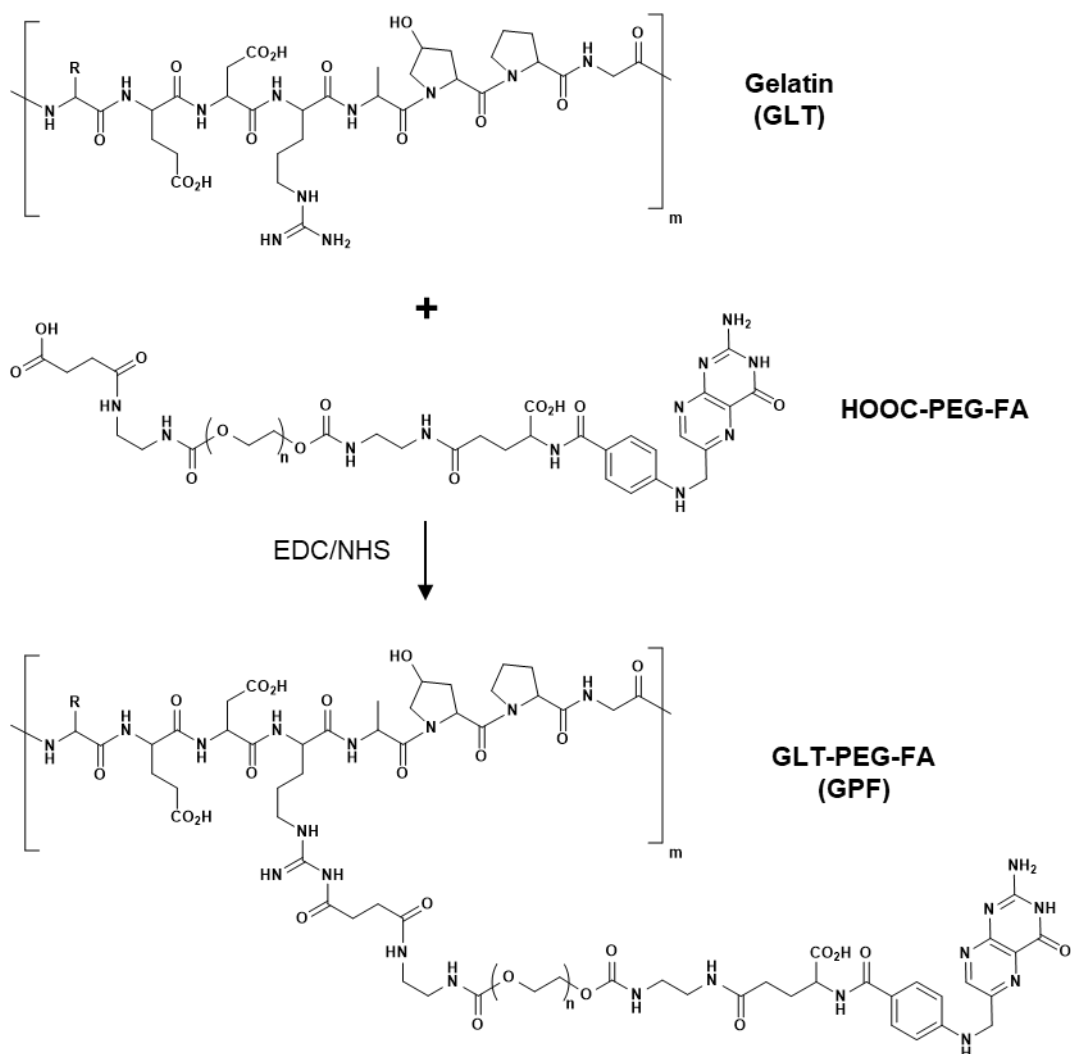


Figure 2.7. Synthesis process of GPF

2.2.4.3. Synthesis of Chitosan-PEG-FA

Chitosan-PEG-FA (CPF) was synthesized through a reaction of chitosan (CTS) with HOOC-PEG-FA, following a modified procedure previously reported [122].

CTS was completely dissolved in distilled water with slowly adding of HCl, and pH adjusted to 4-5 by adding of NaOH 0.1M to obtain solution A. Solubilization of FA-PEG-COOH was employed ($n_{\text{FA-PEG-COOH}} / n_{\text{CTS}} = 5:1, 10:1, 20:1, 30:1, 40:1$) in buffer solution (pH = 4.5), followed by the addition of EDC (2 equivalents relative to FA-PEG-COOH) to acquire solution B. Then, solution B was slowly dropped into solution A under magnetic stirring to conduct 24-hour reaction at room temperature prior the utilization of dialysis (12-14 kDa membrane) in distilled water for 3 days. Finally, the lyophilization was leveraged to achieve CPF.

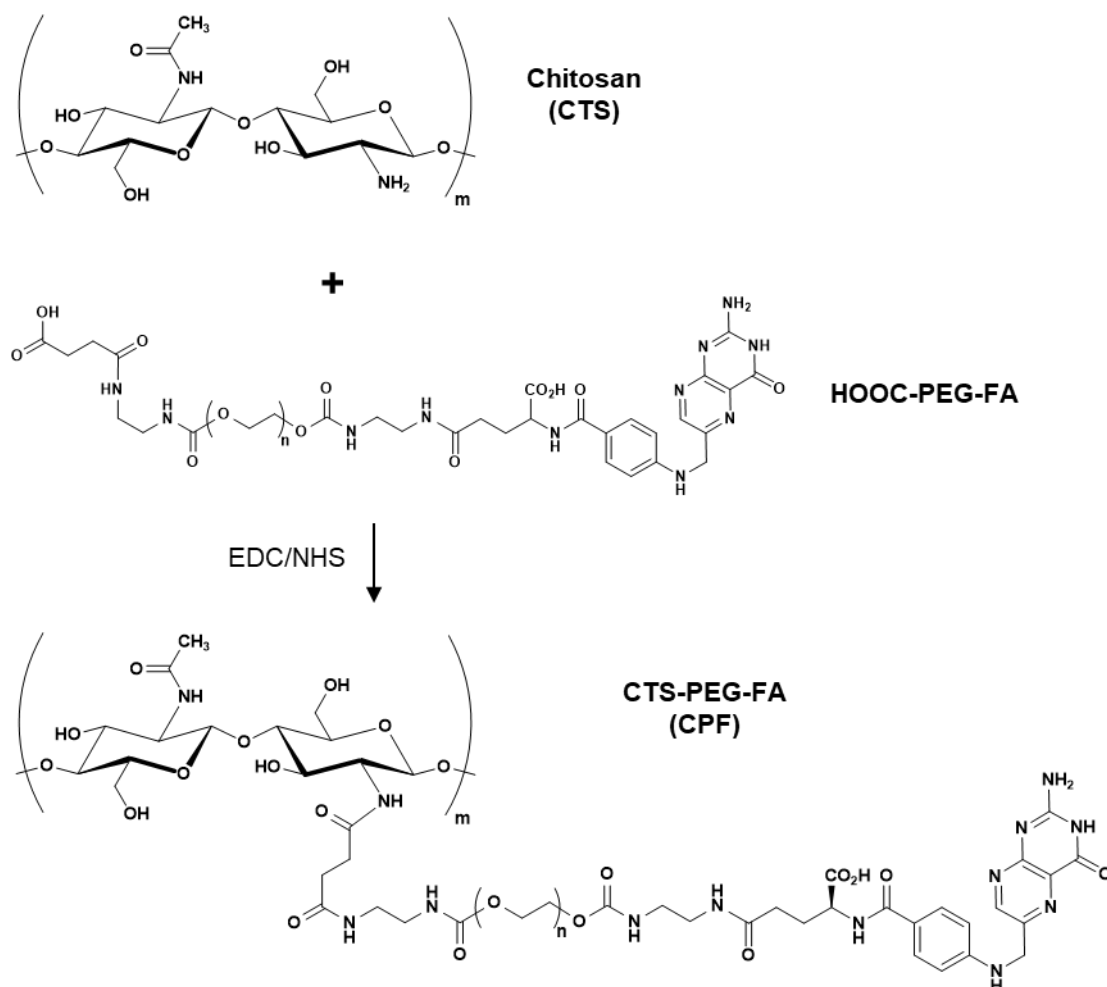


Figure 2.8. Synthesis process of CPF

2.2.5. Synthesis of polymers coated ML

2.2.5.1. Synthesis of Brij-FA coated ML

Firstly, LP-Brij-FA (LBF) was synthesized according to a modified method described in a previous study [120]. Lecithin, cholesterol, and CTAB were weighed in a ratio of 90:10:1 (w/w) in a 100 mL flask. Brij-FA was added to the flask and completely dissolved with 20 mL chloroform. The chloroform was evaporated by rotary evaporation to form a thin lipid membrane over 2 hours. Then, 20 mL of deionized water was added to the flask, stirred for 30 minutes at 60 °C, and homogenized by ultrasound for 30 minutes at 60 °C.

Synthesis of Brij-FA coated ML (MLBF): 10 mg of MSN-NH₂ was dispersed in 2 mL of deionized water, sonicated for 15 minutes, then slowly added to the LBF solution (10 mg/mL) preheated to 60 °C, stirred for 30 minutes while maintaining the temperature at 60 °C. The mixture was sonicated for 30 minutes at 60 °C. The mixture was then centrifuged at 10000 rpm for 10 minutes, the upper layer was removed,

washed with deionized water three times, and then dispersed in deionized water by ultrasound. The synthesis process was illustrated in **Figure 2.9**.

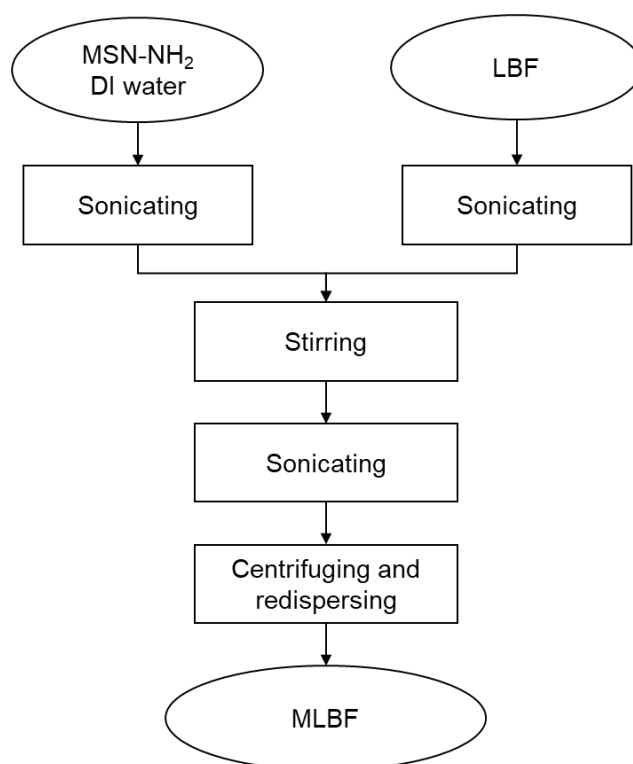


Figure 2.9. Synthesis process of MLBF

2.2.5.2. Synthesis of GPF coated ML

GPF was completely dissolved in distilled water by stirring and sonicating at 50 °C. The ML solution was slowly added into GPF solution stirred at room temperature. The ML to GPF ratios, GPF concentration, and PEG to GLT ratios were investigated. The mixture was sonicated for 15 minutes and centrifuged at 10000 rpm for 10 minutes. The pellet was washed 3 times with distilled water, then re-dispersed in water by ultrasound.

2.2.5.3. Synthesis of CPF coated ML

CPF was completely dissolved in distilled water by stirring and sonicating at 50 °C. The ML solution was slowly added into CPF solution stirred at room temperature. The ML to CPF ratios, CPF concentration, reaction time, and PEG to CTS ratios were investigated. The mixture was sonicated for 15 minutes and centrifuged at 10000 rpm for 10 minutes. The pellet was washed 3 times with distilled water, then re-dispersed in water by ultrasound.

2.3. Analysis methods

The particle size and zeta potential of the nanoparticles were determined by a Zetasizer Nano SZ (SZ-100, Horiba, Japan), with the setting detection angle of 90°, temperature of 25 °C and laser wavelength of 532 nm.

The particles size, morphology and core shell structure of polymer coated ML were evaluated by scanning electron microscope (SEM) and transmission electron microscope (TEM) using FE-SEM S-4800 (HITACHI, Japan) and JEM-1400 (JEOL, Japan), respectively. In addition, Energy dispersive X-ray spectroscopy (EDX) was used for the chemical characterized using FE-SEM S-4800.

To study the component of the synthesized nanoparticle, FT-IR and thermal gravimetric analysis (TGA) were carried out. FT-IR investigation was performed on FT-IR/NIR Spectroscopy Frontier (Perkin Elmer, USA). TGA investigation was performed with a TGA Analyzer (Mettler Toledo, Switzerland) under a nitrogen flow with temperature program from 30-800 °C.

The chemical structure of copolymers was characterized by Nuclear magnetic resonance spectroscopy (NMR) (Bruker Advance 500 MHz, USA).

The drug concentration was quantified by Ultraviolet – visible spectroscopy (UV-Vis) using Microplate reader (Agilent BioTek 800 TS, USA).

2.4. Drug loading and releasing

2.4.1. DOX loading

The DOX was loaded into the MSN-NH₂ by equilibrium method [58]. Initially, 20 mg of MSN-NH₂ was dispersed in 2 mL of appropriate solvents (DI, methanol, or ethanol). The drug solution was prepared by dissolving 4 mg of the drug in 2 mL of the same solvent. Sequentially, the drug solution was added into the MSN solution, the mixture was stirred at room temperature for 24 hours. After that, the drug loaded MSN was collected by centrifuging at 10000 rpm in 30 min. The supernatant was collected to determine the unloaded drug using UV-Vis at 490 nm.

To loading drug into the ML, the drug solution was added into the MSN-NH₂ solution and stirred at room temperature for 24 hours. Then, the mixture was dropped into the heated LP solution, stirred at 60 °C for 30 min. The mixture was sonicated for 30 min, then centrifuged at 10000 rpm in 30 min. The supernatant was collected to determine the unloaded drug. The pellet was washed 3 times, and re-dispersed in water.

The free drug standard curves were established by measuring the absorbance of known concentration drug solutions at corresponding wavelength using UV-Vis. DLE and DLC values for each drug was calculated by equations below:

$$DLE(\%) = \frac{m_{Di} - m_{Du}}{m_{Di}} \times 100\% \quad (\text{Eq.1})$$

$$DLC(\%) = \frac{m_{Di} - m_{Du}}{m_{NP} + m_{Di} - m_{Du}} \times 100\% \quad (\text{Eq.2})$$

Where: m_{Di} (mg) is the initial amount of drug used in the loading experiment
 m_{Du} (mg) is the total amount of unloaded drug detected in the supernatant

m_{NP} (mg) is the dry weight of the nanoparticles

2.4.2. Drug releasing

To investigate the *in vitro* drug release, drug loaded materials was dispersed in 5 mL PBS solution (pH 7.4 or 5.5) and transferred into a dialysis tube with MWCO = 3.5 kDa. The tube was then soaked in 20 mL PBS pH 7.4 (or pH 5.5), stirred at 50 rpm, 37 °C. At time intervals as 1, 3, 6, 12, 24, 48, and 72 hours, 1 mL of releasing solution was collected and replaced by an equivalent volume of fresh medium. The concentration of released drugs was determined by microplate reader at 490 nm [58].

2.5. Stability in FBS

To assess stability in a simulated physiological environment, a modified serum assay was employed [123]. Samples were dispersed in FBS serum, and at intervals of 2, 4, 8, 24, 48, 72, and 96 hours, 200 μ L aliquots were collected and analyzed using UV-visible spectroscopy at a wavelength of 450 nm. Serum stability was evaluated by determining the percentage absorbance of samples over time, calculated using the following equation:

$$A_p = \frac{A_{S(t)} - A_{F(t)}}{A_{S(in)} - A_{F(in)}} \times 100\% \quad (\text{Eq.3})$$

Where A_p is absorbance percentage, $A_{S(in)}$ is initial absorbance of sample, $A_{S(t)}$ is absorbance of sample at interval time t, $A_{F(in)}$ is initial absorbance of control solution, $A_{F(t)}$ is absorbance of control solution at interval time t.

2.6. Cell culture and MTT assay

The HeLa cell line was procured from the American Type Culture Collection (ATCC: CCL-2) and utilized as received. Cells were cultured in DMEM

supplemented with 10% FBS, 100 IU mL⁻¹ penicillin, and 0.1 mg mL⁻¹ streptomycin, maintained in a humidified incubator with 5% CO₂.

To assess in vitro cytotoxicity, cells were seeded into 96-well culture plates at a density of 3×10^4 cells per well. After a 24-hour incubation period to allow cell attachment, the cells were treated with the nanocarriers at concentrations ranging from 10 to 200 µg/mL, or with free and encapsulated drugs at concentrations ranging from 0.1 to 1 µg/mL, for 48 hours. Subsequently, 20 µL of a 5 mg/mL MTT solution was added to each well and incubated for 4 hours at 37 °C. Following incubation, the culture medium was removed, and 100 µL of DMSO was added to each well to dissolve the formazan crystals, with gentle shaking for 2 minutes. Absorbance was then measured at a wavelength of 570 nm using a microplate reader [124]. Cell viability was calculated using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{control}} - \text{Abs}_{\text{blank}}} \times 100\% \quad (\text{Eq.4})$$

2.7. Cellular Uptake Study

HeLa cells were seeded in 6-well plates and cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. After 24 hours of pre-incubation, the culture medium was replaced with fresh medium containing either free DiO or DiO-labeled samples. In the labeled formulation, DiO was incorporated into the LP layer before coating onto MSNs. The cells were incubated at 37 °C for 4 hours, which corresponds to previously reported conditions where LP-incorporated DiO achieves maximal fluorescence intensity, ensuring reliable visualization by confocal laser scanning microscopy [121, 125]. After incubation, the cells were washed three times with PBS and fixed with 4% paraformaldehyde for 30 minutes at room temperature. Nuclei were then stained with DAPI for 5 minutes, followed by additional PBS washes. Cellular uptake was visualized using Dragonfly confocal laser scanning microscopy (Oxford Instruments, UK) with an excitation/emission wavelength of 484/501 nm. Fluorescence images were analyzed using ImageJ software.

2.8. Statistical analysis

Each experiment was performed in triplicate. The calculated results were presented as mean $\pm$ standard deviation (SD). Testing for significant differences between means was carried out using Student's t-test ($p < 0.05$).

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Synthesis and evaluation of MSN-LP

3.1.1. Synthesis of MSN-NH₂

The MSN were synthesized via the modified Stöber sol-gel route, utilizing tetraethyl orthosilicate (TEOS) as the precursor, cetyltrimethylammonium bromide (CTAB) as the structure-directing cationic template, and an aqueous ethanol-ammonia mixture to provide alkaline reaction conditions. Under these basic parameters, electrostatically driven self-assembly occurs between negatively charged silicate species and positively charged CTAB micelles, driving the ordered condensation of the silica framework. Subsequent template removal via dialysis yielded pristine MSN with a mean hydrodynamic diameter of 139.9 nm and high monodispersity. The surface charge was measured at -25.9 mV, reflecting mutual electrostatic repulsion among the particles in aqueous dispersion. This negative surface potential confirms the extensive deprotonation of surface silanol groups (Si-OH) into silanolate species (Si-O⁻) at physiological pH.

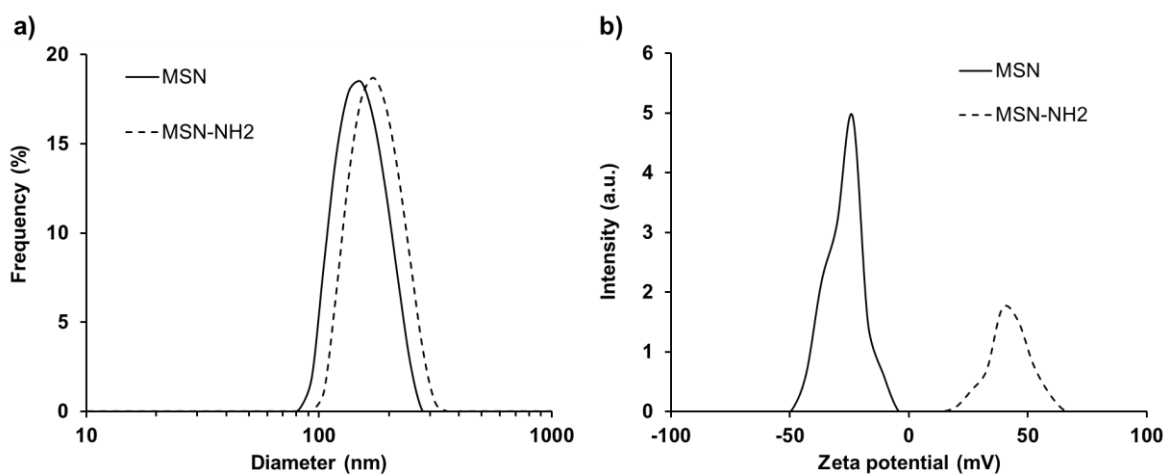


Figure 3.1. DLS particle size (a) and zeta potential (b) of MSNs and MSN-NH₂

Amine-functionalized MSN (MSN-NH₂) were subsequently prepared via a post-synthetic grafting approach by reacting pristine MSN with APTES in anhydrous ethanol. The alkoxy silane moieties of APTES undergo hydrolysis and silanol condensation with the surface hydroxyl groups of the silica framework, leading to the covalent anchoring of aminopropyl chains. Following lyophilization, the resulting MSN-NH₂ was obtained as a fine white powder. Surface modification induced a slight dimensional increase from 139.9 nm to 153.2 nm, attributable to the addition of the organosilane shell. Concurrently, the zeta potential underwent a sharp polarity

reversal from -25.9 mV to +42.2 mV (**Figure 3.1**), providing unambiguous physical evidence for the successful grafting of protonated primary amine moieties (NH_3^+) onto the particle surface.

3.1.2. Synthesis of LP

LP were synthesized by the lipid film hydration method with assistance of ultrasound to generate homogenous LP. The results in **Figure 3.2a** indicated that the LP was high monodispersed nanoparticle with hydrodynamic diameter of 154.85 ± 1.389 nm, and the PDI of 0.245 ± 0.033 . The zeta potential of LP was -63.23 ± 1.169 mV, indicating the stability of LP in physiological conditions (**Figure 3.2b**).

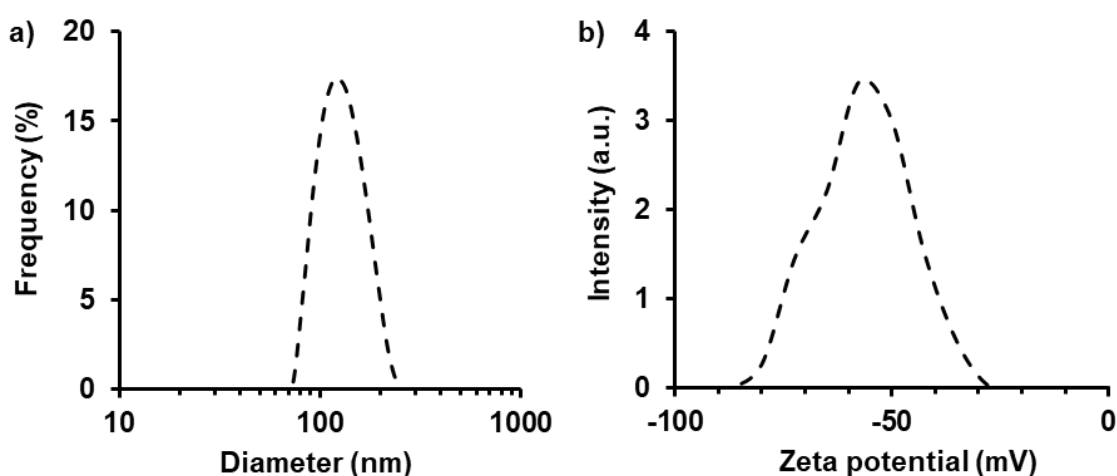


Figure 3.2. DLS particle size (a) and zeta potential (b) of LP

3.1.3. Synthesis of ML

3.1.3.1. Hydration of lipid thin film with MSN solution

The ML was synthesized via hydrating lipid membranes using MSN solution with the ratios of LP to MSN of 10:1 w/w, then homogenized by ultrasound for 30-90 minutes, at 30-90 °C. The results showed that at all LP:MSN ratios and ultrasound conditions, the solution appeared to agglomerate and could not form a stable dispersion. This may be because at the beginning of the hydration process, a small amount of LP was created that covered the MSN surface and formed a bridge that bonded the MSN particles together. The energy of ultrasound (bath) is not enough to break these bridges, so the product agglomerates. In some other studies, using high-energy ultrasound (probe) can help create more stable ML products.

3.1.3.2. Mixing and sonicating LP and MSN solutions

Subsequent lipid coating yielded ML particles with a further increase in hydrodynamic size from 153.2 nm to 178 nm, due to the addition of the lipid bilayer.

The zeta potential shifted significantly from +42.2 mV to -38.2 mV, indicating the outer surface was now dominated by the negatively charged phosphate groups of the phospholipid headgroups (**Figure 3.3**). These findings confirmed the successful formation of a liposome-silica hybrid structure [56, 76].

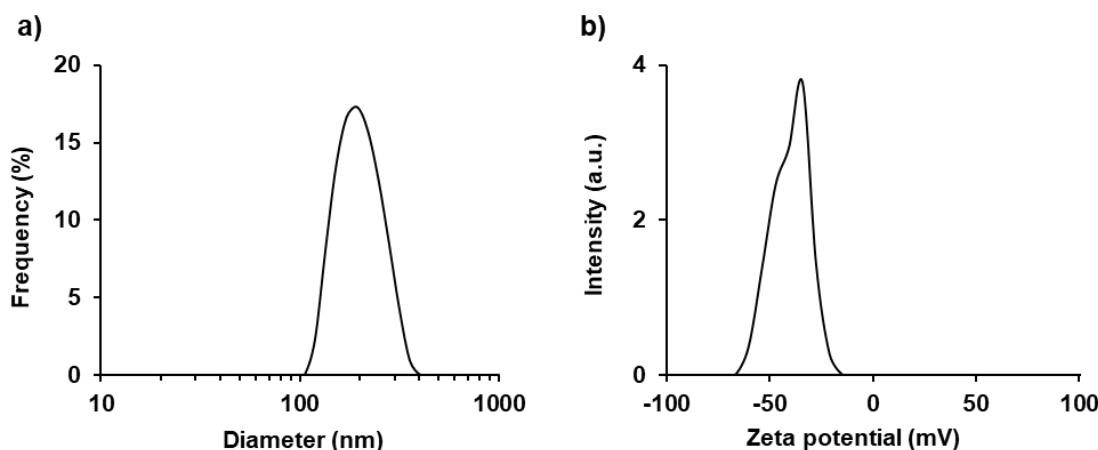


Figure 3.3. DLS particle size (a) and zeta potential (b) of ML

3.1.4. Physicochemical characterization

3.1.4.1. Chemical structure

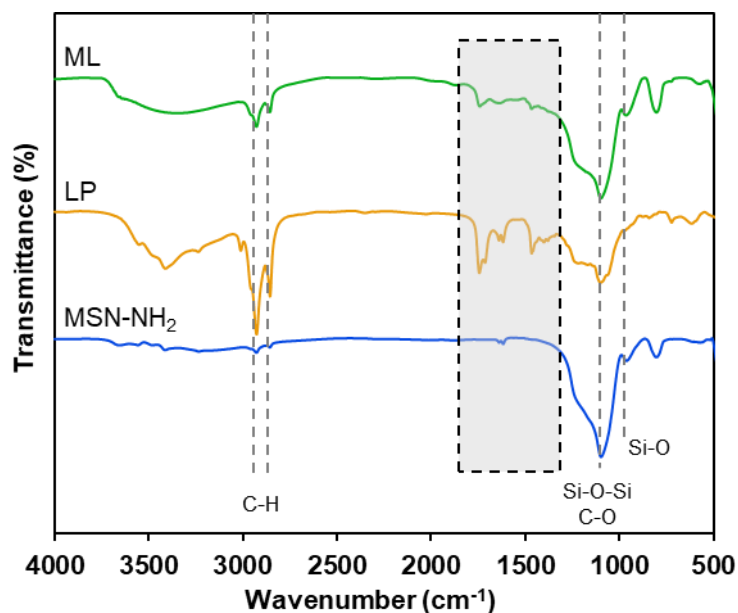


Figure 3.4. FT-IR spectra of MSN-NH₂, LP, và ML

The FTIR spectra of MSN-NH₂, LP, and ML are shown in **Figure 3.4**. The FTIR spectrum of ML (MSN coated with LP) exhibited characteristic peaks from both MSN-NH₂ and lipid components. Prominent peaks included the Si-O-Si asymmetric stretching vibration at 1096 cm⁻¹, Si-O-Si bending at 802 cm⁻¹, and Si-O stretching at 966 cm⁻¹. Signals at 1743 cm⁻¹ (C=O stretching of esters), 1634 cm⁻¹ (C=C), and 1467

cm^{-1} (CH_2 wagging) were attributed to the lipid bilayer. The absorptions between $2890\text{--}2910\text{ cm}^{-1}$ were assigned to C-H stretching. It is worth noting that certain peaks from the lipid bilayer, such as C-O stretching at 1096 cm^{-1} and P=O stretching at 1234 cm^{-1} , overlapped with signals from the silica framework, which is typical for hybrid systems of this nature.

The surface characterization of ML was evaluated by the elemental analysis using the Energy Dispersive X-ray Spectroscopy on the SEM. The spectrum of ML presented the components of the lipid bilayer membrane of LP around the surface with 28.29% carbon, 48.03% oxygen, 5.05% nitrogen, and 0.30% phosphorus. Besides, the atomic percentage of silica has reduced from 29.89% (in MSN) [119] to 18.34%, indicating that the surface of MSNs was coated by LP (**Figure 3.5**).

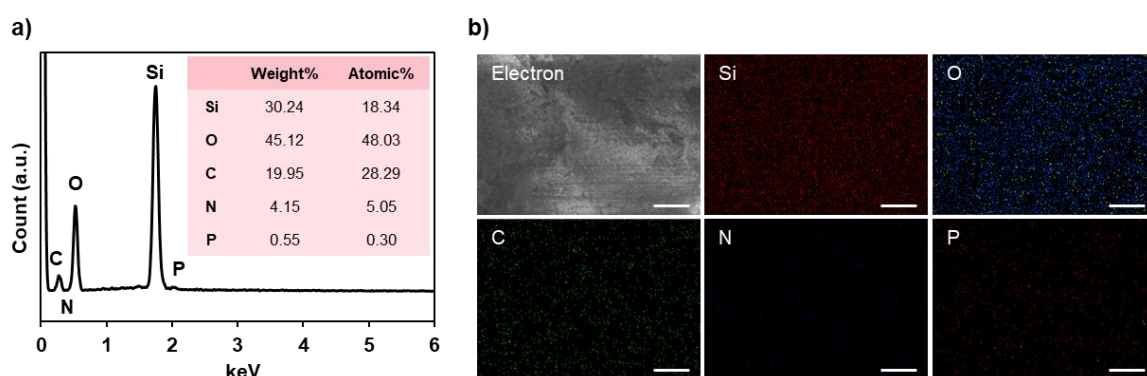


Figure 3.5. EDX spectra (a) and elemental mapping (b) of ML

3.1.4.2. Thermal properties

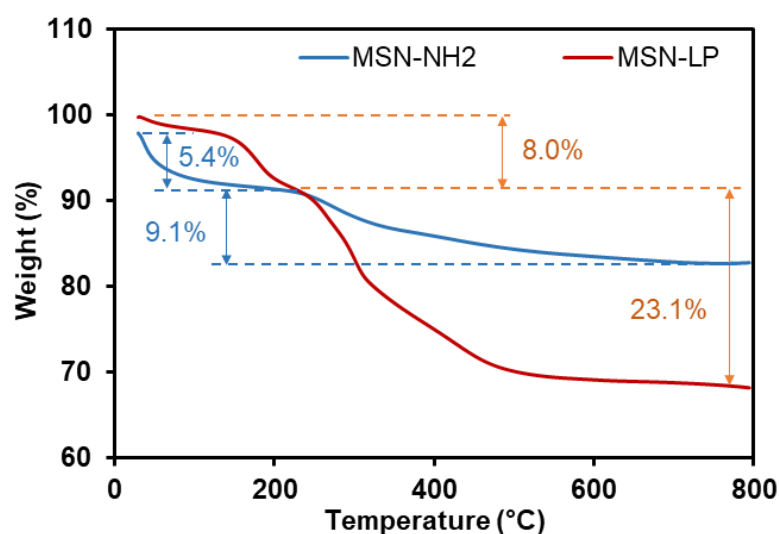


Figure 3.6. Thermograms of MSN-NH₂ and ML

The TGA thermograms of MSN-NH₂ and ML are shown in **Figure 3.6**. For the MSN-NH₂ sample, there was a 5.4% weight loss at 200 °C due to moisture loss, and

a further 9.1% weight loss when increasing the temperature up to 800 °C possibly due to the decomposition of the above amine functional groups on MSN surface. For ML, mass loss (8.0%) also occurred at 200 °C similar to MSN-NH₂. However, the loss of moisture in ML takes place in 2 stages, stage 1 at about 150 °C may be due to moisture loss on the surface, stage 2 from 150-200 °C may be due to water blocked in the pores of MSN by the LP coating on the surface and escapes later. The 23.1% weight loss at 800 °C is due to the decomposition of the LP layer covering on the surface. The above results have contributed to proving the successful coverage of LP on MSN.

3.1.4.3. Morphology

TEM images show that the ML hybrid material has a spherical shape, uniform particle size with a diameter of about 80 nm, was smaller than the size measured by the DLS method because there is no outer solvation layer outside the particles. On the TEM image, the LP layer (paler in color) on the MSN particle surface can be determined (**Figure 3.7**).

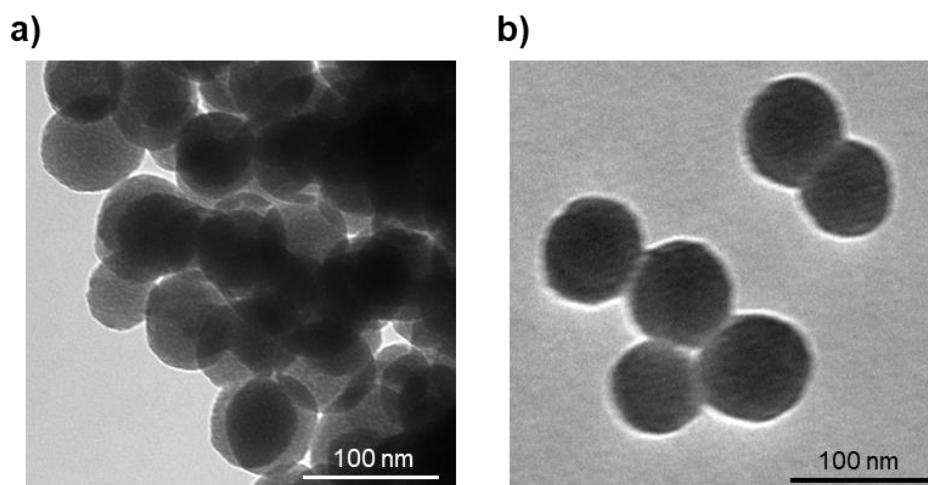


Figure 3.7. TEM images of MSN-NH₂ (a) and ML (b)

3.1.5. Drug loading and releasing

The DOX loaded ML was prepared via diffusion method followed by lipid bilayer formation for capping to keep DOX in MSN pores. The EE and LC were found to be $60.33 \pm 2.5\%$ and $11.12 \pm 0.7\%$, respectively.

The DOX releasing profile of DOX@MSN-NH₂ and DOX@ML were shown in **Figure 3.8**. In a physiological environment (pH 7.4), DOX release from DOX@MSN-NH₂ was markedly faster than from DOX@ML (18.38% at 1 h and 46.45% at 72 h versus 8.67% and 26.90%, respectively). This indicates that the LP coating on the MSN surface effectively suppresses premature drug leakage by

shielding the mesopores [78]. Under tumor-mimicking acidic conditions (pH 5.5), both formulations exhibited increased release relative to pH 7.4. For DOX@MSN-NH₂, the cumulative release rose from 18.38% to 41.23% at 1 h and from 46.45% to 62.44% at 72 h. This behavior is attributed to protonation of DOX amine groups in acidic media, which competes with and weakens hydrogen bonding between DOX and MSN; additionally, imine linkages formed between the DOX ketone and surface amines on MSN are less stable in acid than in neutral/alkaline environments, facilitating dissociation of DOX molecules [22, 126]. For DOX@ML, the 72 h release reached 56.43% at pH 5.5, a 29.53% increase over its release at pH 7.4. This larger increment compared with DOX@MSN-NH₂ likely reflects not only amine protonation and acid-labile imine cleavage, but also the formation of blisters and defects in the LP layer at lower pH, which provide additional diffusion pathways for DOX [41]. Collectively, these results demonstrate that the liposomal cap on MSN surfaces efficiently limits DOX leakage under physiological conditions while maintaining robust drug release in the tumor-like acidic milieu.

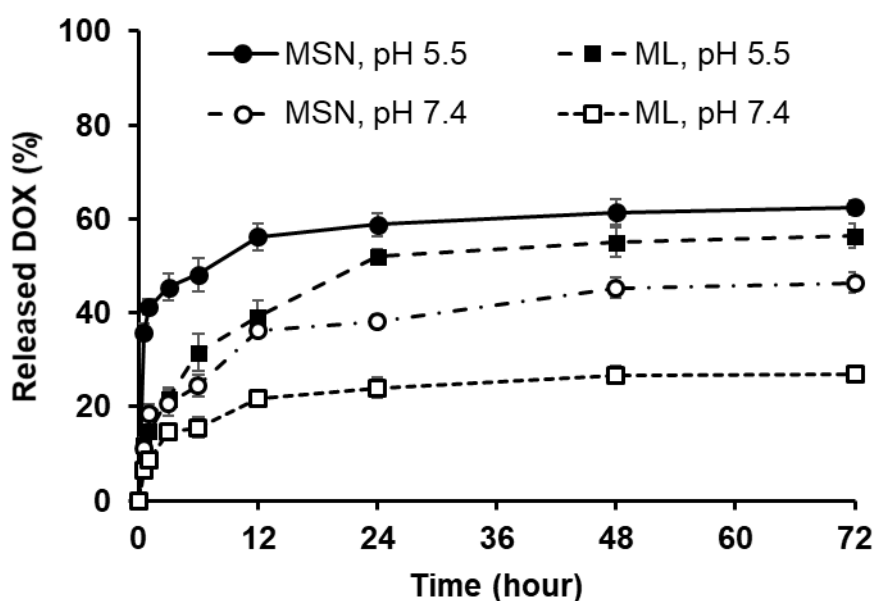


Figure 3.8. DOX release profile of DOX@MSN-NH₂ and DOX@ML in PBS pH 5.5 and 7.4

3.1.6. Cytotoxicity assay

Cell toxicity tests using the MTT method were conducted to evaluate the biocompatibility of ML on HeLa cervical cancer cells. The results presented in **Figure 3.9** show that ML did not induce cytotoxicity on HeLa cells at concentrations up to 200 μ g/mL with cell viability higher than 80%. These findings are consistent

with previous reports, confirming the high biocompatibility of the ML hybrid material [88].

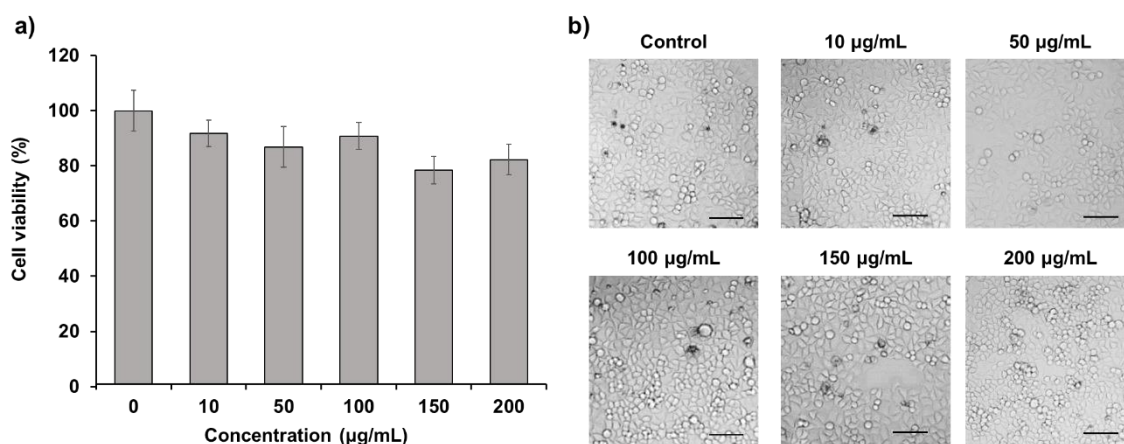


Figure 3.9. Cell viability (a) and images (b) of HeLa cells exposed to ML for 48 hours. The scale bar is 100 μm.

3.1.7. Conclusion

To conclude, the MSN-LP hybrid nanoparticles (ML) was successfully synthesized from MSN-NH₂ and LP via lipid self-assembly process with assistance of ultrasound. The chemical characterization of ML was demonstrated by FT-IR and EDX spectroscopy. The hydrodynamic diameter and zeta potential of ML was 178 nm and -38.2 mV, respectively. The particle size and morphology were confirmed by TEM images. DOX was successfully loaded in ML with EE of $60.33 \pm 2.5\%$ and LC of $11.12 \pm 0.7\%$. The releasing profile of DOX@ML demonstrated that the formation of liposomal cap on MSNs surface had efficiently diminished the burst release of DOX compared to MSN-NH₂.

3.2. Synthesis and evaluation of Brij-FA coated MSN-LP

3.2.1. Synthesis of Brij coated ML

3.2.1.1. Preparation of Brij-coated LP

Brij-modified liposomes (LB) were successfully engineered, displaying hydrodynamic diameters spanning 140 to 180 nm (**Figure 3.10**). Formulations incorporated with Brij at mass ratios between 5% and 30% maintained structural stability, whereas elevating the Brij content to 40% induced particulate aggregation. Hence, LB formulations prepared within the 5-30% (w/w) Brij range were deemed optimal for subsequent surface coating onto MSN.

These observations agree with earlier reports demonstrating that integrating Brij surfactants up to specific threshold concentrations improves both the stability and pharmacokinetic behavior of liposomal carriers. For example, Fazel et al. established that formulating liposomes with 5% Brij yields nanoscale dimensions (82-97 nm) and uniform size distributions, thereby enhancing systemic pharmacokinetics. Conversely, surplus Brij concentrations compromise liposomal membrane integrity, ultimately triggering particle coalescence [127].

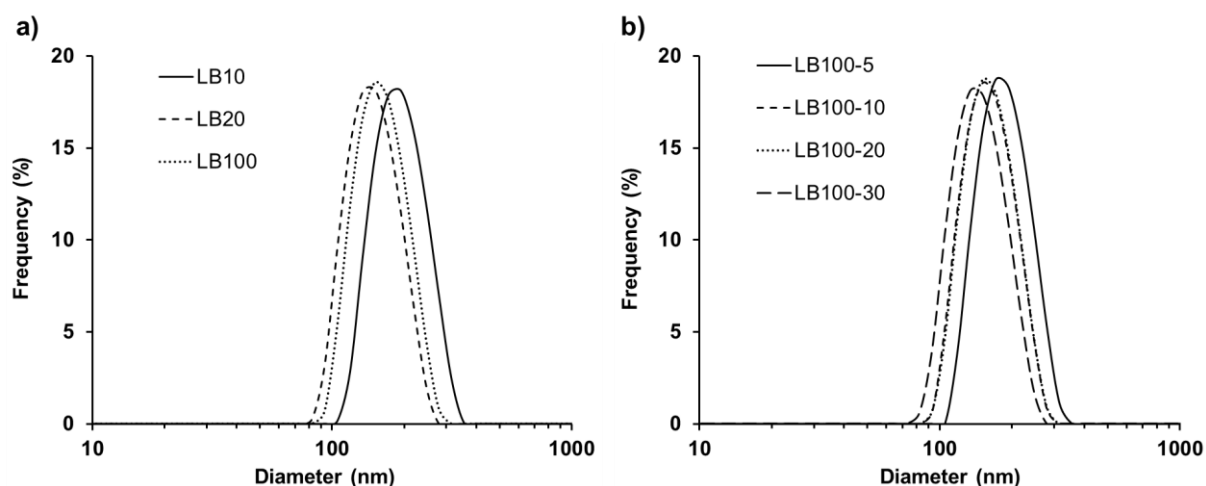


Figure 3.10. Particle size distribution of LB with different types of Brij (a) and Brij ratios (b)

3.2.1.2. Preparation of MLB

The mechanism governing the assembly of MSN-LP hybrid (ML) platforms through the integration of MSN and LP has been thoroughly investigated. In the primary phase, liposomes adsorb onto the MSN framework via non-covalent interactions, predominantly van der Waals forces and electrostatic attractions,

between the hydrophilic headgroups of the phospholipid constituents and the silica surface [48]. As the interfacial interactions intensify or as the surface density of adsorbed LP exceeds a critical threshold, the accumulation of lateral tension induces membrane deformation and subsequent rupture. This structural collapse drives the spreading, fusion, and continuous alignment of lipid bilayers across the MSN topography [44, 50]. The introduction of PEG moieties onto the liposomal surface further amplifies these interfacial interactions, promoting membrane disruption and facilitating lipid spreading on the silica matrix even at lower temperature [128]. Upon initial binding, LP undergo conformational restructuring to maximize PEG tethering onto the silica substrate, a process coupled with the desolvation and displacement of water molecules from the MSN-LP interfacial domain [129]. Nevertheless, an overabundance of PEG chains can occlude active adsorption sites on the MSN surface. Moreover, high local concentrations of surface-bound PEG can trigger steric instability and particulate aggregation, thereby destabilizing the resulting ML platform [128].

a) MLB with different types of Brij

The molecular weight and grafting density of surface-bound PEG chains are well-established factors dictating protein adsorption resistance [130-132]. Consequently, this work explored various Brij derivatives featuring distinct PEG chain lengths (defined by varying numbers of ethylene oxide (EO) units) conjugated onto the MLB matrix to assess how PEG length governs serum stability. Simultaneously, the influence of PEG chain density on overall stability was systematic evaluated by adjusting the Brij mass fraction during lipid bilayer assembly prior to coating onto the MSN surface.

The results presented in **Figure 3.11a** indicate that the particle sizes of MLB₁₀, MLB₂₀, and MLB₁₀₀ were 184.4 nm, 179.7 nm, and 194.2 nm, respectively. This suggests that an increase in the Brij chain length leads to a slight increase in MLB particle size. Additionally, as the Brij chain length increased from 10 to 20 and 100 EO units, the zeta potential of MLB increased from -44.7 mV to -38.1 mV and -36.4 mV, respectively. After 30 days of storage, the particle size of MLB₁₀₀ remained relatively unchanged, whereas the sizes of MLB₁₀ and MLB₂₀ increased to 240.4 nm and 219.3 nm, respectively. These findings suggest that B₁₀₀ effectively stabilizes MLB compared to B₂₀ and B₁₀. This stability can be attributed to the longer PEG chains in Brij, which form a thicker fixed aqueous layer around the particles, effectively preventing aggregation [113, 115].

The circulatory stability of nanocarriers serves as a pivotal determinant of their therapeutic efficacy, as non-specific opsonization, driven by serum proteins such as fibrinogen and immunoglobulins, prompts rapid recognition and clearance by the RES [131]. Furthermore, serum protein adsorption can induce particulate aggregation, resulting in enlarged hydrodynamic diameters and subsequent sedimentation [133, 134]. To assess the physiological stability of MLB, FBS was utilized to simulate the biological milieu. The association of serum proteins with the nanocarriers causes particle aggregation and precipitation, which is monitored via a corresponding decrease in the optical absorbance of the suspension.

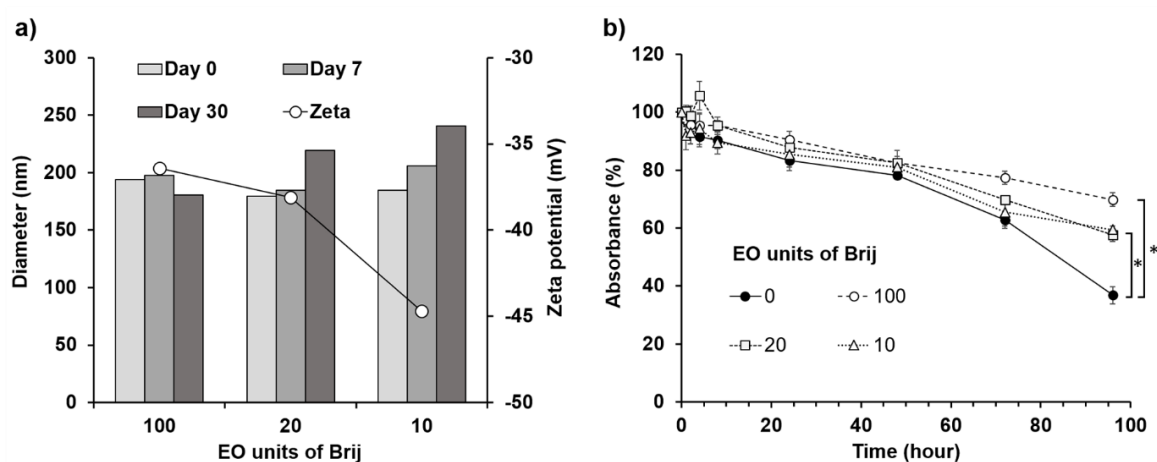


Figure 3.11. Particle size and zeta potential after 30 day storing (a) and stability in FBS (b) of MLB with different types of Brij. Each point is shown as the mean $\pm$ SD ($n = 3$). Statistical significance was evaluated using Student's t-test (* $p < 0.05$)

The stability of the materials in FBS environment is illustrated in **Figure 3.11b**. The data reveal that all materials displayed markedly reduced stability in FBS relative to their dispersion stability in aqueous media. For the pristine ML system (lacking Brij modification, 0% Brij), the relative absorbance experienced a modest decline to 78.2% after 48 h, followed by a dramatic drop to 59.1% at 72 h and 36.9% at 96 h, driven by pronounced particle aggregation and precipitation. Conversely, the Brij-modified MLB formulations maintained higher absorbance retention across equivalent timeframes, confirming improved colloidal stability. These findings align with earlier reports establishing that surface functionalization of ML architectures with PEG chains mitigates protein-induced aggregation [43]. Extending the Brij hydrophilic segment from 10 to 20 EO units yielded comparable 96 h absorbance values (59.4% and 57.6%, respectively). In contrast, incorporating a longer Brij chain with 100 EO units elevated the 96 h absorbance to 69.8%, demonstrating that MLB₁₀₀

confers superior serum stability compared to its MLB₁₀ and MLB₂₀ counterparts. This enhanced resistance to protein-driven destabilization is primarily attributed to the extended configuration of the higher-molecular-weight PEG chains, which generate a more robust steric barrier that minimizes protein opsonization on the lipid interface, thereby suppressing downstream aggregation and sedimentation [131, 133].

b) MLB with different ratio of Brij

The hydrodynamic dimensions and surface potential of the MLB formulations were characterized across varying surfactant ratios (**Figure 3.12a**). Incorporation of Brij at a 5 wt% ratio initially expanded the mean particle size from 180.6 ± 3.1 nm (pristine ML) to 202.6 ± 3.1 nm. However, progressively increasing the Brij mass fraction to 10%, 20%, and 30% induced a subtle contraction in hydrodynamic diameter to 192.6 ± 2.2 nm, 190.2 ± 2.3 nm, and 188.9 ± 5.1 nm, respectively. This non-monotonic dimensional trend closely mirrors findings reported by Durfee et al. [43]. Across all formulation conditions, the dimensional variations were constrained within a narrow window (~ 20 nm), demonstrating the robust colloidal integrity of the hybrid assembly. Concurrently, the zeta potential remained largely unperturbed upon Brij integration, exhibiting no statistically significant deviation from the uncoated ML baseline. This constancy in surface potential is primarily governed by the spatial arrangement and surface grafting density of the neutral PEG segments introduced by the surfactant chains.

As illustrated in **Figure 3.12b**, the MLB formulations consistently maintained higher relative absorbance values than the unfunctionalized ML system, confirming that Brij integration effectively mitigates protein-induced nanocarrier aggregation in FBS. Progressive elevation of the Brij mass fraction from 5% to 30% led to a stepwise increase in 96 h absorbance values from 58.4% to 69.81%, 70.78%, and 73.1%, respectively. These results indicate that higher Brij ratios reinforce colloidal stability by suppressing particulate agglomeration.

At a low Brij concentration of 5%, the relatively sparse surface coverage of PEG chains imparts insufficient steric repulsion to block protein opsonization. Increasing the Brij content to 10% elevates the PEG grafting density, establishing a denser steric shield that more effectively repels serum proteins. This trend accords with prior studies demonstrating that increasing surface PEG density directly minimizes protein adsorption and enhances nanocarrier stability within complex biological media.

However, beyond a specific concentration threshold, demonstrated here between 10% and 20% Brij mass fractions, further increases in PEG grafting density yield diminishing returns regarding colloidal stability. This plateauing effect can be attributed to the PEG chains adopting a dense, brush-like conformation once critical coverage is achieved, thereby optimizing steric repulsion. Consequently, raising the PEG density further provides marginal protective advantage, given that the particle surface is already saturated to hinder protein adsorption [135]. Thus, a 10% Brij ratio represents the optimal balance between establishing adequate surface coverage for enhanced MLB stability and maintaining material efficiency.

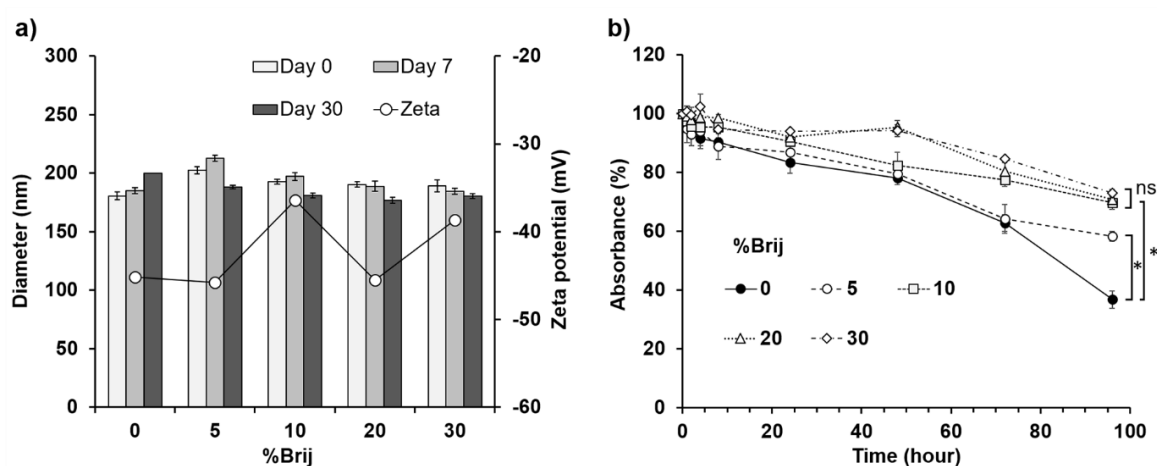


Figure 3.12. Particle size and zeta potential after 30 day storing (a), and stability in FBS (b) of MLB with different Brij percentage. Each point is shown as the mean $\pm$ SD ($n = 3$). Statistical significance was evaluated using Student's t-test (* $p < 0.05$)

3.2.2. Synthesis and characterization of Brij-FA coated ML

Based on the aforementioned investigations, Brij S100 with 100 EO units was selected for conjugation with FA to form Brij-FA (BF). Subsequently, BF was utilized to synthesize BF-coated LP (LBF) and BF-coated ML (MLBF) with a BF content of 10%.

3.2.2.1. Characterization of BF

^1H -NMR was used to characterize the conjugation of Brij and FA, with the chemical structures illustrated in **Figure 3.13a**. The spectra were recorded on a Bruker 500 MHz spectrometer at ambient temperatures and proton chemical shifts δ were presented in ppm. The purified samples were dissolved in D_2O (δ 4.79 ppm) for calibration. The NMR spectrum of BF (**Figure 3.13b**) showed the peaks of FA with chemical shift δ of 6.85 ppm, 7.71 ppm, and 9.20 ppm, which were attributed to the

aromatic protons and labeled j, i, and k, respectively. Moreover, characteristic chemical shift signals of Brij also appeared in the spectrum at peak a (0.75 ppm) of -CH_3 , peak b (1.13 ppm) of $\text{-CH}_2\text{-}$, and peaks c and d (3.59 - 3.73 ppm) of $\text{-O-CH}_2\text{-CH}_2\text{-OCH}_2\text{-}$. These results demonstrate that BF was successfully synthesized [120].

The FT-IR spectroscopy was used to identify the chemical adsorption of the functional group on the material. **Figure 3.13c** showed the FT-IR spectra of the reactants and the final material. The broad peak between 1200 and 1000 cm^{-1} was ascribed to the C-O stretching vibration of Brij. The peaks that appeared at 3524 cm^{-1} and 3444 cm^{-1} were ascribed to the N-H bond of FA. The signal oscillating at around 1580 cm^{-1} and 1466 cm^{-1} illustrated for the NH bending and double bond C=C (phenyl and pteridin ring) of FA, respectively [120, 136]. The presence of most of these characteristic functional groups of the reactants in the FT-IR spectrum confirmed the success of BF synthesis.

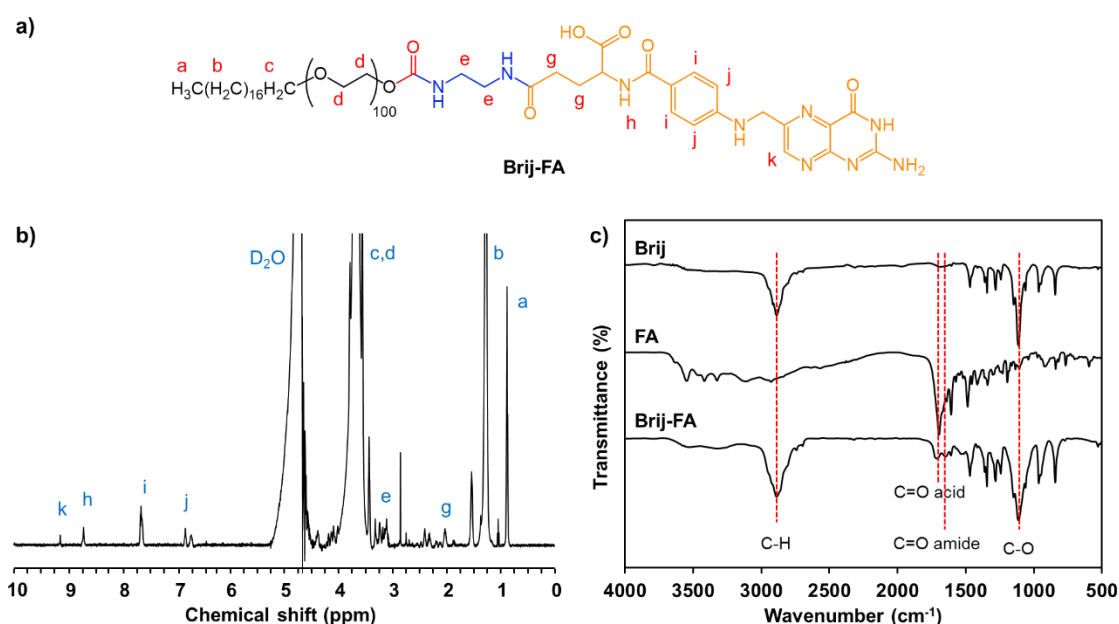


Figure 3.13. Chemical structure of BF (a). $^1\text{H-NMR}$ spectrum of BF (b). FT-IR spectra of Brij, FA, and BF (c)

3.2.2.2. Characterization of MLBF

The MLBF hybrid system was successfully constructed, displaying a hydrodynamic diameter of $191.8 \pm 3.2\text{ nm}$ (**Figure 3.14a**). This dimensional increase relative to unmodified ML (178 nm) is attributable to the surface integration of amphiphilic BF chains, which extend outward and thicken the hydration shell, thereby enlarging the effective hydrodynamic radius. Additionally, the zeta potential shifted from -38.2 mV for ML to -24.4 mV for MLBF (**Figure 3.14b**). This surface

charge attenuation stems from the outer BF layer partially shielding the underlying negative functional groups of the liposomal membrane. Taken together, these concomitant shifts in particle size and surface charge confirm the successful assembly of the BF-functionalized ML nanostructure.

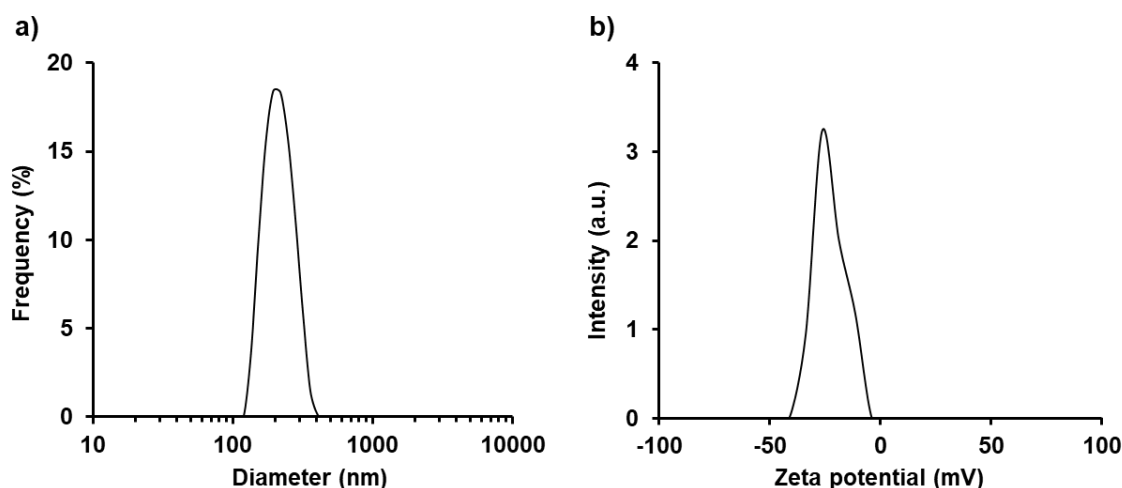


Figure 3.14. Particle size distribution (a) and zeta potential (b) of MLBF

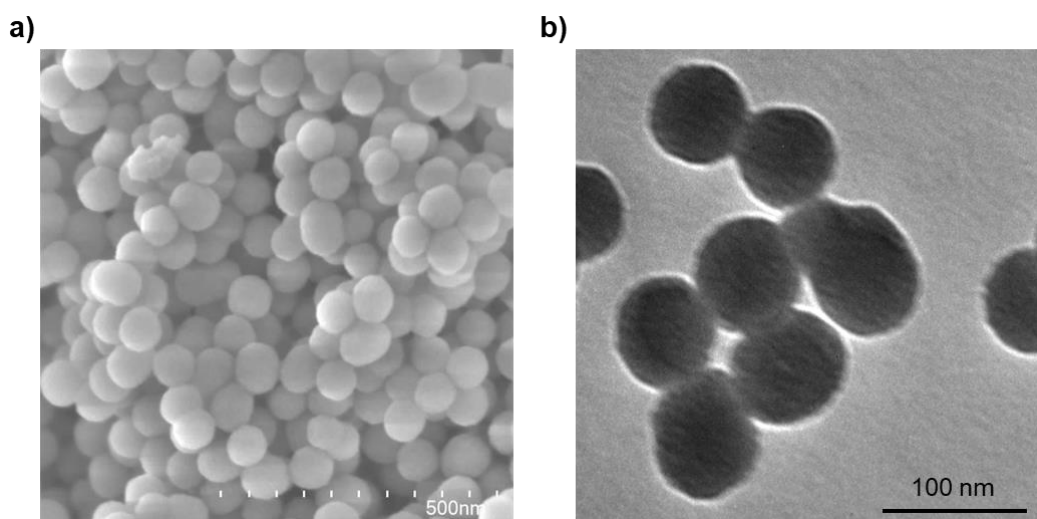


Figure 3.15. SEM (a) and TEM (b) images of MLBF

Scanning electron microscopy (SEM) analysis (**Figure 3.15a**) reveals that the MLBF nanoparticles exhibit a distinct spherical morphology with a narrow size distribution, primarily falling within the 60-90 nm range. This physical core size is substantially smaller than the hydrodynamic diameter of 191.8 nm recorded via dynamic light scattering (DLS) (**Figure 3.14a**). This dimensional variance between SEM and DLS is characteristic of surface-bound, PEG-containing BF moieties. In aqueous dispersion, these polymeric segments become extensively hydrated and assume extended chain conformations, expanding the effective hydrodynamic

boundary detected by DLS. Conversely, SEM analysis requires dry-state preparation, causing the flexible polymer coating to collapse onto the particle core and yielding a smaller measured size. Furthermore, transmission electron microscopy (TEM) micrographs (**Figure 3.15b**) substantiate these structural attributes, where the semi-transparent electron contrast reflects the intrinsic porous framework of the hybrid MLBF architecture.

The surface characterization of ML and MLBF were evaluated by the elemental analysis using the Energy Dispersive X-ray Spectroscopy (**Figure 3.16**). The spectrum of MLBF shown the weight percentage of Si as 10.06%, lower than the percentage of Si in ML (19.65%), whereas the percentage of C is higher (37.99% compared to 28.73% in ML), indicating the presence of the BF coating layer outside the LP layer (**Figure 3.16a and c**). The elemental mapping shows that O, C, P, and N are evenly distributed alongside Si, verifying the successful and uniform coating of organic components on the MSN surface (**Figure 3.16b and d**).

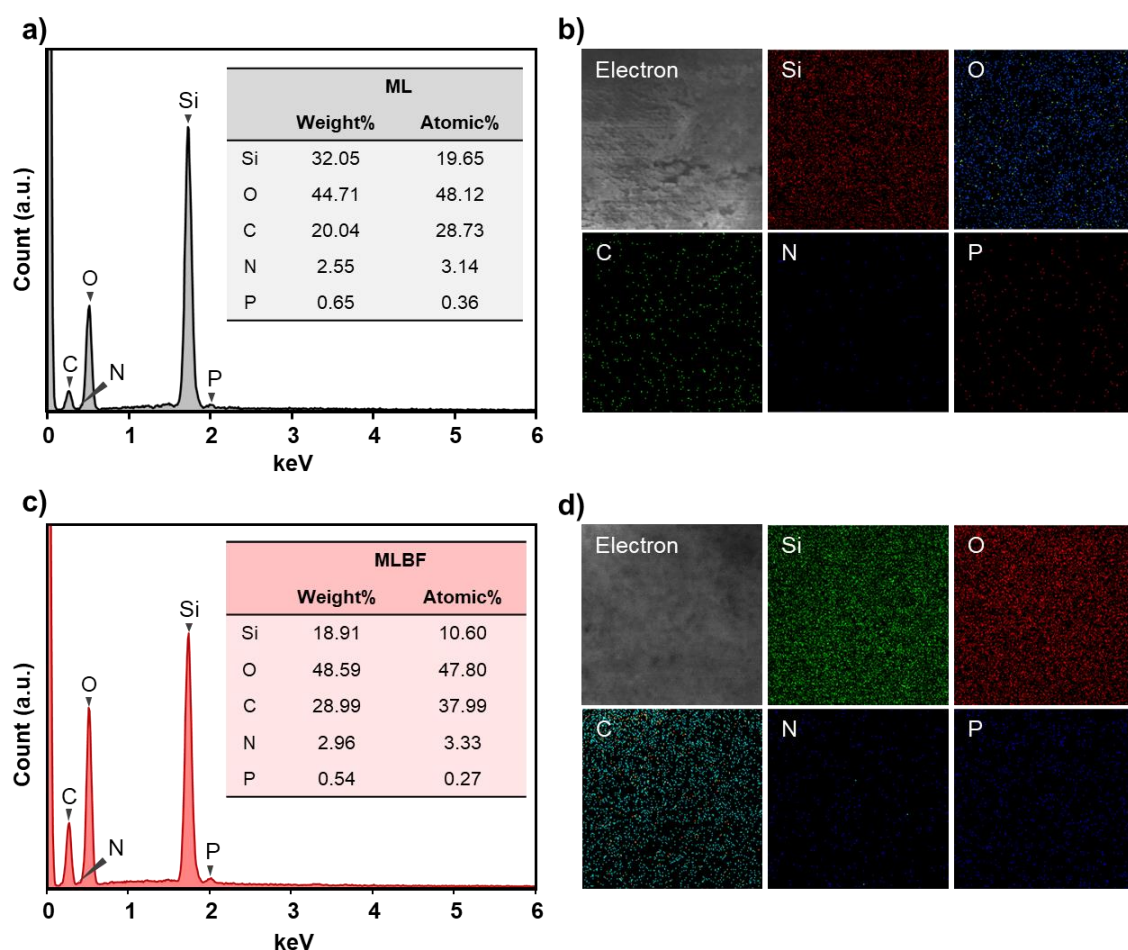


Figure 3.16. EDX spectra (a and c) and elemental mapping (b and d) of ML and MLBF, respectively

TGA thermograms of MSN-NH₂, ML, and MLBF are illustrated in **Figure 3.17**. The primary MSN-NH₂ matrix exhibits a cumulative mass loss of approximately 14.5% up to 800 °C, originating from the desorption of physically bound moisture alongside the pyrolytic decomposition of surface amine moieties. In comparison, the ML hybrid displays a higher overall weight loss of 31.6% at 800 °C, reflecting the combined thermal degradation of physisorbed water, amine functional groups, and the liposomal shell enveloping the silica core. The MLBF composite demonstrates a further mass loss increment to nearly 34.9%, marginally exceeding that of ML. This supplemental mass loss is attributed to the thermal breakdown of the outer BF coating on the liposomal surface, thereby verifying the successful integration of the BF shell onto the nanocarrier platform.

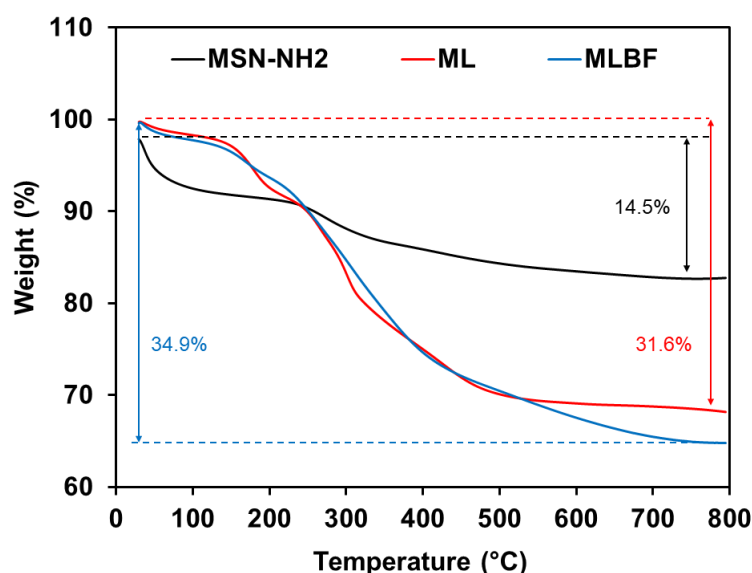


Figure 3.17. Thermograms of MSN-NH₂, ML, and MLBF

3.2.3. Drug loading and releasing

To evaluate the drug-delivery potential of the MLBF, DOX was employed as a model anticancer drug. Quantitative analysis revealed that MLBF exhibited an encapsulation efficiency of $58.4 \pm 2.9\%$ and a loading capacity of $10.45 \pm 0.2\%$.

Figure 3.18 depicts the *in vitro* release dynamics and cumulative kinetic profile of DOX. Under simulated physiological conditions (pH 7.4), the DOX-loaded MLBF platform exhibits minimal premature drug leakage, with cumulative release reaching only 21.57% at 24 h and 25.26% after 72 h. This sustained retention stems from the dual-anchoring mechanism: chemical immobilization of DOX within the nanocarrier matrix through dynamic imine linkage with amine-functionalized silica mesopores, combined with the physical barrier provided by the lipid bilayer shell, which restricts

aqueous solvent influx into the pore channels and hinders outward drug diffusion [22, 137]. When the pH is reduced to 5.5, a pronounced increase in release is observed, with cumulative values of approximately 42.91% at 24 h and 49.74% at 72 h. The accelerated release under acidic conditions is primarily associated with pH-induced defect and blister formation in the lipid bilayer, creating diffusion pathways for DOX [41], as well as proton-mediated cleavage of imine linkages between DOX and the MSN surface [22, 126]. Overall, these findings confirm the pH-responsive and controlled drug-release capability of the MLBF hybrid nanocarriers.

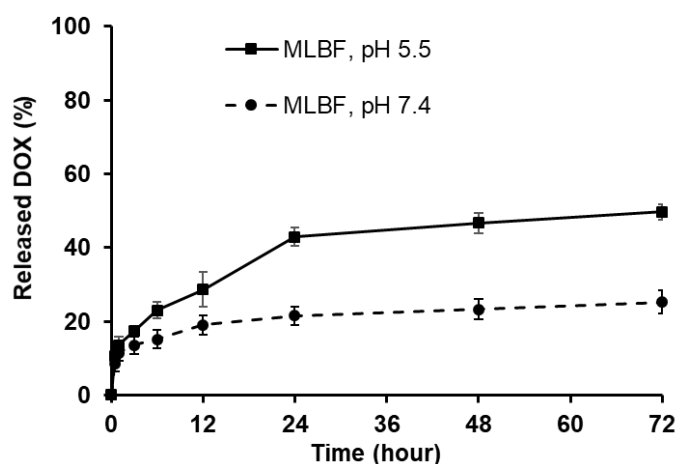


Figure 3.18. DOX release profile of MLBF in PBS pH 5.5 and 7.4

3.2.4. Cytotoxicity assay

The biocompatibility of MLBF toward cervical cancer HeLa cells was evaluated by the MTT assay, and the corresponding results are shown in **Figure 3.19a**. The MLBF formulation alone exhibited negligible cytotoxicity, maintaining cell viabilities above 80% across the tested concentration range up to 150 $\mu\text{g/mL}$, indicating its favorable cytocompatibility.

In contrast, treatment with either free doxorubicin (DOX) or DOX-loaded MLBF (DOX@MLBF) for 48 h resulted in a clear concentration-dependent reduction in cell viability (**Figure 3.19b**). Notably, no statistically significant difference was observed between the cytotoxic effects of free DOX and DOX@MLBF at equivalent concentrations, suggesting that DOX encapsulated within MLBF retains anticancer efficacy comparable to that of the free drug (**Figure 3.19c**).

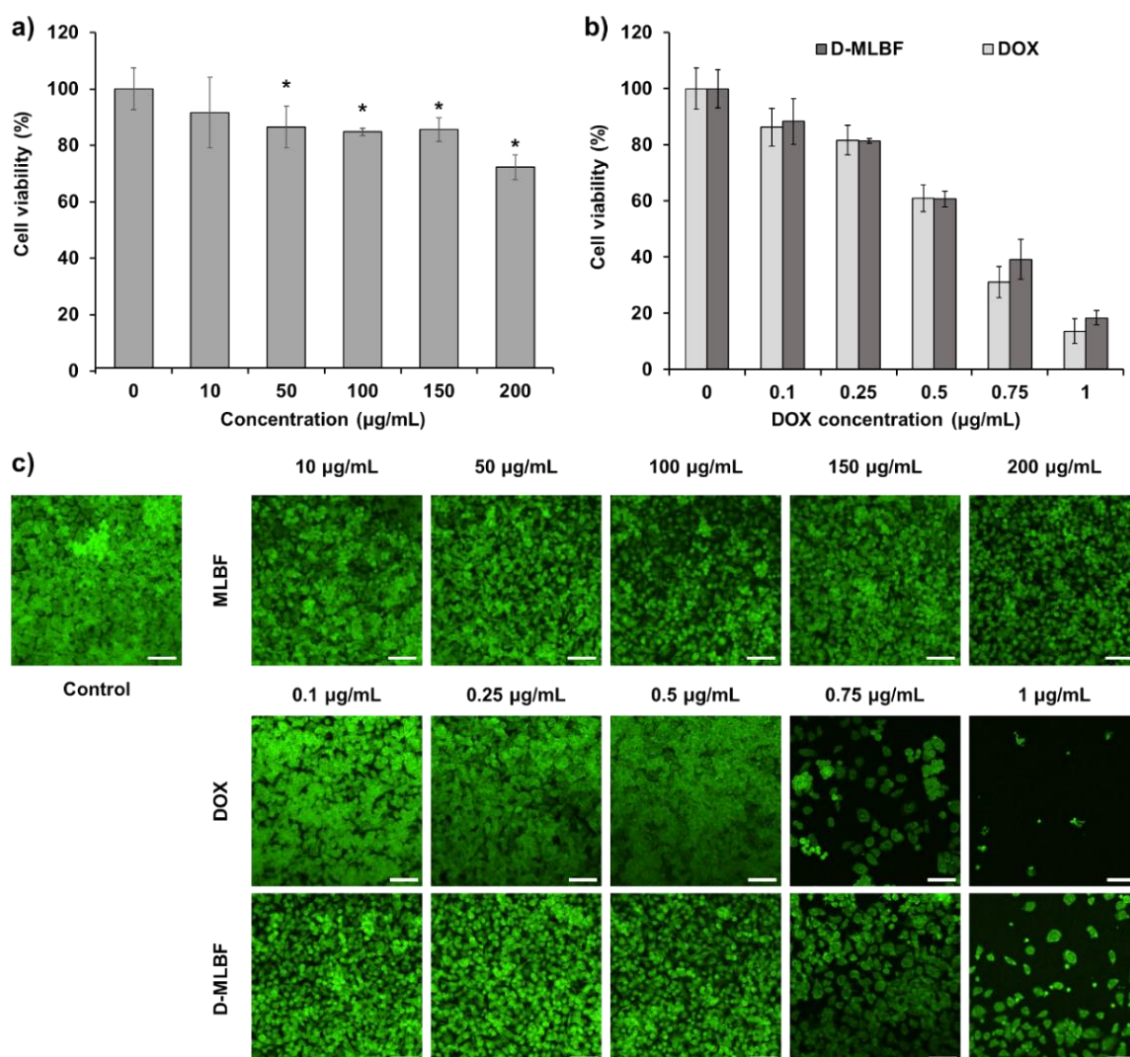


Figure 3.19. *In vitro* cellular viability (a and b) and fluorescence images (c) of HeLa cells exposed to MLBF and DOX@MLBF for 48 hours, respectively. The scale bar of all images is 100 μm .

3.2.5. Cellular uptake

The intracellular accumulation of ML and MLBF was examined in HeLa cells to evaluate their respective membrane penetration capacities and active targeting efficiencies. To facilitate fluorescence imaging, the nanocarriers were hydrophobic-labeled using DiO dye, generating DiO-ML and DiO-MLBF, and incubated with HeLa cells for 4 h. As depicted in **Figure 3.20**, both nanostructures underwent successful cellular internalization, evidenced by strong green fluorescent signals localized within the intracellular compartment [125]. Merged confocal images revealed a broad cytoplasmic distribution of the DiO signal surrounding the nuclear envelope, confirming effective cytosolic delivery. Notably, HeLa cells treated with DiO-MLBF displayed substantially stronger fluorescence intensity compared to those

exposed to DiO-ML, demonstrating superior cellular uptake driven by Brij-FA surface modification. This enhancement is predominantly attributed to the high-affinity binding between the surface-exposed folic acid moieties of Brij-FA and folate receptors overexpressed on cancer cell membranes, which triggers rapid receptor-mediated endocytosis [138].

In a related study, Li et al. functionalized an ML hybrid platform using folate-conjugated DSPE-PEG2000 (ML-DSPE-PEG-FA) and evaluated its cellular internalisation in NB4 cells. Their findings demonstrated an uptake hierarchy of ML-PEG-FA > ML-PEG > MSN, confirming that liposomal encapsulation accelerates cellular entry relative to bare silica cores, while FA conjugation further amplifies uptake through target-specific recognition [82]. Although evaluated across distinct cancer models (HeLa vs. NB4), the MLBF platform developed in this study demonstrates robust cell-penetrating capabilities and targeted uptake efficiency fully comparable to established ML-DSPE-PEG-FA architectures.

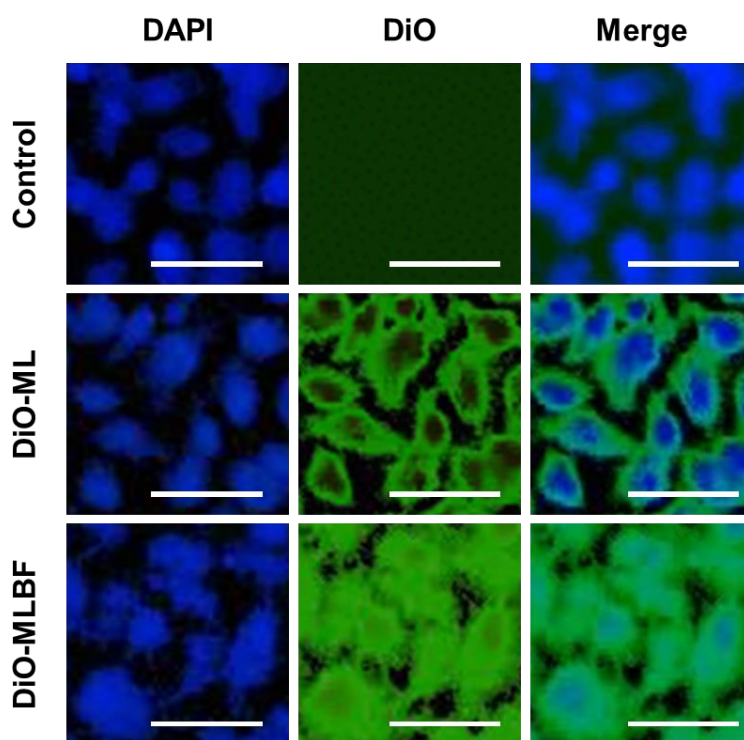


Figure 3.20. The fluorescence images of HeLa cells after exposed to DiO-ML and DiO-MLBF for 4 hours. The scale bar of all images is 50 μm .

3.3. Synthesis and evaluation of GLT-PEG-FA coated MSN-LP

3.3.1. Synthesis of GLT-PEG-FA

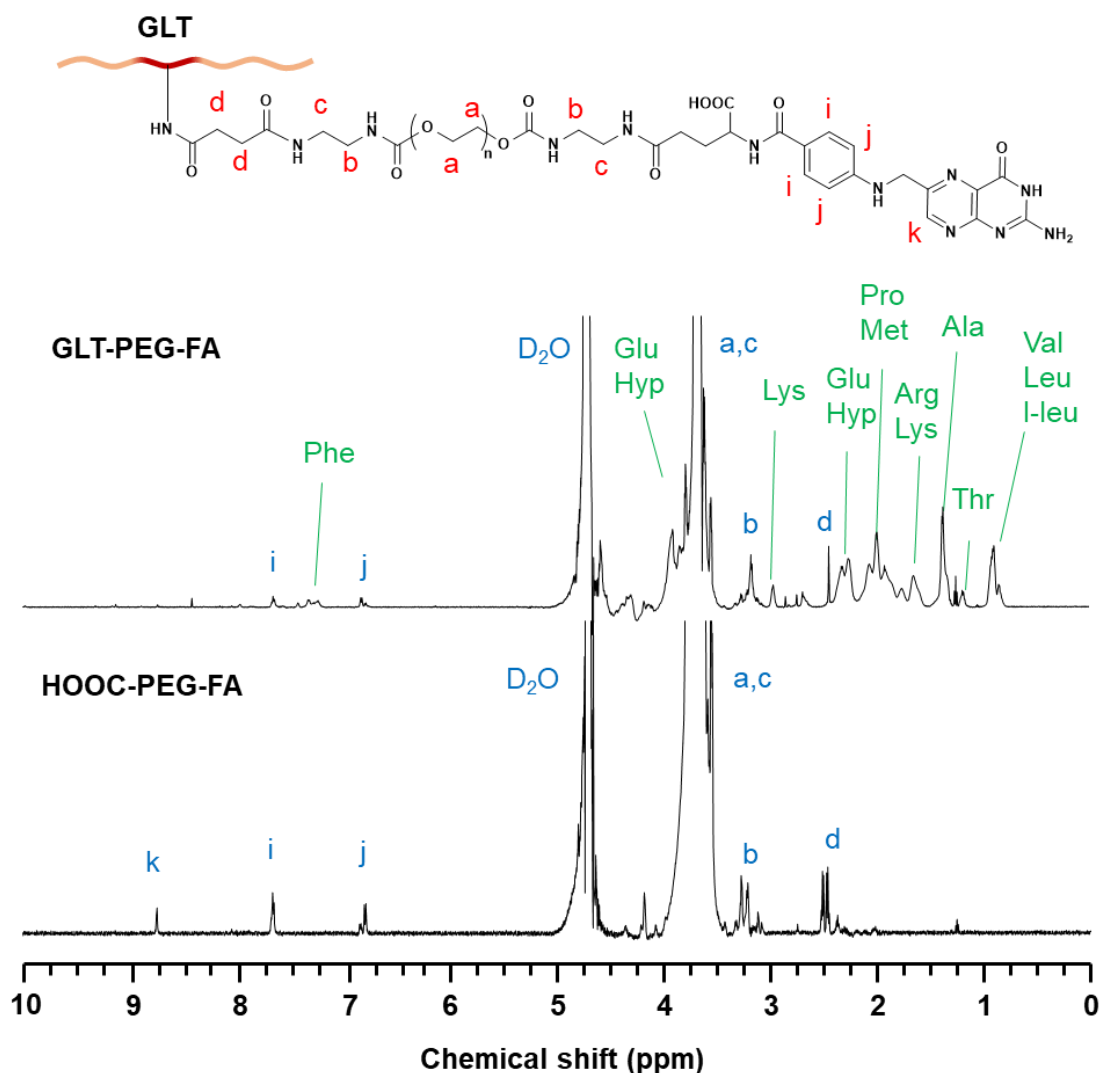


Figure 3.21. ^1H -NMR spectra of HOOC-PEG-FA and GPF

^1H -NMR was used to characterize the GPF (**Figure 3.21**). The spectra were recorded on a Bruker 500 MHz spectrometer at ambient temperatures and proton chemical shifts δ were presented in ppm. The purified samples were dissolved in the protic solvent of D_2O (δ 4.79 ppm) for calibration. The ^1H -NMR spectrum of FA-PEG-COOH showed the FA signal at peak k (8.76 ppm), peak i (7.71 ppm), and peak j (6.85 ppm) [139]. Moreover, characteristic chemical shift signals of PEG were also appeared in the spectrum at peak a (3.49 - 4.03 ppm) of $-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-$. Signals of $-\text{C}(=\text{O})-\text{CH}_2-\text{CH}_2-\text{C}(=\text{O})-$ of succinic acid appeared at 2.5 ppm (peak d). These results demonstrated that the FA-PEG-COOH was successfully synthesized. The ^1H -NMR spectrum of GPF exhibits signals corresponding to the presence of gelatin and

FA-PEG-COOH, which are the active components. The distinctive signals of FA-PEG-COOH seen at $\delta H = 3.76$ ppm (a) correspond to the proton signals of the $-\text{OCH}_2\text{-CH}_2\text{-O}-$ group. The signals at $\delta H = 6.9$ ppm (j), 7.4 ppm (i), and 8.5 ppm (k) are indicative of FA signals on FA-PEG-COOH. The distinctive signals of gelatin are derived from specific amino acids. For instance, the signal observed at $\delta H = 0.9$ ppm corresponds to the proton signal of γ - and δ - CH_3 groups found in valine (Val), leucine (Leu), and isoleucine (I-leu). Similarly, the signal at $\delta H = 1.22$ ppm represents the proton signal of the γ - CH_3 group in threonine (Thr). The signal at $\delta H = 1.4$ ppm corresponds to the $-\text{CH}_3$ group in alanine (Ala). Additionally, the proton signal at $\delta H = 1.6$ - 1.7 ppm is attributed to the β - and γ - CH_2 groups in arginine (Arg), as well as the β -, γ -, and δ - CH_3 groups in lysine (Lys). The signal for proline (Pro) and methionine (Met) occurs at a chemical shift of 2 ppm (parts per million) in the ^1H NMR spectrum. The signal for glutamic acid (Glu) and hydroxyproline (Hyp) shows at a chemical shift of 2.3 ppm. The signal for lysine (Lys) appears at a chemical shift of 2.99 ppm. The signal for glutamic acid (Glu) and hydroxyproline (Hyp) appears at a chemical shift of 3.92 ppm. The signal for phenylalanine (Phe) appears at a chemical shift of 7.25 ppm [140]. The examination of the ^1H -NMR spectrum revealed the successful synthesis of grafted gelatin FA-PEG-COOH (GPF).

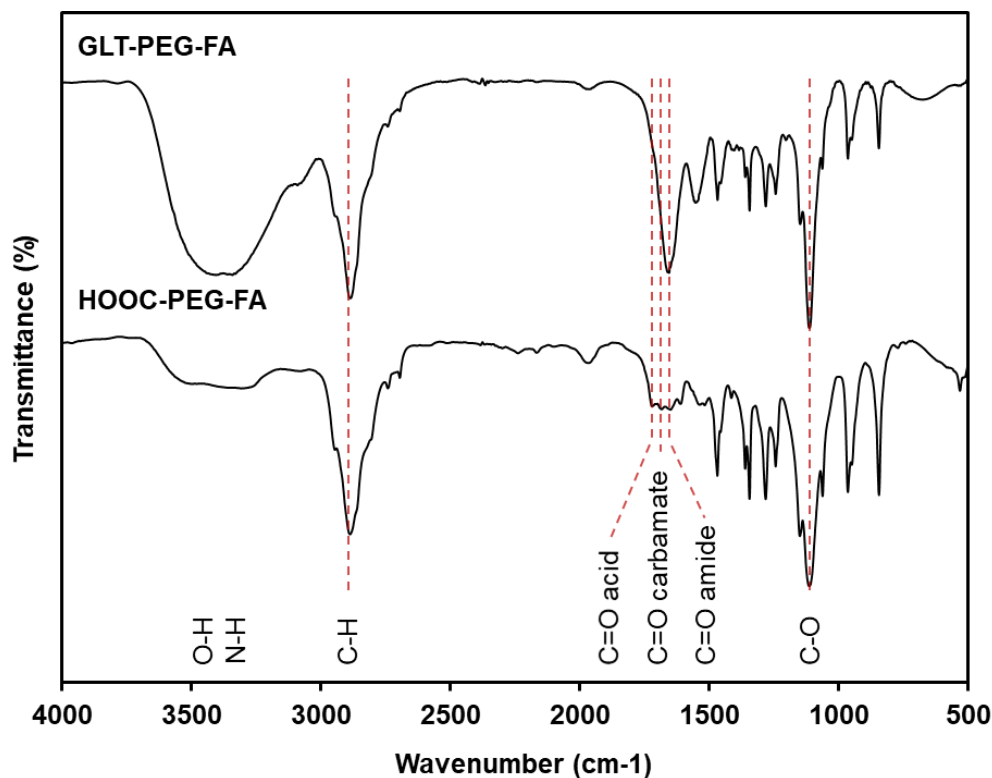


Figure 3.22. FT-IR spectra of HOOC-PEG-FA and GPF

The FT-IR spectrum analysis for FA-PEG-COOH and GPF is displayed in **Figure 3.22**. The FT-IR spectrum of FA-PEG-COOH reveals specific signals indicating the presence of certain chemical bonds. The signal at 1121 cm^{-1} corresponds to the vibrations of the C-O-C bond in PEG. The signal at 1711 cm^{-1} represents the C=O acid bond vibration in both FA and SA. Additionally, the signals at 1644 cm^{-1} , 1642 cm^{-1} , and 1602 cm^{-1} indicate the presence of C=O amide vibrations, which are created when FA and SA are linked with PEG. The analytical findings above indicate the effective synthesis of FA-PEG-COOH. The FT-IR spectrum findings of GPF indicate that the signals at 1121 cm^{-1} correspond to the C-O-C bond vibrations of PEG. Additionally, there are distinctive signals of GLT seen at wave numbers 1591 cm^{-1} for the C=O amide I group and 1502 cm^{-1} for the N-H amide II group [141]. The findings indicated an effective synthesis of GPF. When the binding ratio of PEG on GLT is raised, the ratio between the signal of PEG at 1121 cm^{-1} and the signals of GLT at 1591 cm^{-1} and 1502 cm^{-1} likewise increases proportionally.

3.3.2. Synthesis of GLT-PEG-FA coated ML

The investigation of the ML:GPF ratio is summarized in **Table 3.1**. The results indicated that high ratios of ML to GPF (2:1 and 1:1) led to instability and agglomeration, preventing accurate measurement of particle size. However, increasing the GPF:ML ratios to 2:1, 3:1, and 4:1 resulted in stable dispersions with particle sizes ranging from approximately $240.1 \pm 9.1\text{ nm}$ to $361.8 \pm 12.2\text{ nm}$ and zeta potentials between -25.9 mV and -37.6 mV . This instability at lower GPF ratios may be due to insufficient GPF molecules to adequately cover the surface of ML, leading to aggregation. By increasing the GPF ratio, the surface of ML becomes fully coated, resulting in stable dispersions.

Table 3.1. MLGPF with different precursors ratios and concentrations

	Observation	Size (nm)	Zeta potential (mV)
ML to GPF ratios (w/w)			
2:1	Aggregation	-	-
1:1	Aggregation	-	-
1:2	Dispersion	361.8 ± 12.2	-37.6
1:3	Dispersion	240.1 ± 9.1	-25.9
1:4	Dispersion	256.4 ± 4.5	-32.6
GPF concentration ($\mu\text{g/mL}$)			

500	Dispersion	857.8	-
1000	Dispersion	240.1 ± 9.1	-25.9
2000	Dispersion	218.8 ± 2.7	-23.8
3000	Dispersion	270.7 ± 19.4	-24.1
4000	Aggregation	-	-
GLT to PEG ratios			
1:5	Dispersion	203.5 ± 2.2	-42.6
1:10	Dispersion	209.6 ± 4.9	-37.8
1:20	Dispersion	218.8 ± 2.7	-23.8
1:30	Dispersion	653.5 ± 38.1	-47.6
1:40	Dispersion	796.7 ± 19.1	-44.9

At a low concentration of GPF (500 µg/mL), the product exhibits a significant increase in particle size (approximately 857 nm). As the concentration of GPF increases to 1000 and 2000 µg/mL, the particle size decreases to 240.1 ± 9.1 nm and 218.8 ± 2.7 nm, respectively. The particle size of MLGPF increases to 270.7 ± 19.4 nm when the GPF concentration is raised to 3000 µg/mL. The zeta potential shows no significant change with varying GPF concentrations, ranging from -23.8 to -25.9 mV. This may be explained by the fact that increasing the concentration of GPF enhances the ability to fully coat the surface of the ML, thereby improving the stability of the particles. However, upon raising the GPF concentration to 4000 µg/mL, agglomeration occurs, indicating that the optimal concentration of GPF for coating on ML is approximately 1000-3000 µg/mL, with an optimal concentration of 2000 µg/mL.

When varying the GPF to PEG ratio from 1:5, 1:10, to 1:20, the particle size of MLGPF slightly increased from 203.5 nm to 218.6 nm, while the zeta potential rose from -42.6 mV to -23.8 mV. However, when the GPF to PEG ratio was increased to 1:30 and 1:40, the particle size of MLGPF surged to over 600 nm, indicating that GPF₃₀ and GPF₄₀ are unsuitable for the synthesis of MLGPF.

Stability assessments revealed that at the time of synthesis (day 0), MLGPF₅, MLGPF₁₀, and MLGPF₂₀ exhibited no significant difference in particle size, ranging from approximately 203 to 218 nm. However, at days 7 and 30, MLGPF₂₀ showed a smaller increase in particle size compared to MLGPF₅ and MLGPF₁₀, demonstrating the highest stability (**Figure 3.23a**).

Based on these results, the optimal synthesis process for MLGPF was determined to utilize GPF₂₀, with a GPF to ML ratio of 3:1 (w/w), and a GPF concentration of 2000 µg/mL.

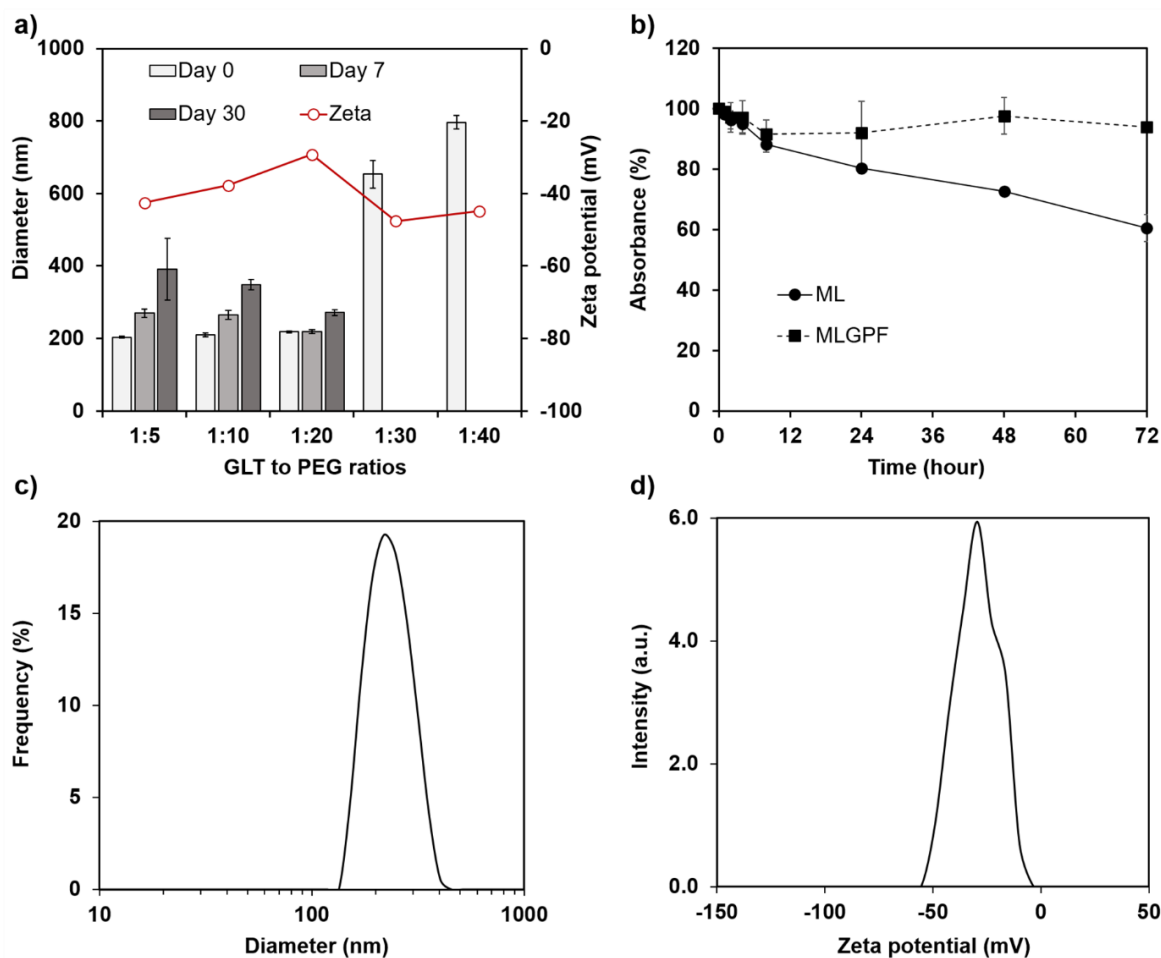


Figure 3.23. The stability of MLGPF with different GLT to PEG ratios (a). The stability in FBS media of ML and MLGPF (b). Particle size (c) and zeta potential (d) of MLGPF₂₀

The stability test results in FBS media are presented in **Figure 3.23b**. In the first 6 hours, both ML and MLGPF exhibited similar absorbance percentages, about 95%. At 24, 48, and 72 hours, the absorbance percentage of MLGPF showed no significant change, whereas the absorbance percentage of ML decreased to 80.26%, 72.56%, and 60.43%, respectively. This decline may be attributed to the interaction of proteins in FBS with the surface of ML, leading to the formation of clusters that sediment more easily [142]. These results indicate that the stability of ML in FBS media was improved by coating with GPF.

The particle size distribution and zeta potential of the optimized sample (MLGPF₂₀) are shown in **Figures 3.23c and d**.

3.3.3. Physicochemical characterization

3.3.3.1. Chemical structure

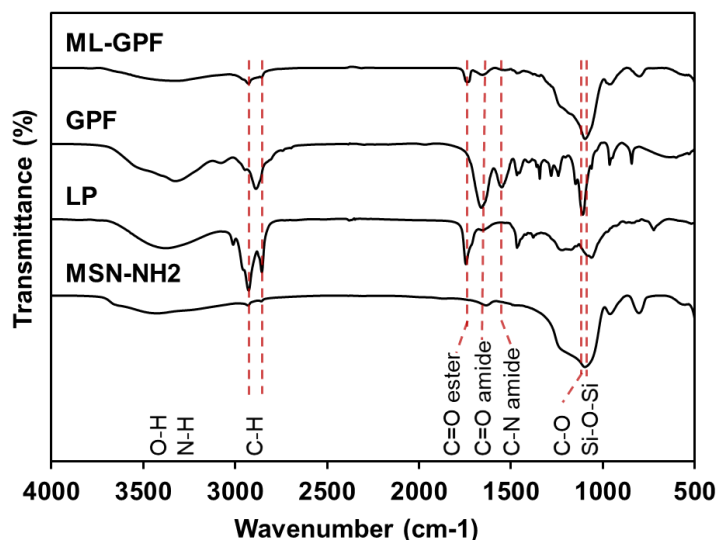


Figure 3.24. FT-IR spectra of MSN-NH₂, GPF, LP, and ML-GPF

The IR spectra of MLGPF and its precursors are shown in **Figure 3.24**. It can be observed that in the IR spectrum of MLGPF, although the signals in the range of 950-1300 cm⁻¹ are obscured by the strong characteristic signal of Si-O-Si vibrations, the characteristic signals of LP appear at 1747 cm⁻¹ and GPF at 1654 cm⁻¹ [143]. These results demonstrate the successful coating of LP and GPF layers on MSN.

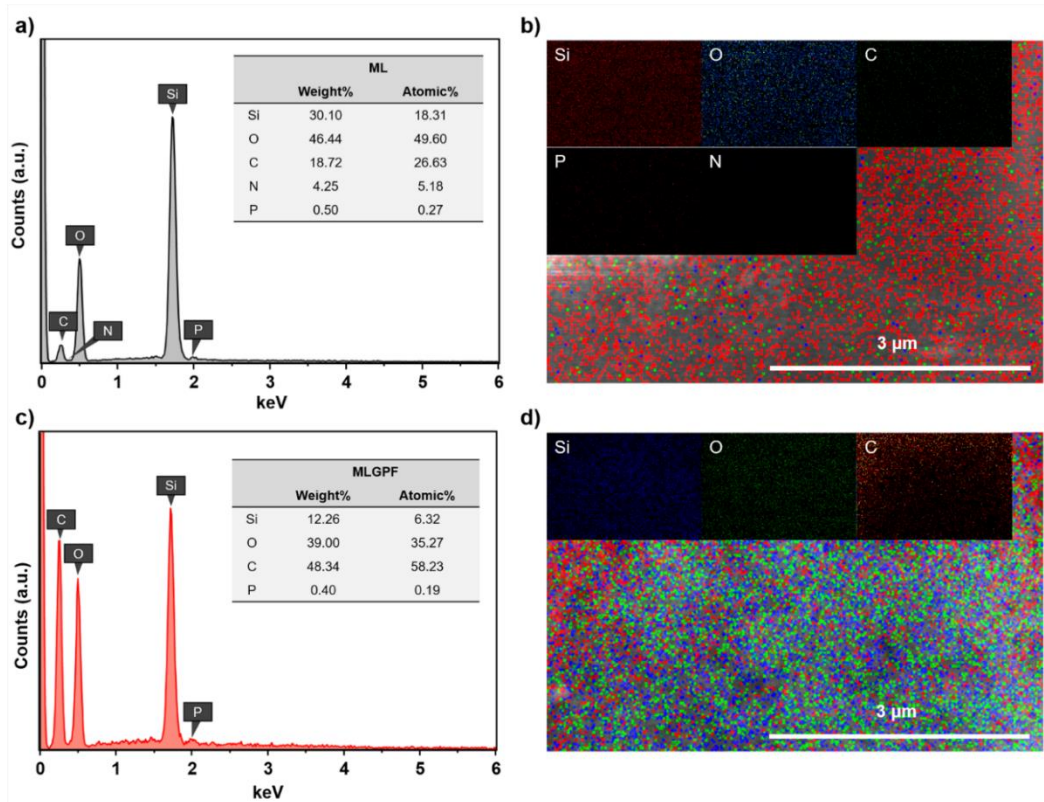


Figure 3.25. EDX results (a and c) and elemental mapping (b and d) of ML and MLGPF

The surface characterization of ML and ML-GPF were evaluated by the elemental analysis using the Energy Dispersive X-ray Spectroscopy on the SEM. The spectrum of ML-GPF shown the weight percentage of Si and O as 6.32% and 35.27%, lower than the percentage of Si and O in ML (18.31% and 49.60%, respectively), whereas the percentage of C is higher (58.23% compare to 26.63%), indicating the increase of the coating layer due to GPF (**Figure 3.25**).

3.3.3.2. Thermal properties

The TGA curve of ML-GPF is depicted in **Figure 3.26**. Both ML and MLGPF samples exhibit mass reduction in two stages: stage 1 at around 150 °C, possibly due to loss of water molecules in pores and surface, and stage 2 from 150-800 °C, possibly due to the decomposition of organic layers [56, 75]. Based on the differences in weight loss among the MSN-NH₂, ML, and MLGPF samples at 800 °C, the organic composition coating the surface of MSN can be inferred. In our previous study, the weight loss of MSN-NH₂ at 800 °C was determined to be 7.7%, attributed to the degradation of APTES [119]. The weight loss of ML was 31.5%, resulting from the decomposition of APTES and the lipid bilayer [86]. The weight loss of ML-GPF reached 39.1%, which is higher than that of ML (31.5%), likely due to the decomposition of GPF [144]. This also confirms the presence of the GPF coating layer on the surface of ML.

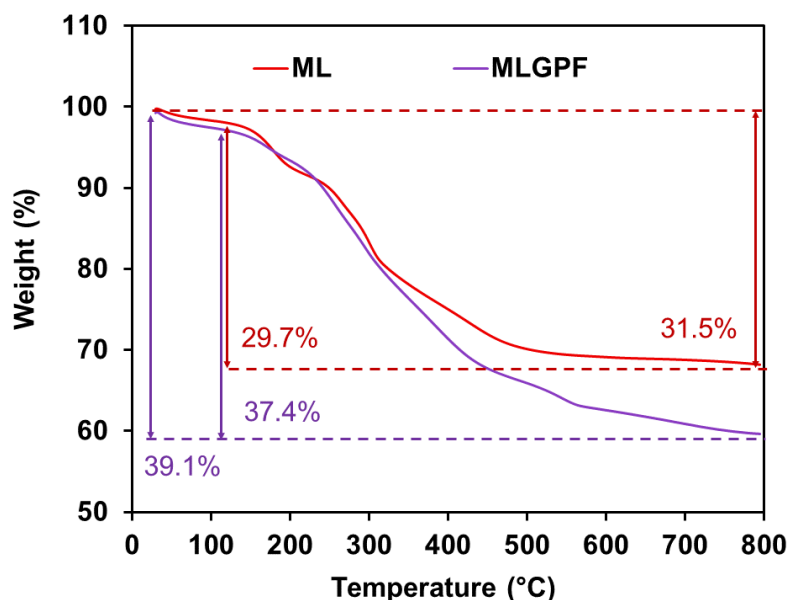


Figure 3.26. TGA thermograms of ML and ML-GPF

3.3.3.3. Morphology

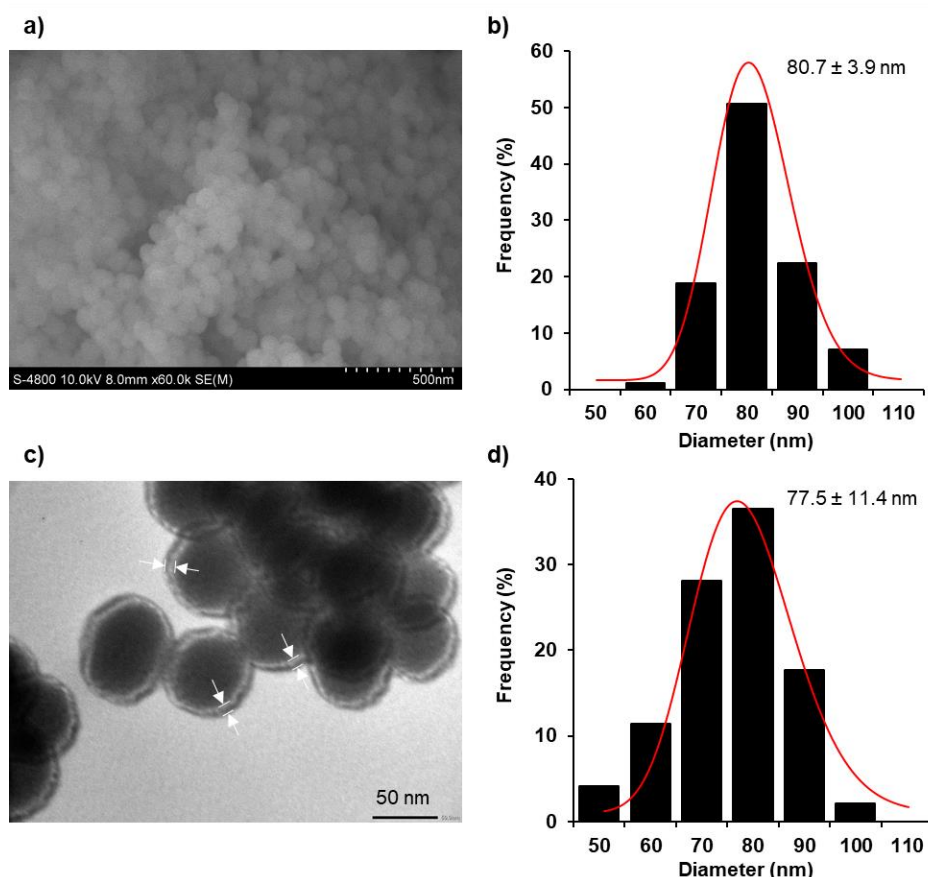


Figure 3.27. SEM (a) and TEM (c) images of ML-GPF with respective statistical size distributions (b and d)

The results of SEM, TEM, and particle size distribution statistics from the SEM and TEM images of MLGPF are presented in **Figure 3.27**. Both SEM and TEM results indicate that MLGPF has a spherical shape, with a size of approximately 50-100 nm and a relatively narrow distribution (**Figure 3.27a, b, and d**). TEM images also reveal the GPF coating layer on the surface of ML (white arrows in **Figure 3.27c**), with a thickness of about 5-10 nm. In the study by Carmona et al., the gelatin coating on MSN also exhibited a thickness of approximately 4-6 nm when observed via TEM imaging [143].

3.3.4. Drug loading and releasing

To evaluate the drug-delivery potential of the MLGPF, DOX was employed as a model anticancer drug. Quantitative analysis revealed that MLGPF exhibited an encapsulation efficiency of $52.5 \pm 1.6\%$ and a loading capacity of $9.82 \pm 0.4\%$. The favorable loading performance is plausibly explained by the high surface area and accessible mesoporous architecture of the silica core, together with surface functional moieties capable of forming stabilizing interactions with DOX.

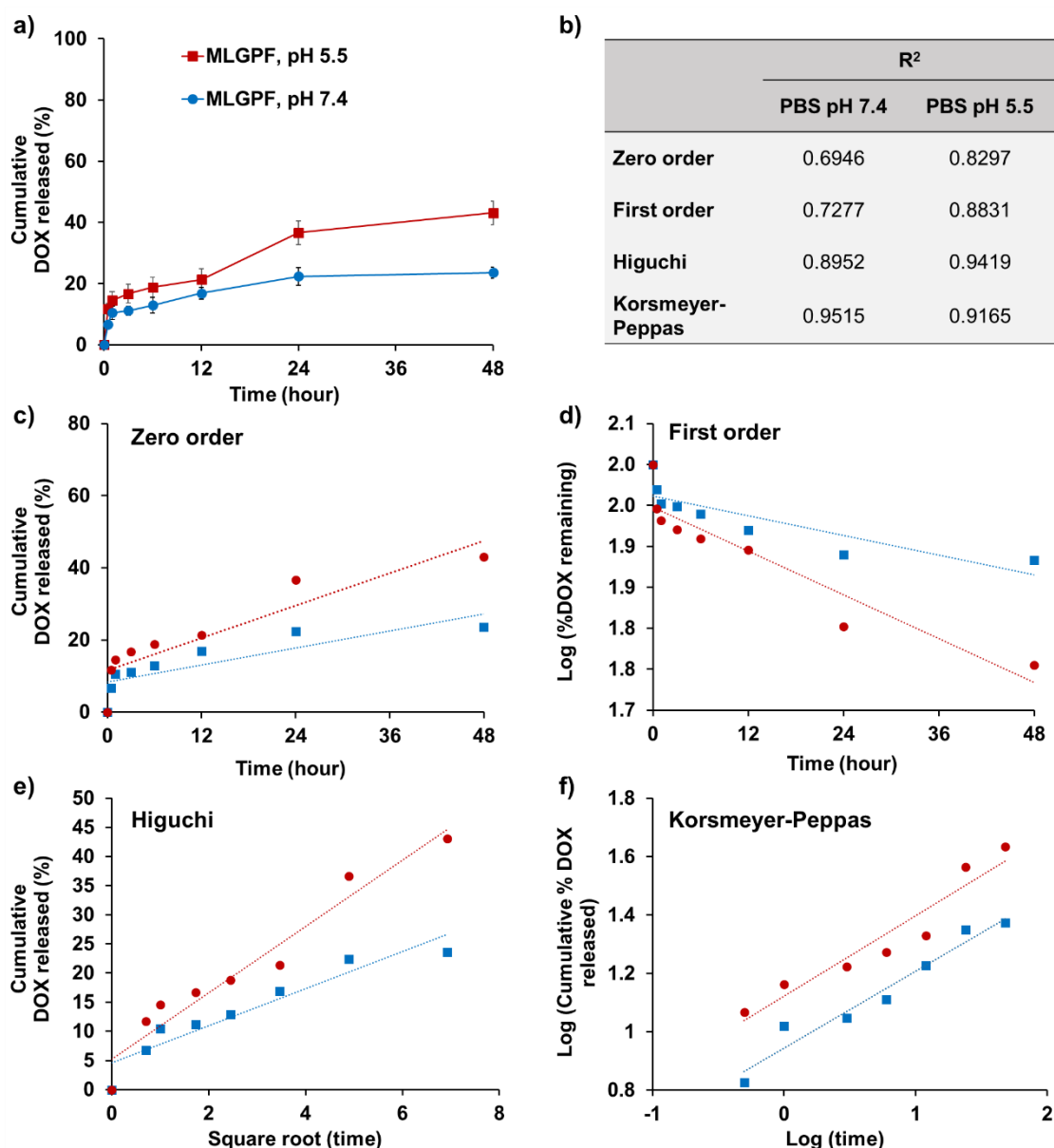


Figure 3.28. DOX releasing profile in PBS pH 7.4 and pH 5.5 of DOX loaded in ML-GPF (a). Fitting of DOX releasing profile by Zero order (c), First order (d), Higuchi (e), and Korsmeyer-Peppas (f) mathematic models, with the correlation coefficient (R^2) (d).

The *in vitro* release profile and kinetics of DOX are presented in **Figure 3.28a**. The drug release results indicate that at pH 7.4, DOX-loaded MLGPF exhibits low release rates of less than 20% after the first 12 hours and about 23.3% after 72 hours. This behavior is ascribed to (i) imine bond formation between DOX and surface amine groups on MSN at pH 7.4 and (ii) the presence of the LP bilayer and GPF coating, which shield the mesopores and hinder DOX diffusion [22, 137]. In an acidic environment (pH 5.5), the DOX release rate increases markedly, reaching about

36.63% at 24 hours and 44.29% at 72 hours. This enhancement is attributed to blister formation within the lipid bilayer at pH 5.5, which generates openings that accelerate DOX diffusion [41]. Additionally, acid-triggered cleavage of imine linkages between DOX and MSN surface amines further promotes release [22, 126]. These results underscore the pH-responsive, controlled-release behavior of MLGPF.

To further elucidate the release mechanism, the release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. The corresponding correlation coefficients (R^2) at pH 7.4 and 5.5 were 0.6946 and 0.8297 (zero-order), 0.7277 and 0.8831 (first-order), 0.8952 and 0.9419 (Higuchi), and 0.9515 and 0.9165 (Korsmeyer-Peppas), respectively (**Figure 3.28b,c,d,e,f**). At pH 7.4, the Korsmeyer-Peppas model provided the best fit ($R^2 = 0.9515$), suggesting a combined diffusion- and matrix-controlled release mechanism, which is consistent with the presence of multiple structural barriers in the multilayer system (**Figure 3.28f**). In contrast, at pH 5.5, the Higuchi model showed the highest correlation ($R^2 = 0.9419$), indicating that drug release is predominantly governed by diffusion (**Figure 3.28e**). This shift in release mechanism reflects the partial destabilization of the outer layers and cleavage of imine linkages under acidic conditions, which facilitate drug diffusion [44-46]. Such pH-dependent kinetic behavior is consistent with previous reports on mesoporous silica-based hybrid nanocarriers, where structural relaxation under acidic conditions promotes diffusion-dominated release [7, 46].

3.3.5. Cytotoxicity assay

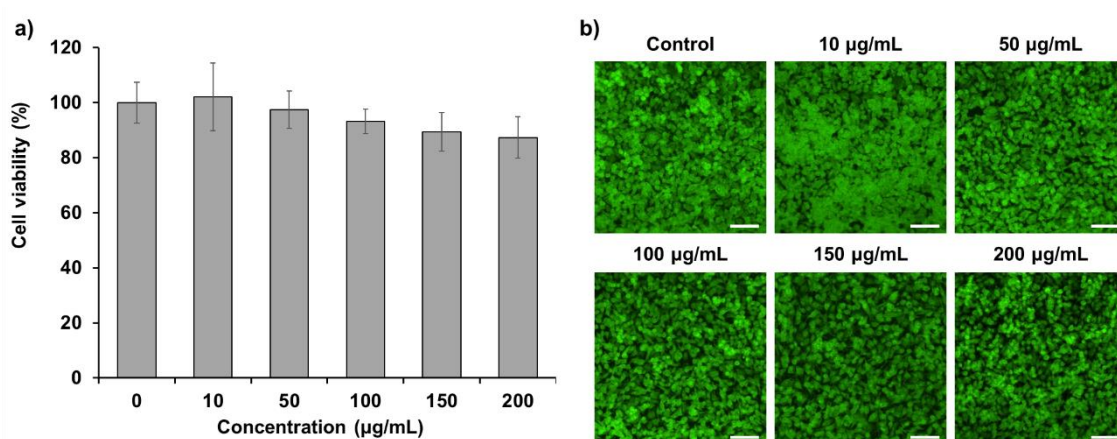


Figure 3.29. *In vitro* cellular viability (a) and fluorescence images (b) of HeLa cells exposed to MLGPF for 48 hours. The scale bar of all images is 100 μm.

Cytotoxicity evaluation using the MTT method was conducted to assess the biocompatibility of ML-GPF on cervical cancer HeLa cells. The results presented in

Figure 3.29 indicate that ML-GPF does not induce cytotoxicity, with cell viability remaining above 80% at concentrations of up to 200 $\mu\text{g/mL}$.

The cell viability of HeLa cells exposed to free DOX and DOX-loaded MLGPF (DOX@MLGPF) for 48 hours demonstrated a concentration-dependent inhibition of cell growth (**Figure 3.30**). At concentrations of 0.1 and 1 $\mu\text{g/mL}$, there was no statistically significant difference in viability between cells treated with free DOX and those treated with DOX@MLGPF (**Figure 3.30a**). However, at concentrations of 0.25, 0.5, and 0.75 $\mu\text{g/mL}$, the viability of cells treated with DOX@MLGPF was lower than that of cells treated with free DOX. The IC_{50} value for DOX@MLGPF was 0.47 $\mu\text{g/mL}$, which is lower than that of free DOX at 0.67 $\mu\text{g/mL}$ (**Figure 3.30b**).

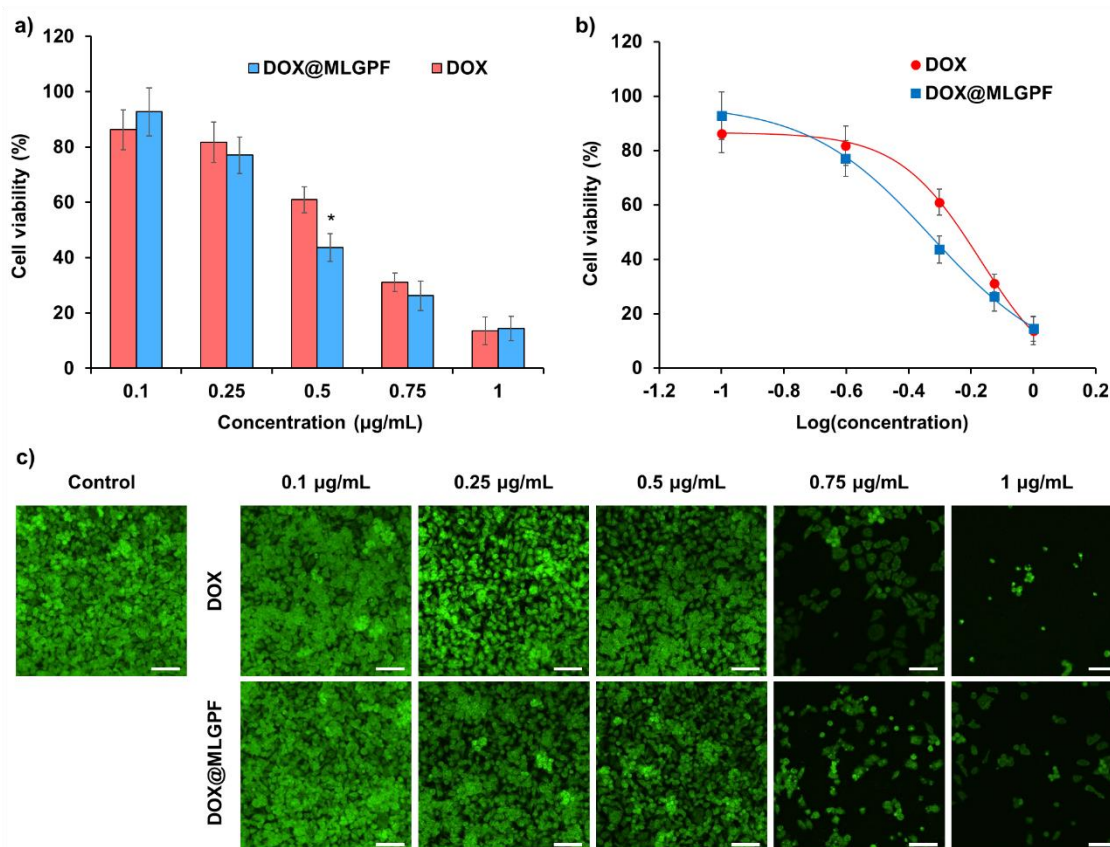


Figure 3.30. *In vitro* cellular viability (a), IC_{50} (b), and fluorescence image of HeLa cells exposed to free DOX and DOX-loaded MLGPF. The error bars in the graph represent standard deviations ($n = 3$). (*) $p \leq 0.05$. The scale bar of all images is 100 μm .

These results indicate that DOX@MLGPF exhibits greater cytotoxicity than free DOX, likely due to the higher affinity of MLGPF for cancer cells. This is consistent with the pharmacological action of DOX, which triggers apoptosis through

three primary mechanisms: (i) intercalating between nitrogenous base pairs to distort the double helix and inhibit replication/transcription; (ii) stabilizing the DNA-topoisomerase II complex to prevent DNA re-ligation, leading to double-strand breaks; and (iii) inducing the generation of reactive oxygen species (ROS), which causes lethal oxidative stress [145]. Since these mechanisms require DOX to internalize into the cell, the superior cellular uptake and adhesion promoted by MLGPF facilitate more efficient intracellular delivery of DOX, thereby enhancing its overall therapeutic efficacy.

When compared to other gelatin-modified or bioresponsive MSN systems reported in recent literature, DOX@MLGPF exhibits highly competitive biological activity. In a study by Zou et al. using the DOX/MSN@Gelatin system on Hep-G2 hepatoma cells, the reported IC₅₀ was 17.27 ± 0.63 $\mu\text{g/mL}$. Although this system offers pH-responsive drug release, its IC₅₀ is substantially higher than that of DOX@MLGPF, likely due to differences in cell lines and the lack of active targeting ligands like folic acid [146]. Zhang and Xu developed the DOX/MHGP (MSN@HA-gelatin-PEG) system targeting MDA-MB-231 breast cancer cells. Their data indicated that at a DOX concentration of approximately 1.56 $\mu\text{g/mL}$, cell viability remained around 40-45%, suggesting an IC₅₀ in the 1.3-1.5 $\mu\text{g/mL}$ range. This value is higher than the 0.47 $\mu\text{g/mL}$ of DOX@MLGPF, underscoring the efficient cellular internalization of the MLGPF carrier in HeLa cells [147]. The DOX@MSN-SS-KLA/BSA system by Zhang et al. was designed for synergistic therapy targeting the mitochondria and nucleus of HeLa cells. This study reported that at a carrier concentration of 40 $\mu\text{g/mL}$ (equivalent to approximately 2 $\mu\text{g/mL}$ DOX), HeLa cell viability decreased dramatically to ~8%. This indicates a very high potency that may be comparable to or exceed the efficiency of DOX@MLGPF depending on incubation time, facilitated by the synergistic effect between the KLA apoptotic peptide and DOX [148]. Xu et al. evaluated the DOX-MSNs@Gel system against human colon carcinoma cells, finding an IC₅₀ of 0.098 $\mu\text{mol/mL}$ (approximately 53.2 $\mu\text{g/mL}$). While this represented an improvement over free DOX (IC₅₀: 0.117 $\mu\text{mol/mL}$) in that specific study, the required concentration for 50% inhibition is notably higher than that observed for the DOX@MLGPF system [149].

The DOX@MLGPF system possesses an IC₅₀ (0.47 $\mu\text{g/mL}$) that is lower than most traditional gelatin-coated MSN systems or those relying solely on single-enzyme responsiveness. This high efficacy is attributed to precise targeting via folate receptors and integrins, which are typically overexpressed on cancer cell membranes,

thereby enhancing receptor-mediated endocytosis and intracellular drug delivery [138, 150, 151].

3.3.6. Cellular uptake

The cellular uptake of ML and MLGPF was evaluated on HeLa cells to assess their cell penetration ability and targeting efficiency. ML and MLGPF were labeled with the fluorescent agent DiO (DiO-ML and DiO-MLGPF, respectively) and incubated with the cells for 4 hours. The results presented in **Figure 3.31** indicate that both DiO-ML and DiO-MLGPF successfully penetrated the cells, resulting in green fluorescence [125]. Merged images demonstrated that the DiO-loaded nanocarriers infiltrated the entire cytoplasm surrounding the cell nucleus. The higher fluorescence intensity observed in cells exposed to MLGPF suggests that MLGPF is taken up by cancer cells more efficiently than ML. This enhanced uptake may be attributed to the affinity of GPF for folate receptors and integrins that are overexpressed on the membranes of cancer cells [138, 150, 151].

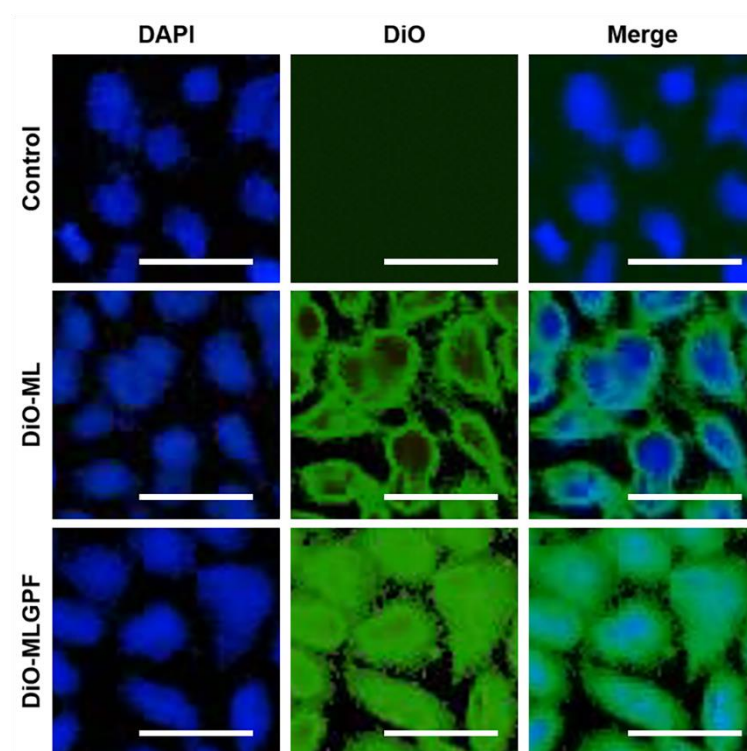


Figure 3.31. The fluorescence images of HeLa cells after exposed to DiO-ML and DiO-MLGPF for 4 hours. The scale bar of all images is 50 μm .

The findings regarding the enhanced cellular uptake of MLGPF in HeLa cells align with several studies investigating ligand-targeted gelatin nanocarriers. Singh et al. demonstrated that DOX-loaded folate-conjugated gelatin nanoparticles exhibited

higher uptake of DOX on HeLa cells compared to non-folated nanoparticles. The biodistribution of DOX in the lungs of Wistar rats was higher in conjugated nanoparticles as compared to unconjugated nanoparticles [152]. Similarly, Xia et al. demonstrated that FA functionalization on gelatin nanoparticles (GNPs) significantly enhances cellular internalization via receptor-mediated endocytosis. In MCF-7 cells, FA-modified GNPs exhibited significantly faster drug uptake and higher mean fluorescence intensity compared to non-targeted GNPs [153]. Furthermore, Wang et al. reported that folic acid-modified succinylated gelatin micelles showed significantly greater cellular uptake and cytotoxicity against receptor-positive cells than their unmodified counterparts [154]. These collective results corroborate the observations for MLGPF, confirming that the affinity of specific moieties for receptors overexpressed on cancer cell membranes, such as folate receptors and integrins, is a primary mechanism for increasing the penetration efficiency and targeting accuracy of nanocarriers.

3.4. Synthesis and evaluation of CTS-PEG-FA coated MSN-LP

3.4.1. Synthesis of CTS-PEG-FA

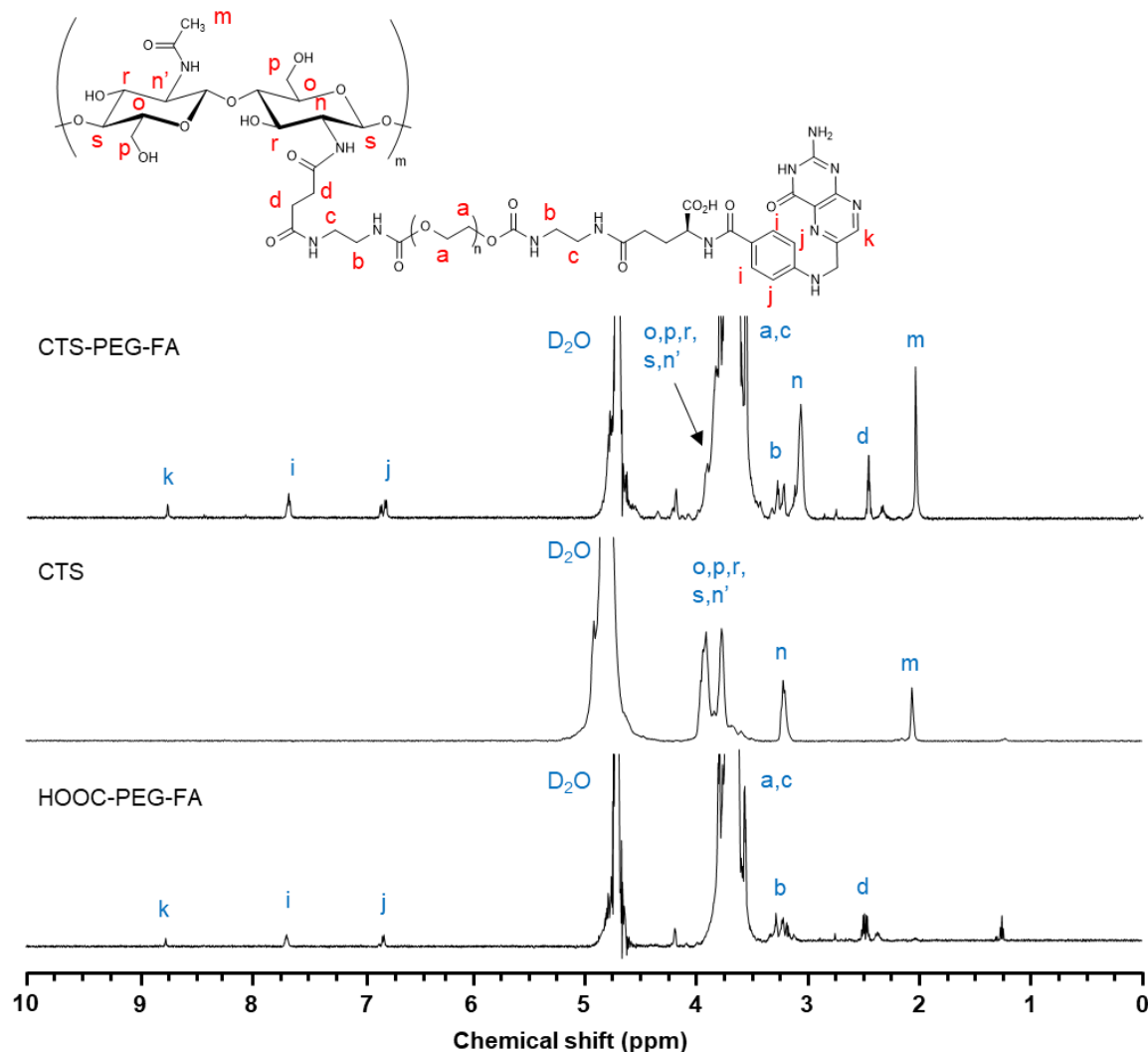


Figure 3.32. ^1H -NMR result of CTS, FA-PEG-COOH, and CTS-PEG-FA

The composition and chemical structure of CTS-PEG-FA were examined using a ^1H -NMR spectrum (500 MHz, D_2O , ppm) and shown in **Figure 3.32**. The ^1H -NMR spectrum of CTS-PEG-FA exhibits signals corresponding to the presence of CTS and FA-PEG-COOH, which are the active components. The distinctive signals of FA-PEG-COOH seen at $\delta\text{H} = 3.76$ ppm (a) correspond to the proton signals of the $-\text{OCH}_2\text{-CH}_2\text{-O}-$ group. The signals at $\delta\text{H} = 6.9$ ppm (j), 7.4 ppm (i), and 8.5 ppm (k) are indicative of FA signals on FA-PEG-COOH. The distinctive signals of CTS is $-\text{NH-CO-CH}_3$ (e) appeared at $\delta\text{H} = 2.12$ ppm (singlet), proton of $-\text{CH-NH}_2$ (f) at $\delta\text{H} = 3.12$ ppm (triplet), and protons (g,h,k,m,n) at $\delta\text{H} = 3.5\text{-}4.1$ ppm (multiplet) [51]. The signal of proton (a) is overlapping with the signals of protons (g, h, k, m, n), however the signal of (a) has a significantly higher intensity so it can still be determined. The

examination of the ^1H -NMR spectrum revealed the successful synthesis of CTS-PEG-FA [52, 53].

The successful synthesis of CPF and the stepwise formation of hybrid nanocarriers were further verified by FTIR spectroscopy (**Figure 3.35**). The FTIR spectrum of CPF revealed characteristic absorption bands corresponding to chitosan, PEG, and folic acid moieties. Specifically, symmetric and asymmetric stretching vibrations of $-\text{CH}_2$ groups were observed at 2853 and 2921 cm^{-1} , respectively. A strong band at 1638 cm^{-1} was attributed to the stretching vibration of carbonyl groups ($\text{C}=\text{O}$), while the broad absorption between 3200-3600 cm^{-1} and peaks at 3311 and 1412 cm^{-1} were assigned to O-H and N-H stretching vibrations. Additionally, the spectrum showed strong absorptions in the 1000-1120 cm^{-1} region, corresponding to C-O-C stretching vibrations from the PEG backbone. These features are consistent with previous findings, confirming the reproducible synthesis of CPF [155].

3.4.2. Synthesis of CTS-PEG-FA coated ML

3.4.2.1. Effect of CPF Content on Physicochemical Properties

The effect of CPF copolymer loading on the hydrodynamic diameter and surface charge of the hybrid nanocarriers was evaluated across various ML:CPF mass ratios (2:1, 1:1, and 1:2). As depicted in **Figure 3.33a**, the hydrodynamic diameter of MLCPF exhibited a subtle upward trend with increasing CPF mass fractions, yielding values of 236 nm (2:1), 249 nm (1:1), and 241 nm (1:2). This enlargement reflects the assembly of a denser, thicker polymeric envelope around the inorganic-organic hybrid core. These findings mirror prior reports, where thickening of the surface polymer shell systematically expands the effective hydrodynamic radius [125, 134, 156]. Concurrently, the zeta potential shifted toward increasingly positive values, progressing from +21.4 mV to +25.6 mV and +32.4 mV as the CPF proportion was raised (**Figure 3.33b**). This behavior accords with observations by Liao et al. (2021), who reported that functionalizing MSN with a CTS layer enlarged the hydrodynamic diameter from ~ 155 nm to ~ 199 nm, accompanied by a sharp polarity reversal from -25.0 mV (pristine MSN) to +26.4 mV owing to abundant protonated amine groups (which subsequently moderated to -1.66 mV following PEGylation) [157]. In the MLCPF platform, the pronounced surface charge escalation is driven by the dense accumulation of ionized primary amines along the chitosan backbone, alongside peripheral folate residues. These tuned interfacial properties confirm the successful

deposition of the copolymer shell while conferring an electrostatic advantage for binding negatively charged cell membranes [158].

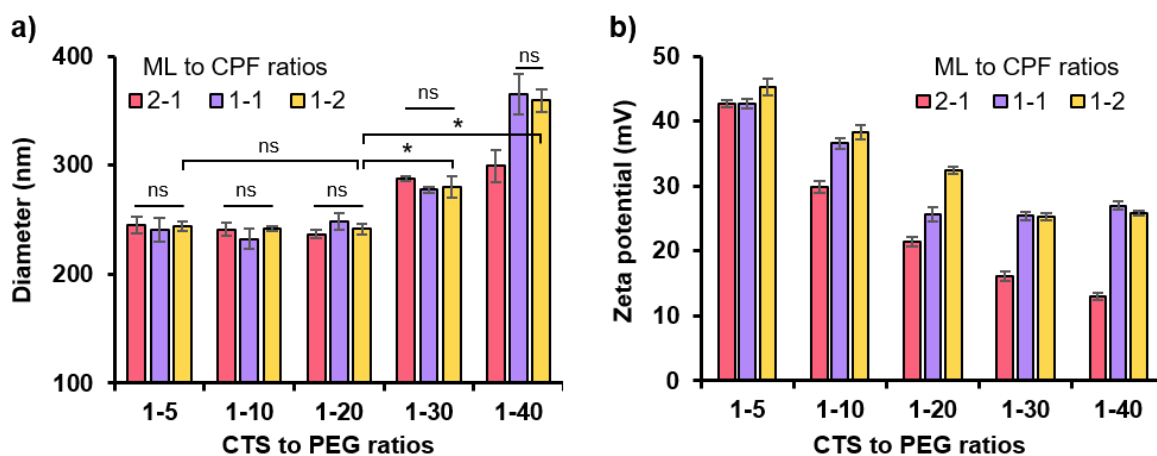


Figure 3.33. (a) Particle size and (b) zeta potential of MLCPF nanoparticles with different ML to CPF mass ratios (2:1, 1:1, and 1:2) and CTS to PEG molar ratios (1:5, 1:10, 1:20, 1:30, and 1:40). The error bars in the graph represent standard deviations ($n = 3$). (*): significant difference, (ns): no significant difference with $p \leq 0.05$.

3.4.2.2. Influence of CTS:PEG Molar Ratio in CPF Copolymer

To further refine surface properties, CPF copolymers were synthesized across a series of CTS-to-PEG molar ratios ranging from 1:5 to 1:40. As depicted in **Figure 3.33a**, the hydrodynamic diameter exhibited relative stability at lower PEG grafting densities (1:5 to 1:20); however, a pronounced dimensional increase was observed at higher PEG proportions (1:30 and 1:40), likely driven by steric repulsion and the spatial extension of flexible PEG chains outward into the aqueous phase. Conversely, the zeta potential displayed a continuous decline as the PEG mass fraction increased (**Figure 3.33b**). This attenuation in positive charge stems from the electrostatic shielding effect imparted by the neutral PEG coating, which effectively screens the underlying cationic primary amines of the chitosan backbone [157, 159]. Such PEG-induced surface passivation is well recognized for minimizing non-specific serum protein interactions and improving colloidal stability under physiological conditions, representing a highly desirable feature for systemic drug delivery platforms [159].

3.4.2.3. Colloidal Stability During Storage and in Biological Media

The long-term storage stability of MLCPF formulations was monitored over 7 and 30 days. As depicted in **Figure 3.34a**, CPF-coated systems featuring CTS-to-

PEG molar ratios from 1:5 to 1:20 maintained remarkably consistent hydrodynamic diameters, confirming superior colloidal resistance against degradation over extended periods. Conversely, formulations prepared with lower PEG ratios underwent gradual size expansion, signifying a tendency toward particle agglomeration. To evaluate physiological robustness, MLCPF nanocarriers were incubated in 50% fetal bovine serum (FBS) at 37 °C for 72 h. As shown in **Figure 3.34b**, CPF-functionalized particles exhibited significantly higher relative absorbance retention than untreated ML cores, indicating suppressed protein corona acquisition and mitigated protein-mediated aggregation. This enhanced biocompatibility is directly attributable to the PEG-rich polymeric envelope, which forms a dense interfacial hydration layer that shields the nanocarrier core from non-specific serum protein binding [131, 160]. In a related context, Zhang et al. (2016) demonstrated that PEG grafting reduced the positive zeta potential from +26.4 mV to +2.2 mV, underscoring how this protective shell minimizes undesired non-specific interactions with bio-components in systemic circulation [161]. Similarly, Claudia et al. (2020) highlighted that surface-anchored, highly hydrated PEG chains establish a steric repulsion barrier that prevents nanoparticle-protein opsonization and self-aggregation [160]. They further noted that PEGylated nanostructures often maintain smaller hydrodynamic sizes compared to unmodified chitosan formulations because the bulky, hydrated PEG domains impede higher-order particulate assembly.

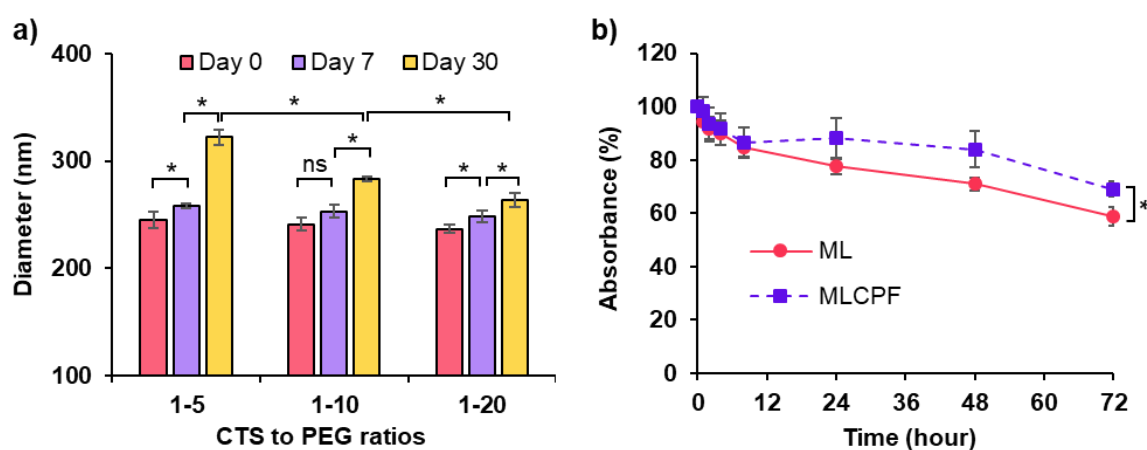


Figure 3.34. (a) Particle size of MLCPF nanoparticles with different CTS to PEG molar ratios (1:5, 1:10, and 1:20) after storage for 0 day, 7 days, and 30 days. (b) Stability of ML and MLCPF (1:20) in FBS. The error bars in the graph represent standard deviations ($n = 3$). (*): significant difference, (ns): no significant difference with $p \leq 0.05$.

Collectively, these findings confirm that surface modification with CPF, particularly at an optimized CTS-to-PEG ratio, endows the hybrid nanocarrier with favorable physicochemical properties for systemic nanomedicine, combining tailored dimensions, moderated surface potential, and exceptional stability across both prolonged storage and simulated biological environments [159, 161].

3.4.3. Physicochemical characterization

3.4.3.1. Chemical structure

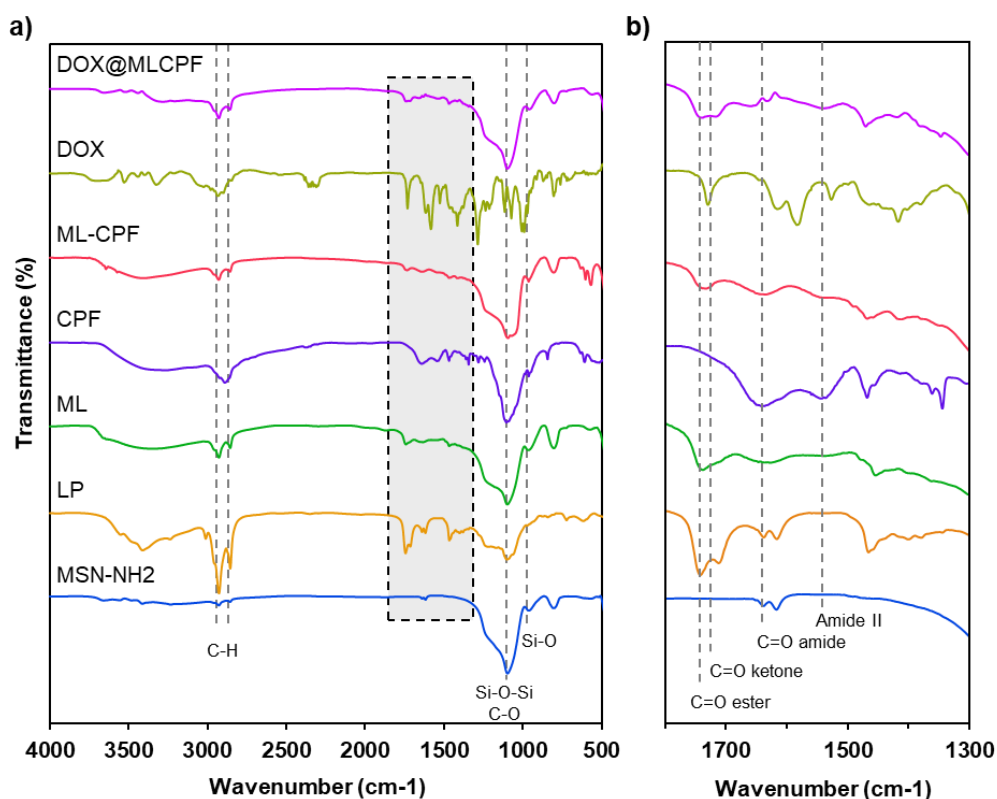


Figure 3.35. (a) FT-IR spectra of MSN-NH₂, LP, ML, CPF, MLCPF, free DOX, and DOX@MLCPF. (b) Zoomed spectra from 1300 cm⁻¹ to 1800 cm⁻¹

The structural features of the hybrid carriers and their precursors were also examined and showed in **Figure 3.35a**. The FTIR spectrum of ML (MSN coated with LP) exhibited characteristic peaks from both MSN-NH₂ and lipid components. Prominent peaks included the Si-O-Si asymmetric stretching vibration at 1096 cm⁻¹, Si-O-Si bending at 802 cm⁻¹, and Si-O stretching at 964 cm⁻¹. Signals at 1743 cm⁻¹ (C=O stretching of esters), 1634 cm⁻¹ (C=C), and 1467 cm⁻¹ (CH₂ wagging) were attributed to the lipid bilayer. The absorptions between 2890-2910 cm⁻¹ were assigned to C-H stretching. It is worth noting that certain peaks from the lipid bilayer, such as C-O stretching at 1096 cm⁻¹ and P=O stretching at 1234 cm⁻¹, overlapped with signals from the silica framework, which is typical for hybrid systems of this nature. Upon

coating ML with CPF to obtain MLCPF, the FTIR spectrum retained most characteristic bands from ML. However, in the magnified region of the spectrum (**Figure 3.35b**), a notable increase in the relative intensity of the peaks at 1631 cm^{-1} and 1734 cm^{-1} was observed. The enhancement of the 1631 cm^{-1} band, particularly, was attributed to the amide C=O stretching vibration introduced by the CPF layer. This increase serves as additional evidence for the successful surface functionalization of ML with the CPF copolymer. Importantly, the FTIR spectrum of DOX@MLCPF, in addition to the characteristic bands of MLCPF, exhibited a distinct absorption band at 1717 cm^{-1} corresponding to the C=O stretching of the ketone group in DOX. The appearance of this peak, albeit with relatively low intensity due to partial shielding of DOX molecules encapsulated within the mesopores, confirms the successful incorporation of DOX into the hybrid carrier. Similar spectral features have been reported in recent studies using FTIR to verify DOX loading into nanocarriers [58, 162].

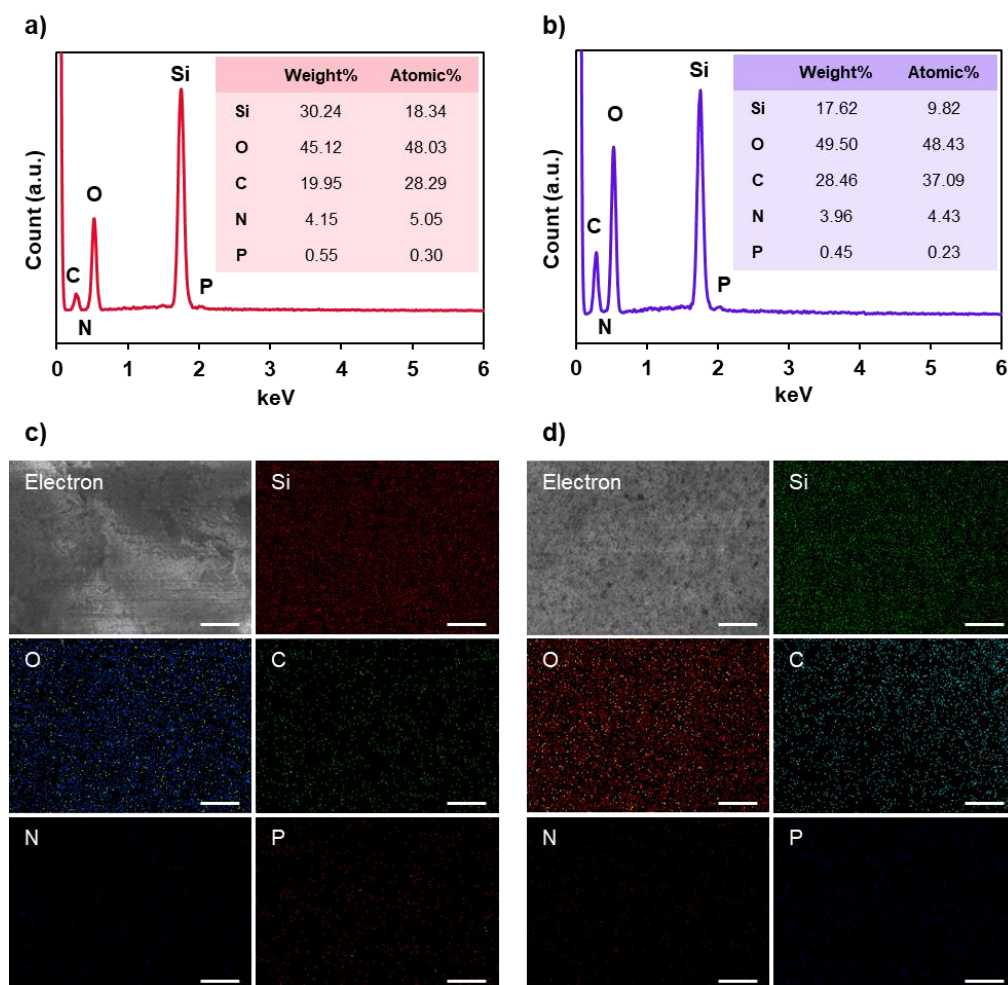


Figure 3.36. EDX results of ML (a) and MLCPF (b) with respective element mapping (c and d). The scale bar is 1 μm

To confirm the successful surface modification of ML with the CPF copolymer, energy-dispersive X-ray spectroscopy (EDX) was conducted to characterize the elemental profiles of ML and MLCPF (**Figure 3.36**). The resulting EDX spectra and quantitative elemental analysis demonstrated distinct compositional shifts between the two formulations. Specifically, for MLCPF, both the weight and atomic percentages of silicon (Si) decreased substantially relative to pristine ML, dropping from 30.24% to 17.62% (wt%) and from 18.34% to 9.82% (at%), respectively (**Figure 3.36a and b**). This signal attenuation of the inorganic Si core reflects the shielding effect of the outer organic CPF envelope, which partially attenuates X-ray emission from the underlying silica framework. Concurrently, the carbon (C) content rose significantly from 19.95 wt% and 28.29 at% in ML to 28.46 wt% and 37.09 at% in MLCPF, directly corresponding to the carbon-dense backbone of the CPF copolymer (comprising chitosan, PEG, and folic acid). The oxygen (O) mass fraction also exhibited a moderate increase, rising from 45.12 wt% in ML to 49.50 wt% in MLCPF, consistent with the oxygen-rich ether and amide/carboxyl linkages within the PEG and FA segments.

3.4.3.2. Thermal properties

TGA was conducted to characterize the thermal degradation behavior and quantify the organic fraction of the hybrid nanocarrier platforms. As illustrated in **Figure 3.37**, the TGA thermograms of both ML and MLCPF displayed a typical two-stage pyrolytic weight-loss profile. The initial mass loss phase, occurring between 50 °C and 150 °C, registered at approximately 5% for both formulations and corresponds to the evaporation of physisorbed water and bound moisture. The primary decomposition stage spanned from 150 °C to 800 °C, reflecting the thermal breakdown of the organic coatings anchored to the mesoporous silica core. Notably, MLCPF exhibited a significantly higher cumulative weight loss at 800 °C (41.6%) relative to ML (32.6%). This mass loss differential of ~9.0 wt% is directly attributable to the thermal degradation of the outer CPF copolymer coating (incorporating chitosan, PEG, and folic acid moieties), corresponding to an estimated CPF coating content of approximately 9.4% within the MLCPF system. These thermogravimetric data provide quantitative verification of successful CPF grafting onto the ML surface, corroborating the FTIR and EDX spectroscopic results. Furthermore, the TGA profile of the drug-loaded formulation, DOX@MLCPF, was integrated for comparison (**Figure 3.37**). DOX@MLCPF demonstrated a total cumulative weight loss of 45.2% at 800 °C, highlighted by a prominent derivative thermogravimetric (DTG)

decomposition peak centered at 536 °C, characteristic of the pyrolytic cleavage of DOX molecules. [162]. From the differential mass loss between MLCPF and DOX@MLCPF, the DOX loading was estimated to be ~5 wt%. This value is lower than the DOX content determined by UV-Vis spectroscopy (9.7 wt%), which may be attributed to the incomplete volatilization of DOX under TGA conditions, as part of the drug undergoes carbonization and remains as char residue in the crucible [163]. Indeed, pure DOX exhibits only ~55% weight loss at 700 °C [164], supporting this interpretation. Similar discrepancies between TGA- and spectroscopic-based drug content analyses have been reported in recent studies [165, 166].

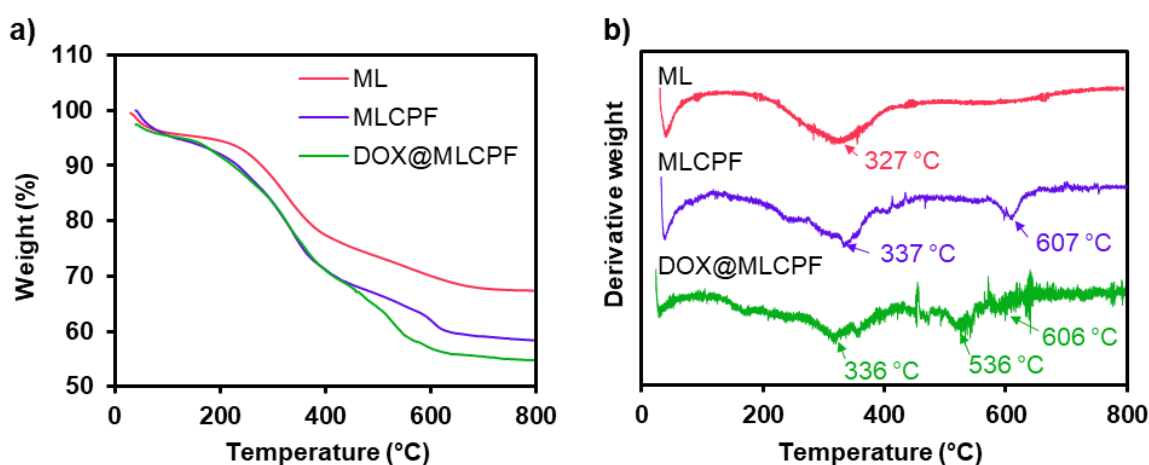


Figure 3.37. TGA thermograms (a) and derivative of weight to temperature calculated from TGA thermograms (b) of ML, MLCPF, and DOX@MLCPF

3.4.3.3. Morphology

The morphological features and structural architecture of the fully assembled hybrid nanocarriers were comprehensively examined via scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (**Figure 3.38a and b**). As depicted in **Figure 3.38c**, the MLCPF nanoparticles displayed a well-defined spherical morphology with a mean physical diameter of approximately 80 nm and a narrow size distribution. Notably, high-resolution TEM imaging clearly resolved a distinct outer polymeric shell with a uniform thickness of roughly 5-10 nm enveloping the particle surface (indicated by white arrows), corresponding to the deposited CPF copolymer layer. This well-delineated shell structure provides direct visual confirmation of successful CPF surface functionalization, fully supporting the spectroscopic and thermal findings from FTIR, EDX, and TGA analyses. The dimensional disparity observed between TEM characterization and DLS measurements, where DLS yields larger hydrodynamic diameters, is well-

documented for coated mesoporous silica systems, reflecting the fully hydrated, extended state of the surface-bound polymer and lipid layers in aqueous media [167]. These electron microscopy observations confirm the successful establishment of a distinct core-shell architecture, wherein the MSN-LP hybrid core is uniformly encapsulated within a functional CPF shell, a structural motif highly advantageous for maintaining colloidal stability and enabling stimuli-responsive drug release dynamics.

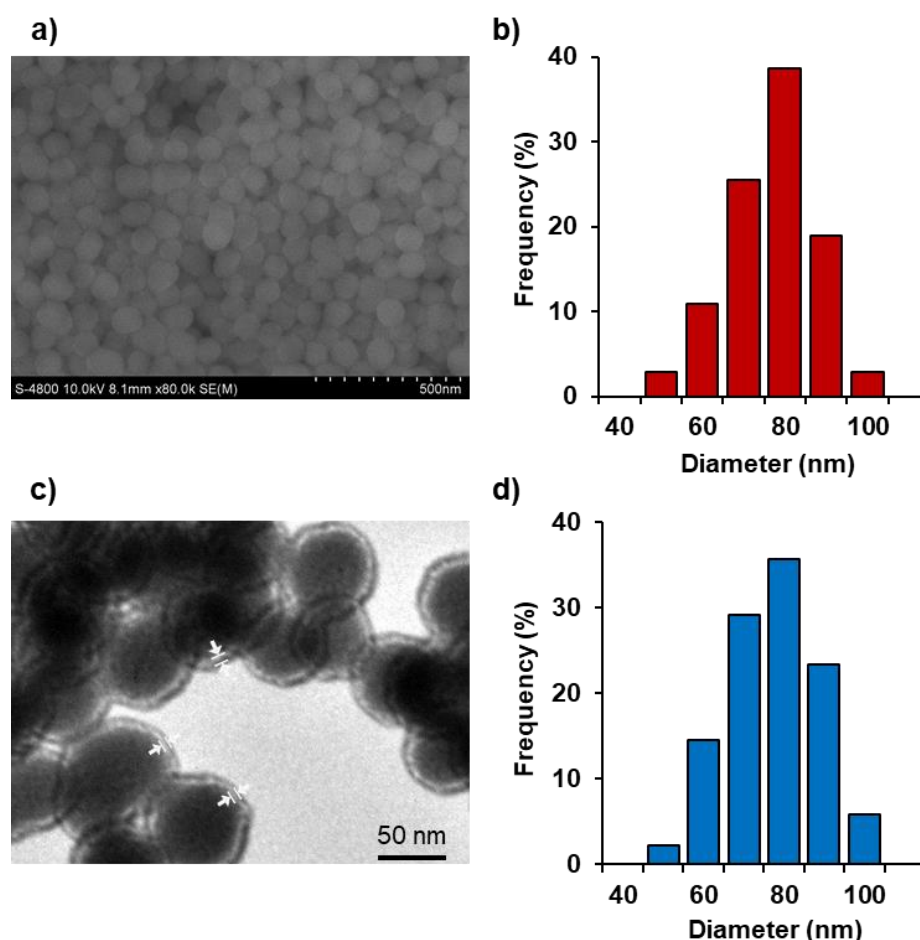


Figure 3.38. (a, b) SEM and (c, d) TEM images of MLCPF with respective statistical size distributions.

3.4.4. Drug loading and releasing

The drug encapsulation efficiency and release behavior of the fully integrated hybrid nanocarrier platform were evaluated using doxorubicin (DOX) as a model chemotherapeutic agent. The entrapment efficiency (EE) and loading capacity (LC) of DOX within MLCPF were quantified at 59.5% and 9.7%, respectively. These metrics demonstrate the high drug-accommodating capacity of the hybrid matrix, facilitated by the abundant internal volume of the mesoporous silica core alongside

favorable electrostatic and non-covalent interactions between DOX molecules and functional surface moieties. To assess the stimuli-responsive performance of the platform, the *in vitro* DOX release dynamics from MLCPF were monitored across two distinct physiological regimes: pH 7.4 (simulating systemic circulation and healthy tissues) and pH 5.5 (mimicking the acidic microenvironment of tumor endosomes and lysosomes). As depicted in **Figure 3.39a**, DOX@MLCPF displayed minimal premature leakage at pH 7.4, releasing only ~20% of its drug payload over 48 h. This sustained retention is primarily attributed to the structural barrier provided by the lipid bilayer and the outer CPF copolymer coating, which effectively restrict solvent penetration and minimize passive DOX diffusion. Conversely, exposure to acidic conditions (pH 5.5) triggered a pronounced acceleration in drug liberation, reaching approximately 26% within the first 6 h and progressing to roughly 48% at 48 h. This acid-stimulated burst behavior stems predominantly from the protonation of ionizable groups along the polymeric backbone, which induces matrix swelling and structural loosening via charge repulsion, coupled with the partial destabilization of the underlying lipid membrane under endo/lysosomal pH conditions [41]. This combination enhances matrix permeability, thereby accelerating outward drug diffusion. Similar protonation-induced destabilization of polymeric shells has been documented in related MSN-based architectures; for instance, PDMAEMA-grafted MSNs demonstrated minimal premature leakage at physiological pH while exhibiting dramatically accelerated drug liberation under acidic conditions due to shell protonation and matrix swelling [168]. Such pH-triggered release kinetics are highly advantageous for targeted cancer chemotherapy, as they suppress off-target drug exposure in normal tissues while selectively driving payload release within the acidic intracellular microenvironments of malignant cells [169]. Overall, these findings demonstrate that the MLCPF hybrid nanocarrier successfully couples high drug-loading efficiency with pH-responsive release dynamics, underscoring its promise as a stimuli-sensitive platform for controlled, site-specific tumor therapy.

To further elucidate the release mechanism of DOX from MLCPF, the *in vitro* drug release data were fitted to four commonly used kinetic models: zero-order, first-order, Higuchi, and Korsmeyer-Peppas. The coefficient of determination (R^2) values at pH 7.4 were found to be 0.6348, 0.6669, 0.8511, and 0.9601, respectively. At pH 5.5, the corresponding R^2 values were 0.7657, 0.8199, 0.9444, and 0.9832 (**Figure 3.39b**). The poor fit with zero-order and first-order models, particularly at pH 7.4,

supports the notion that drug release is not governed by simple constant-rate or concentration-dependent kinetics (**Figure 3.39c and d**).

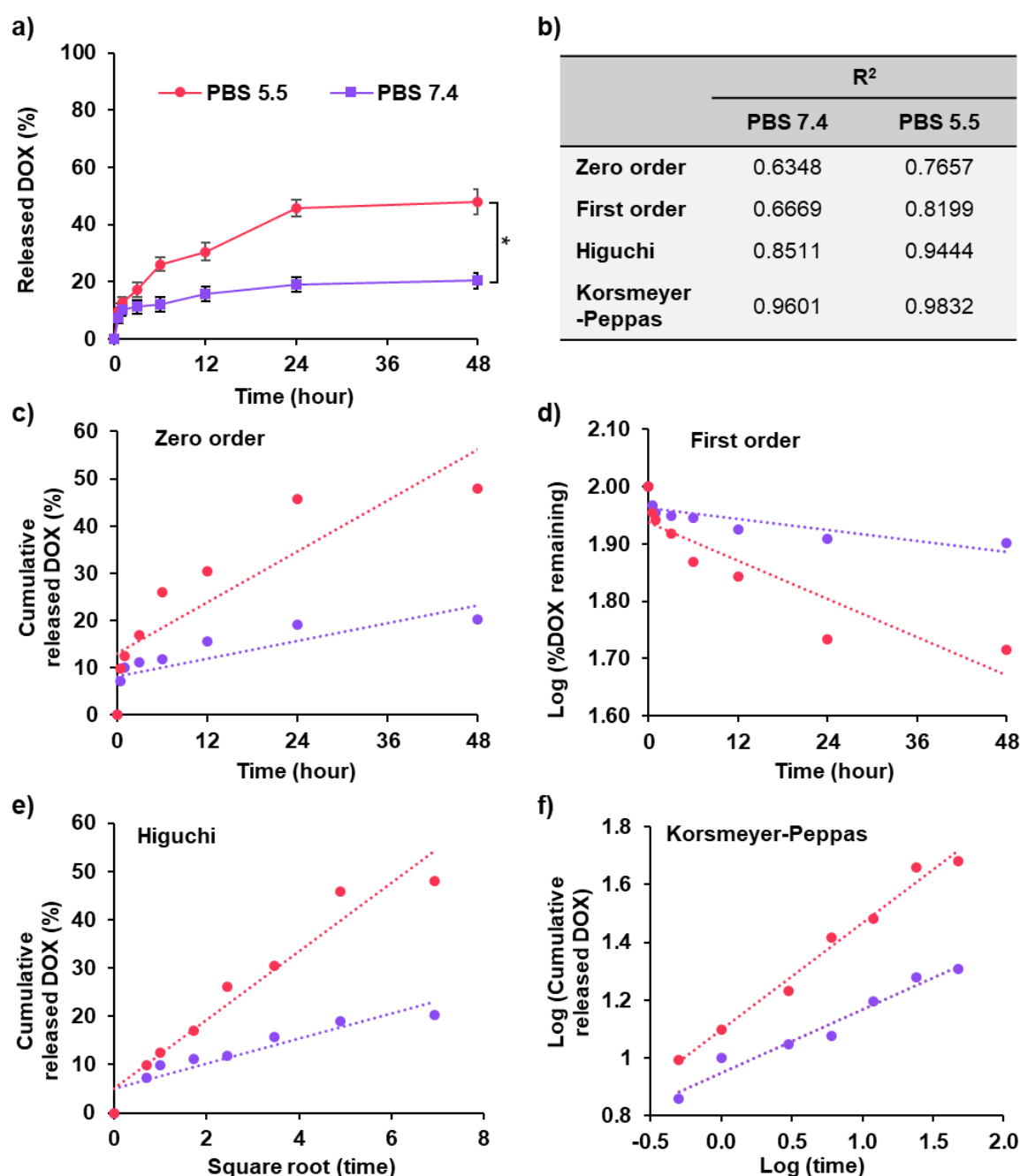


Figure 3.39. (a) *In vitro* DOX release profiles from MLCPF in PBS at pH 7.4 and pH 5.5. Cumulative release data fitted to kinetic models, including (c) zero-order, (d) first-order, (e) Higuchi, and (f) Korsmeyer-Peppas models. (b) Corresponding correlation coefficients (R^2) for model fitting.

Mathematical modeling of the *in vitro* release kinetics revealed that the Korsmeyer-Peppas model provided the highest correlation across both physiological and acidic conditions, indicating a release mechanism governed by coupled diffusion

and matrix relaxation, characteristic of non-Fickian anomalous transport (**Figure 3.39f**). This behavior underscores the synergistic role of the lipid membrane and the outer polymeric CPF shell in dictating a complex, multi-stage liberation process. Concurrently, the Higuchi model exhibited strong linearity, particularly at pH 5.5 ($R^2 = 0.9444$), confirming that mass transport through the porous, hydrated matrix remains a key driving component of the overall release profile (**Figure 3.39e**). To further elucidate the underlying mass transfer mechanism, the release exponent (n) derived from the Korsmeyer-Peppas equation was analyzed. The calculated n values were 0.2201 at pH 7.4 and 0.3692 at pH 5.5. For spherical geometry, an exponent $n < 0.45$ signifies a quasi-Fickian diffusion-dominated mechanism (**Figure 3.39f**) [170]. Consequently, DOX liberation from the MLCPF hybrid platform is primarily dictated by Fickian diffusion under both pH regimes, with accelerated transport kinetics observed at acidic pH. These empirical kinetic parameters directly reflect the structural setup of the nanocarrier: the mesoporous channels of the silica core promote core-to-surface diffusion, whereas the surrounding lipid bilayer and CPF polymer envelope serve as barrier coatings. The upward shift in n at pH 5.5 captures the protonation-induced swelling of the polymer shell alongside the partial structural perturbation of the lipid membrane in acidic media, which lowers transport resistance and accelerates outward drug flux. Collectively, these kinetic data confirm the pH-responsive, diffusion-controlled drug release behavior of the MLCPF platform, demonstrating its suitability for targeted delivery within acidic microenvironments [171, 172].

3.4.5. Cytotoxicity assay

The in vitro cytotoxicity and biocompatibility of the hybrid nanocarrier system were evaluated using the MTT assay on HeLa cervical cancer cells. As illustrated in **Figure 3.40**, blank MLCPF nanoparticles exhibited high biocompatibility across a broad concentration range (up to 200 $\mu\text{g/mL}$), maintaining cell viability above 80% after 48 h of exposure. A 48-hour exposure duration was selected to mirror the in vitro release timeline, which demonstrated sustained DOX release through this point, thereby facilitating a direct correlation between release kinetics and cellular safety profiles. These results confirm the low intrinsic toxicity of the individual nanocarrier building blocks, including the mesoporous silica core, the lipid membrane, and the outer CPF copolymer, underscoring their biocompatibility and potential for safe systemic drug delivery.

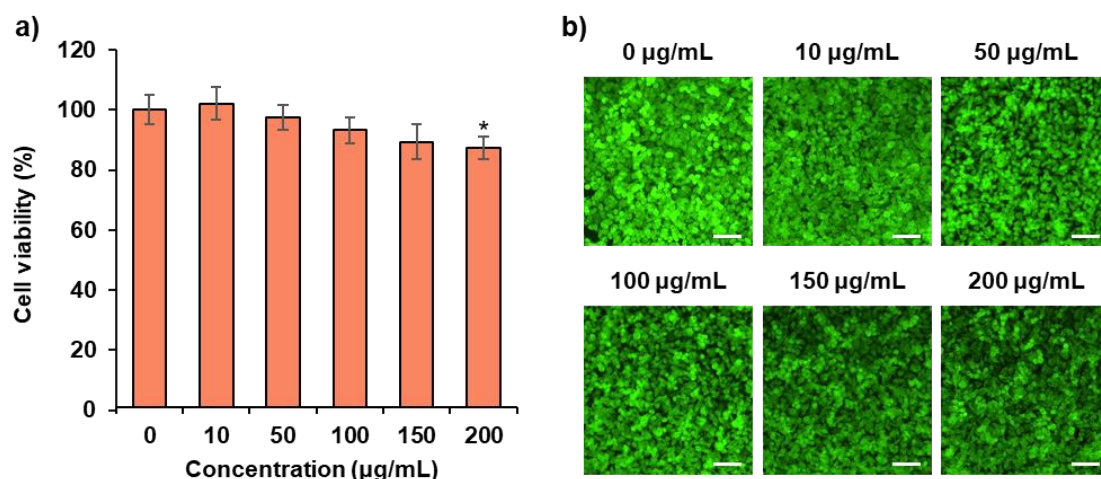


Figure 3.40. Cellular viability (a) and fluorescence image (b) of MLCPF testing on HeLa cells for 48 hours. The scale bar is 100 μm . The error bars in the graph represent standard deviations ($n = 3$). (*): significant difference, (ns): no significant difference with $p \leq 0.05$ when comparing to control.

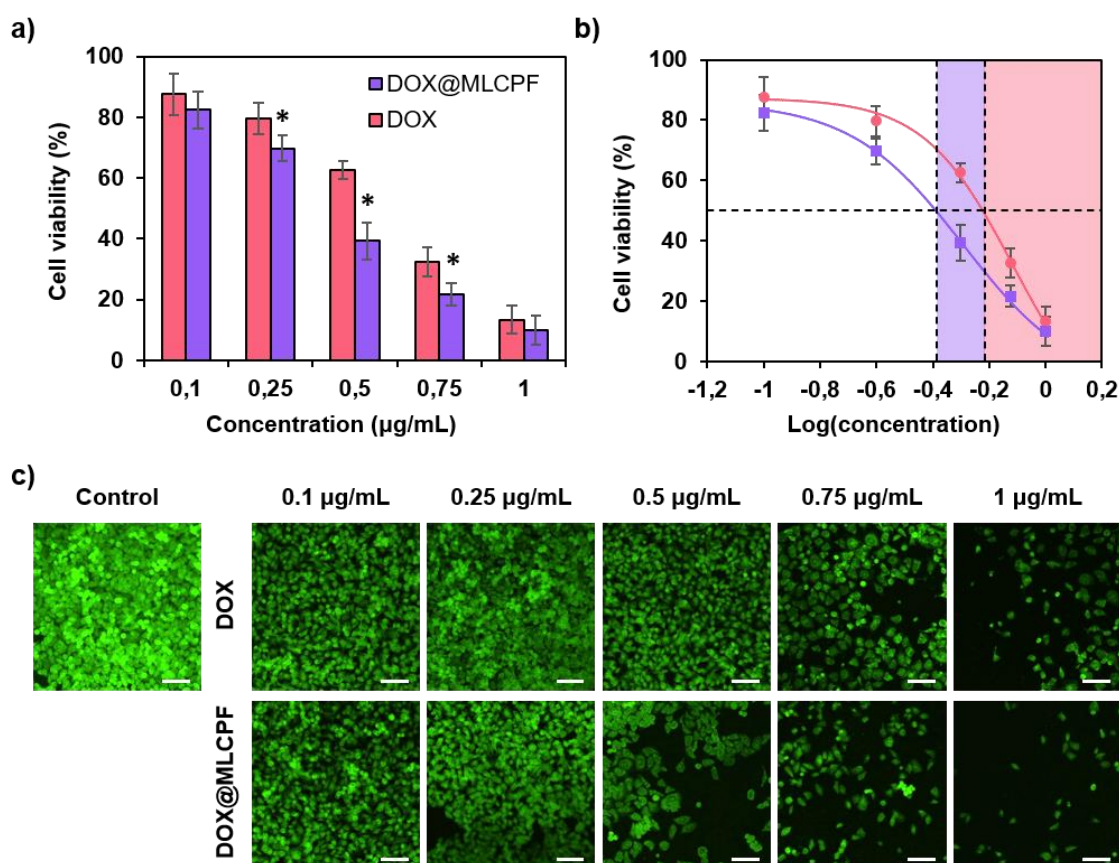


Figure 3.41. Cellular viability (a), IC_{50} (b), and fluorescence image of DOX and DOX@MLCPF testing on HeLa cells. The error bars in the graph represent standard deviations ($n = 3$). (*) significant difference with $p \leq 0.05$ when comparing to free DOX at the same concentration. The scale bar is 100 μm .

Conversely, both free DOX and DOX@MLCPF elicited marked, concentration-dependent antiproliferative effects (**Figure 3.41**). HeLa cell viability dropped sharply with increasing equivalent DOX concentrations (0.25-1.0 $\mu\text{g/mL}$), confirming efficient intracellular internalization and therapeutic potency across both formulations. Notably, within the lower dosage window (0.25-0.75 $\mu\text{g/mL}$), DOX@MLCPF demonstrated significantly higher cytotoxicity than free DOX ($p < 0.05$); however, at 1.0 $\mu\text{g/mL}$, the difference between the two treatments ceased to be statistically significant. The IC_{50} of DOX@MLCPF was calculated to be 0.410 $\mu\text{g/mL}$, representing a meaningful potency enhancement relative to free DOX ($\text{IC}_{50} = 0.599 \mu\text{g/mL}$). This cytotoxicity enhancement is primarily ascribed to augmented cellular uptake and sustained intracellular drug accumulation mediated by the hybrid nanocarrier. Specifically, the multifunctional CPF shell, enriched with cationic chitosan and targeting folic acid moieties, facilitates electrostatic and receptor-mediated engagement with HeLa cell membranes, thereby promoting rapid endocytosis followed by pH-triggered endo/lysosomal drug liberation.

Notably, DOX@MLCPF demonstrates superior therapeutic potency when compared with previously reported antibody-based delivery vectors [173]. In a study evaluating IgG-DOX, FA-IgG-DOX, and FA-PEG-IgG-DOX in HeLa cells, free DOX yielded an IC_{50} of 0.2 $\mu\text{g/mL}$, whereas IgG-DOX exhibited significantly compromised cytotoxicity ($\text{IC}_{50} \sim 23.9 \mu\text{g/mL}$). Although folate functionalization moderately restored antiproliferative activity with FA-PEG-IgG-DOX reaching an IC_{50} of 3.22 $\mu\text{g/mL}$, its potency remained substantially lower than that of DOX@MLCPF ($\text{IC}_{50} = 0.410 \mu\text{g/mL}$). This marked performance differential underscores the structural advantages of the MLCPF platform, which synergizes active folate-mediated targeting, pH-triggered release kinetics, and high-capacity non-covalent payload loading to circumvent key limitations of antibody-drug conjugates, such as steric hindrance and delayed intracellular liberation. Furthermore, comparative evaluation against cucurbituril-based nanocarriers (Dox@CB[7]NPs) reinforces the general efficacy of engineered nanoparticle platforms in overcoming systemic therapeutic barriers [174]. In that study, Dox@CB[7]NPs outperformed free DOX across multiple cancer cell lines (HeLa, MCF-7, A2780, and Dox-resistant A2780/AD). Notably, the IC_{50} of Dox@CB[7]NPs in multidrug-resistant A2780/AD cells ($\sim 0.6 \mu\text{M}$) was comparable to that observed in chemosensitive A2780 cells ($\sim 0.8 \mu\text{M}$), demonstrating the ability of nanocarriers to bypass P-glycoprotein-mediated efflux through alternative endocytic entry pathways. Additionally, Dox@CB[7]NPs

exhibited significantly attenuated cytotoxicity toward non-cancerous HEK293 cells, contrasting sharply with free DOX, which imparted severe off-target damage to healthy controls. Collectively, these comparative findings highlight that DOX@MLCPF not only achieves enhanced cytotoxicity against HeLa cancer cells relative to free drug and conventional antibody conjugates, but also shares fundamental nanomedicine advantages, including site-specific selectivity, efficient subcellular transport, and potential utility in overcoming multidrug resistance. These integrated attributes position the MLCPF platform as a promising candidate for targeted oncology applications.

3.4.6. Cellular uptake

To evaluate the cellular internalization efficiency of the nanocarrier system, ML and MLCPF platforms were labeled using the green lipophilic fluorophore DiO, which was incorporated into the ML matrix prior to CPF deposition, yielding DiO-ML and DiO-MLCPF formulations. HeLa cells were incubated with the labeled nanostructures for 4 h, counterstained with nuclear DAPI (blue), and imaged via fluorescence microscopy. As depicted in **Figure 3.42**, robust green fluorescent signals were detected within the cytoplasmic domain surrounding DAPI-stained nuclei, confirming effective intracellular accumulation of the labeled nanoparticles. Notably, the fluorescent signal from DiO-MLCPF was uniformly dispersed across the cytoplasm, signifying efficient trans-membrane penetration and cytosolic distribution rather than superficial membrane binding. Superimposed fluorescence images further illustrate that the majority of internalized nanoparticles concentrated in the perinuclear region, a spatial distribution pattern characteristic of endocytic cellular entry followed by intracellular trafficking toward endo-lysosomal pathways. The augmented cellular uptake observed for MLCPF can be attributed to a combination of distinct factors: (i) the functional presentation of folic acid moieties within the CPF shell, which potentially promotes specific interactions with overexpressed folate receptors on HeLa cells (though this mechanism remains hypothetical in the absence of receptor-blocking assays); and (ii) optimized physicochemical attributes, notably the nanoscale hydrodynamic diameter (~250 nm) and positive surface potential, which drive favorable electrostatic interactions with the negatively charged plasma membrane to facilitate endocytosis. These findings align with reports on FA-PEG-CS-coated Fe₃O₄ nanoparticles, which exhibited markedly enhanced intracellular fluorescence in HeLa cells relative to non-targeted CS- or mPEG-CS-coated controls, confirming that folate functionalization

significantly bolsters uptake efficiency in folate receptor-positive malignancies [175]. Overall, these results demonstrate that CPF surface engineering enhances nanoparticle–cell interactions, although the exact extent of receptor-mediated versus charge-driven endocytosis warrants further mechanistic validation.

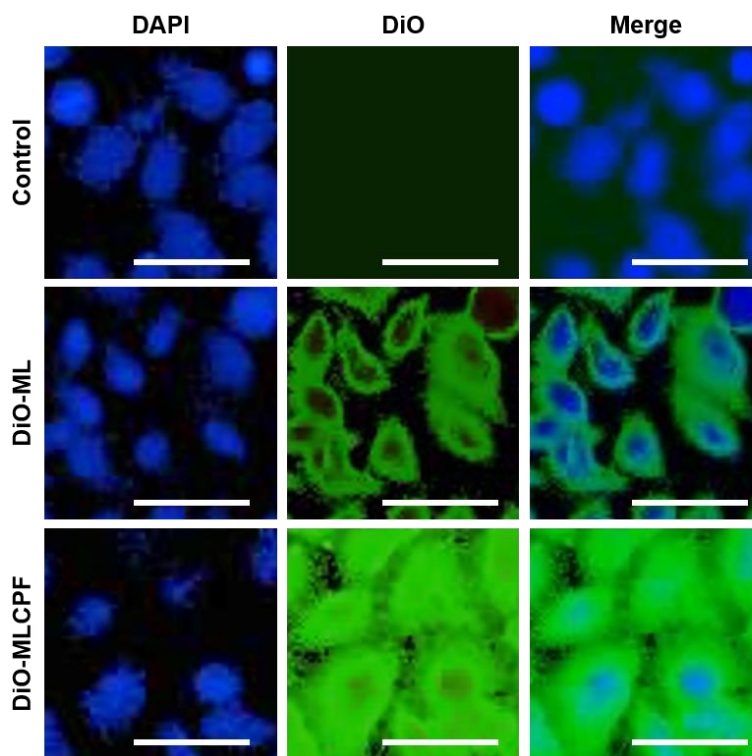


Figure 3.42. Fluorescence microscopy images of HeLa cells after 4 h incubation with free-DiO (Control), Dio-labeled ML, and DiO-labeled MLCPPF nanoparticles. Cell nuclei were stained with DAPI (blue), while DiO was incorporated into ML of the nanocarrier (green). The merged image indicates effective cytoplasmic uptake and intracellular distribution. Scale bar = 50 μm .

3.5. Discussion

3.5.1. Physicochemical properties of ML-based nanoplateforms

The particle size and zeta potential of the ML-based nanoplateforms, including ML, MLBF, MLGPF, and MLCPF, are presented in **Figure 3.43**. The results demonstrate that the surface functionalization process significantly altered both the hydrodynamic diameter and the zeta potential of the systems.

Regarding particle size, the ML system initially exhibited a diameter of 178 nm. Upon functionalization, the MLBF system showed a slight increase to 191.8 nm, which is attributed to the anchoring of Brij-FA molecules characterized by their short and flexible chain structures. In contrast, the particle sizes of MLGPF and MLCPF increased substantially to 218.6 nm and 236.7 nm, respectively. This growth can be explained by the macromolecular nature of gelatin and chitosan, combined with a high degree of PEGylation that creates a thick, bulky hydration shell, thereby increasing the hydrodynamic particle size. Consequently, the particle size increased following the order of $ML < MLBF < MLGPF < MLCPF$, correlating with the structural complexity and molecular weight of the functionalizing agents employed in each strategy. Notably, all developed nanocarriers possessed sizes within the optimal range (50–300 nm) for anticancer drug delivery applications.

The ML system, featuring a lipid bilayer (LP) coating, possessed a strong negative surface charge of -38.2 mV, providing the electrostatic repulsion necessary for a stable dispersed state. Following functionalization with non-ionic Brij-FA and amphoteric GPF, the negative zeta potential values diminished to -24.4 mV and -29.2 mV, respectively. This shift is a direct consequence of the masking effect exerted by the neutral PEG polymer layer. Despite the reduction in zeta potential magnitude, the PEG coating enhances the stability of the dispersion through steric effects. Most notably, a charge reversal from negative to positive was observed in the MLCPF system (+21.4 mV). This phenomenon confirms that the CPF, rich in positively charged amine groups, strongly adsorbed onto and completely encapsulated the negative surface of the lipid bilayer. This positive zeta potential not only confirms the successful coating but also serves as a strategic advantage; it enhances the electrostatic affinity of the MLCPF nanoparticles toward negatively charged cell membranes, thereby promoting cellular internalization and improving therapeutic efficacy.

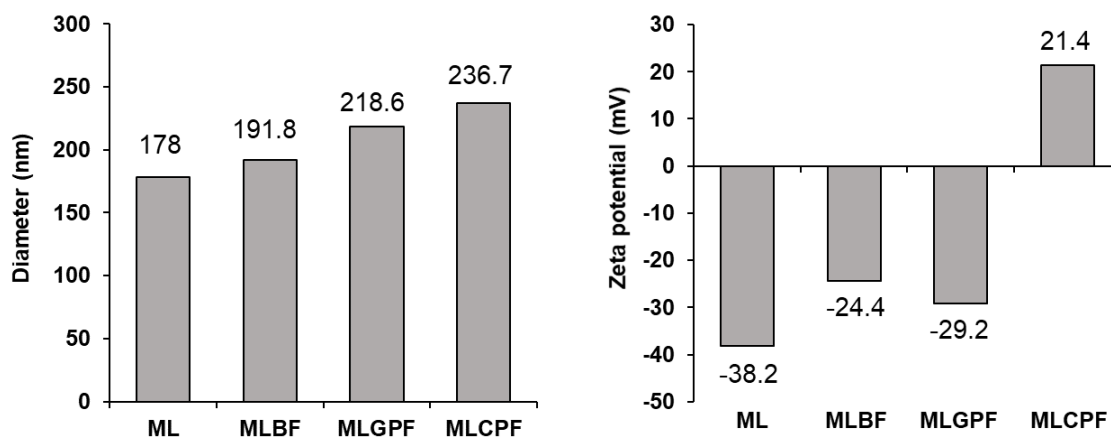


Figure 3.43. Particle size (a) and zeta potential (b) of ML, MLBF, MLGPF, and MLCPF

The summary of the weight loss percentage (%) for MSN-NH₂, ML, MLBF, MLGPF, and MLCPF, as determined by TGA at 800 °C, is presented in **Figure 3.44**. After excluding approximately 5.4% of the weight loss attributed to the evaporation of adsorbed moisture during the initial stage (up to 200 °C), the actual weight loss of MSN-NH₂ was found to be 7.6%, representing the surface amine functional groups. This value increased significantly to 26.1% for the ML, confirming the successful encapsulation by the lipid bilayer with an additional organic content of 18.5%. The weight loss of the MLBF showed a slight increase to 28.8% due to the presence of Brij. Meanwhile, the MLGPF and MLCPF exhibited actual weight loss levels of 33.7% and 36.2%, respectively (corresponding to an increase of 7.6% and 10.1% compared to the ML). These results provide a compelling argument for the presence of GPF and CPF macromolecules coating the particle surfaces. The upward trend in weight loss following the order of MSN-NH₂ < ML < MLBF < MLGPF < MLCPF is highly consistent with the particle size data and confirms the successful establishment of the multifunctional multilayered nanostructures.

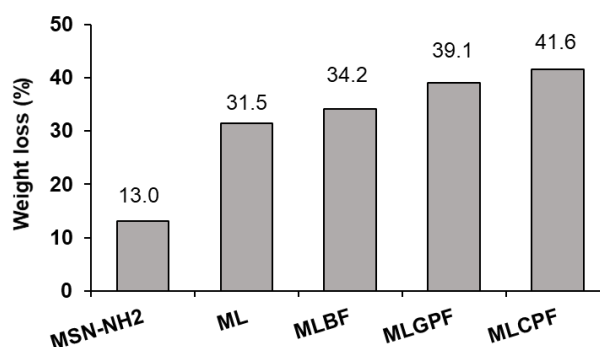


Figure 3.44. Weight loss (%) of MSN-NH₂, ML, MLBF, MLGPF, and MLCPF determined by TGA at 800 °C

3.5.2. Drug loading and releasing profiles of ML-based nanoplatforms

The drug loading and release profiles of the ML-based nanoplatforms are illustrated in **Figure 3.45**. The results indicate that the ML system achieves the highest encapsulation efficiency (EE) and loading content (LC), at approximately 60% and 11%, respectively. This superior performance is attributed to the lipid bilayer directly coating the MSN surface, which acts as a physical barrier preventing the back-diffusion of DOX from the pores. In contrast, the polymer-functionalized systems (MLBF, MLGPF, and MLCPF) show a slight decrease in EE to 52-58%, likely due to the multi-step modification processes and centrifugal washing cycles that cause the loss of weakly bound drug molecules near the pore openings.

The drug release profiles demonstrate that all hybrid systems exhibit effective pH-responsive control. At pH 7.4, these systems retain the drug efficiently with leakage rates of only 23-26%, significantly lower than the 46% observed for MSN-NH₂, thereby minimizing off-target toxicity to healthy tissues.

In an acidic environment at pH 5.5, the release rate escalates to approximately 50-60%. This phenomenon is explained by pH-induced structural alterations in the lipid bilayer, leading to the formation of blebs and pores that facilitate the outward diffusion of DOX. Among the polymer-functionalized materials, MLCPF possesses the thickest polymer coating yet exhibits the highest release rate. This behavior can be attributed to the protonation of amine functional groups, which triggers the swelling of the CPF shell and allows the drug to be released more readily.

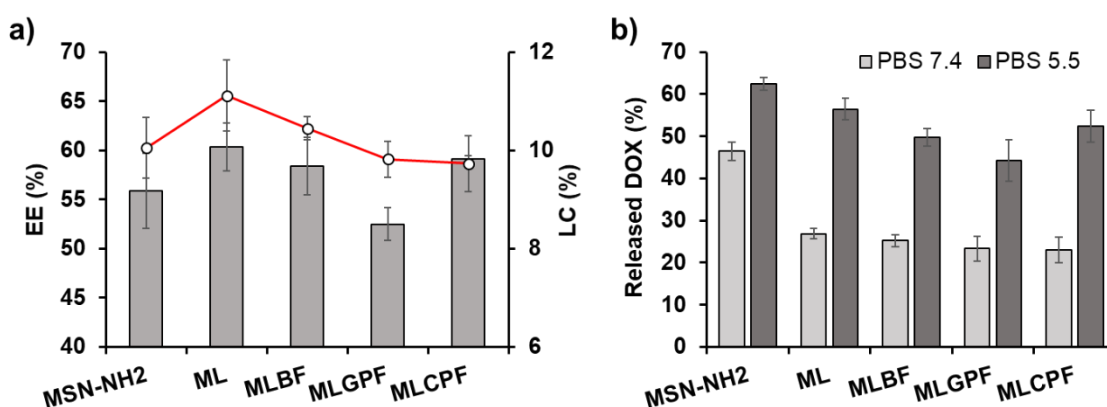


Figure 3.45. Drug loading content (LC) and encapsulation efficiency (EE) (a), and *in vitro* drug release profiles (b) of DOX-loaded nanoplatforms in PBS at pH 7.4 and pH 5.5.

3.5.3. Cytotoxicity

Kết quả khảo sát tính tương hợp sinh học của các hệ ML-based nanoplatfroms được thể hiện trong **Figure 3.46**. Kết quả cho thấy tất cả vật liệu thử nghiệm đều có tính tương thích sinh học cao, với tỉ lệ tế bào sống đạt trên 80% trong khoảng nồng độ khảo sát (10-200 $\mu\text{g/mL}$).

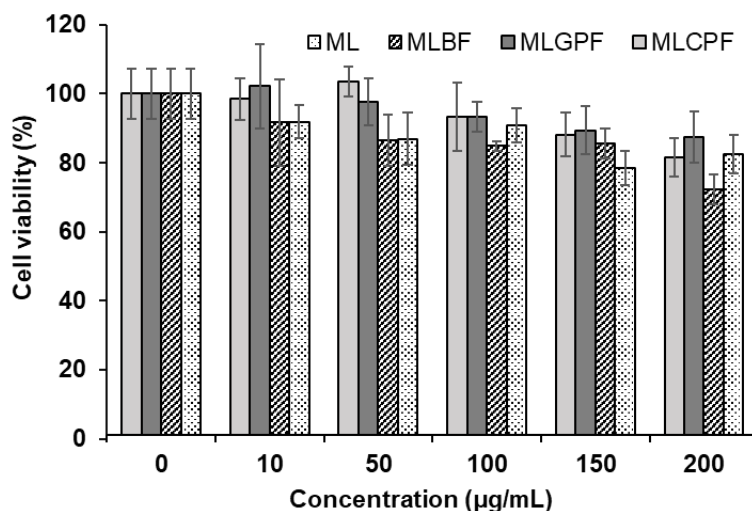


Figure 3.46. Cytotoxicity of ML, MLBF, MLGPF, and MLCPF against the HeLa cell line after 48 hours of incubation.

The comparison of cytotoxicity between free DOX and DOX-loaded ML-based nanoplatfroms is illustrated in **Figure 3.47**. The results demonstrate that the cytotoxicity of all systems is concentration-dependent; however, the variations in IC_{50} values (**Figure 3.47b**) reveal significant differences in their anticancer efficacy. Although DOX@MLBF exhibits lower toxicity compared to free DOX, with an IC_{50} of 0.625 $\mu\text{g/mL}$ versus 0.585 $\mu\text{g/mL}$, it remains more potent than DOX@ML (IC_{50} = 0.738 $\mu\text{g/mL}$). This enhanced performance can be attributed to the FA groups on MLBF, which facilitate stronger binding to the cell membrane and improve cellular uptake via receptor-mediated pathways. In contrast, DOX@MLGPF and DOX@MLCPF display superior cell-killing capabilities compared to free DOX, with IC_{50} values of 0.450 $\mu\text{g/mL}$ and 0.410 $\mu\text{g/mL}$, respectively. This heightened efficacy is likely due to the synergistic effects of the GPF and CPF coatings. Beyond the targeting effect of FA, the GPF coating contains RGD peptide sequences that enable specific adhesion to cancer cells by binding with integrin receptors. Meanwhile, MLCPF possesses a positive surface charge, allowing for more effective electrostatic adhesion to the negatively charged cancer cell membranes. These findings confirm that surface modification of the ML hybrid nanoplatfroms, particularly with CPF, not only enhances nanoparticle stability but also optimizes localized drug concentration

at the target site, significantly increasing therapeutic efficacy compared to traditional free drug therapy.

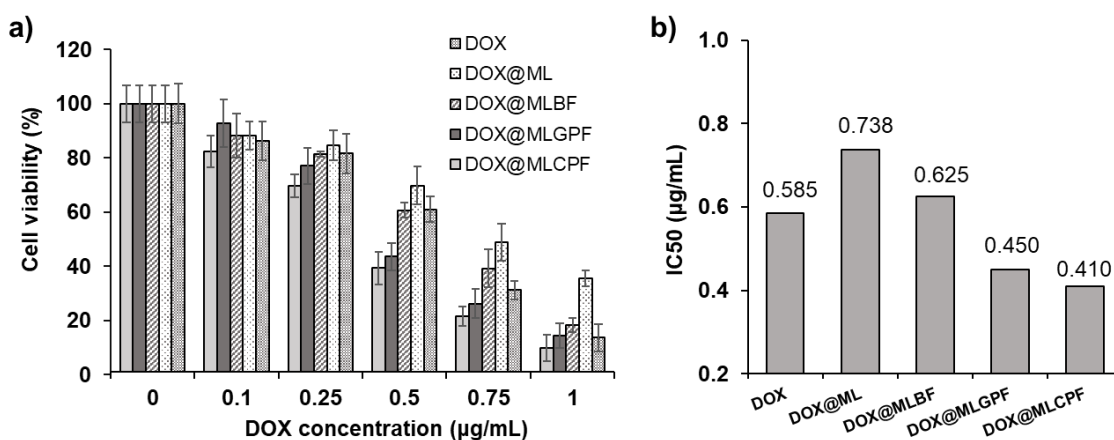


Figure 3.47. Cytotoxicity of HeLa cells exposed to free DOX and DOX-loaded nanoplatforms at various concentrations (a). The corresponding IC₅₀ values (b) of the tested systems after 48 hours of incubation.

3.5.4. Comparison study

A comparative summary highlighting the structural and functional attributes of the MLBF, MLGPF, and MLCPF platforms relative to selected analogous nanocarriers is presented in **Table 3.2**. Compared to previously reported MSN/lipid hybrid delivery systems, the MLBF, MLGPF, and MLCPF constructs display highly advantageous physicochemical and biological properties for targeted nanomedicine applications. Possessing hydrodynamic diameters of 191.8–236.7 nm with core MSN dimensions of 60–90 nm, these materials lie well within the optimal size regime (50–300 nm) required to prevent rapid renal clearance while enhancing passive tumor accumulation through the EPR effect [176]. Crucially, MLBF, MLGPF, and MLCPF exhibit favorable DOX loading capacities (LC) of 10.45%, 9.82%, and 9.70%, respectively, markedly exceeding those of surface-functionalized counterparts such as MSN@LP-PEG (0.27%) and MSN@LP-HA (3.17%) [58, 177]. Regarding folate-targeted systems, Li et al. (2020) reported an MSN@Liposome-PEG-FA platform designed for the dual delivery of paclitaxel (PTX) and tanshinone IIA (TanIIA); nevertheless, that system suffered from poor encapsulation efficiencies (1.8% for PTX and 5.5% for TanIIA) along with rapid release kinetics, losing nearly 90% of both payloads within 8 h [82].

The enhanced cytotoxic efficacy of MLBF, MLGPF, and MLCPF against HeLa cancer cells is equally compelling. These formulations demonstrate exceptionally low

IC₅₀ values (0.41–0.62 µg/mL), reflecting a pronounced increase in therapeutic potency compared to established DOX-loaded nanocarriers such as MSN@LP-PEG (16.87 µg/mL in MCF7 cells) and MSN@LP-HA (3.37 µg/mL in MCF7 cells and 6.50 µg/mL in drug-resistant MCF7/ADR cells) [58, 177]. This superior antitumor performance is predominantly driven by the synergistic integration of tumor microenvironment pH-triggered payload release and folic acid (FA)-mediated active targeting, which accelerates receptor-mediated endocytosis in cancer cells overexpressing folate receptors.

Table 3.2. Comparison of DOX-loaded MLBF, MLGPF, and MLCPF for cancer treatment

Carrier	Particle size	Drug	EE / LC	Drug release behavior	Cell line	IC50	Ref.
MSN@LP-PEG (DSPE-PEG2000)	167 ± 42 nm PDI = 0.31 (Core MSN: 65 nm)	DOX	EE: 67.53% LC: 0.27%	pH responsive	MCF7	16.87 µg/mL	Yang et.al. 2019 [58]
MSN@LP	Core MSN: 80-100 nm	DOX	EE: 42.6% LC: 33.86%	pH responsive	SKBR-3	~7 µg/mL (48h)	Amin et.al. 2021 [88]
MSN@LP-PEG (DSPE-mPEG2000)	174.5 ± 2.41 nm (Core MSN: 145.5 nm)	DTX TMX	DTX: + EE: 86.93% + LC: 1.86% TMX: + EE: 85.91% + LC: 3.8%	pH responsive	4T1 MCF7 A549	1.55 µM (4T1) 1.34 µM (MCF7) 1.65 µM (A549)	Wang et.al. 2024 [178]
MSN@LP-HA	213.3 nm (Core MSN: 120.2 nm)	DOX	EE: 92.98% LC: 3.17%	pH responsive	MCF7 MCF7/ADR	3.37 µg/mL (MCF7) 6.50 µg/mL (MCF7/ADR)	Yang et.al. 2024 [177]

Carrier	Particle size	Drug	EE / LC	Drug release behavior	Cell line	IC50	Ref.
MSN@LP-Lf	102.7 nm (Core MSN: 90 nm)	LEV CUR	LEV: + EE: 10.55% + LC: 21.1% CUR: + EE: 98.09% + LC: 1.96%	pH responsive	---	---	Guo et.al. 2025 [179]
MSN@LP-Brij-FA (MLBF)	191.8 nm (Core MSN: 60-90 nm)	DOX	EE: 58.4% LC: 10.45%	pH responsive	HeLa	0.62 µg/mL	This work
MSN@LP-GLT-PEG-FA (MLGPF)	218.6 nm (Core MSN: 60-90 nm)	DOX	EE: 52.5% LC: 9.82%	pH responsive	HeLa	0.47 µg/mL	This work
MSN@LP-CTS-PEG-FA (MLCPF)	236.7 nm (Core MSN: 60-90 nm)	DOX	EE: 59.5% LC: 9.7%	pH responsive	HeLa	0.41 µg/mL	This work

Where LP: liposome, HA: hyaluronic acid, Lf: lactoferin, DTX: docetaxel, TMX: tamoxifen, LEV: levodopa, CUR: curcumin

CONCLUSIONS

1. MSN-Liposome hybrid nanoparticles (ML) were successfully synthesized from MSN-NH₂ and LP via an ultrasound-assisted lipid self-assembly process, resulting in a hydrodynamic diameter of 178 nm and a zeta potential of -38.2 mV. Polymer-coated ML formulations were also successfully prepared. Brij-FA coated ML (MLBF) displayed a hydrodynamic diameter of 191.8 ± 3.2 nm and a zeta potential of -24.4 mV. ML coated with GLT-PEG-FA (MLGPF) showed a particle size of 218.6 nm with a zeta potential of -29.2 mV, whereas CTS-PEG-FA coated ML (MLCPF) exhibited a larger size of 236.7 nm and a zeta potential of 21.4 mV. Notably, all developed nanocarriers possessed sizes within the optimal range (50-300 nm) for anticancer drug delivery applications.
2. Both ML and polymer-coated ML formulations exhibited pH-responsive controlled drug release behavior. Under simulated physiological conditions (pH 7.4), the cumulative DOX release from DOX-loaded ML, MLBF, MLGPF, and MLCPF remained below 30% after 72 h, with no significant differences observed among the formulations. In contrast, under simulated tumor microenvironment conditions (pH 5.5), a pronounced increase in DOX release was observed for all nanocarriers, with cumulative release after 72 h following the order: ML ($56.4 \pm 2.6\%$) > MLCPF ($52.4 \pm 3.8\%$) > MLBF ($49.7 \pm 2.1\%$) > MLGPF ($44.29 \pm 4.9\%$).
3. ML, MLBF, MLGPF, and MLCPF nanomaterials all exhibited high biocompatibility, maintaining cell viabilities above 80% upon exposure to nanocarrier concentrations of up to 200 µg/mL. Upon DOX loading, DOX@MLBF displayed cytotoxicity statistically indistinguishable from that of free DOX at equivalent concentrations, indicating that the anticancer activity of DOX was well preserved following encapsulation. In contrast, both DOX@MLGPF and DOX@MLCPF demonstrated significantly enhanced cytotoxicity compared with free DOX, with lower IC₅₀ values (0.47 and 0.41 µg/mL, respectively, versus 0.59 µg/mL for free DOX).
4. Fluorescence imaging revealed efficient intracellular delivery, with DiO-MLBF, DiO-MLGPF, and DiO-MLCPF exhibiting substantially higher intracellular fluorescence intensity compared with DiO-ML. This increased cellular uptake is consistent with the presence of folic acid moieties on the nanoparticle surface. Taken together, these findings support MLBF, MLGPF, and MLCPF as promising tumor-targeted nanocarrier platforms.

5. Overall, MLBF, MLGPF, and MLCPF represent promising platforms for anticancer drug delivery, exhibiting high colloidal stability, pH-responsive controlled drug release, and enhanced tumor cell uptake. Notably, among these formulations, MLCPF demonstrated superior DOX release and higher cytotoxicity toward HeLa cells compared with the other nanocarriers.

FUTURE PERSPECTIVES

1. One limitation of the present study is the lack of in vivo evaluation, including systemic toxicity, pharmacokinetics, tissue distribution, clearance time, and antitumor efficacy. Therefore, future investigations should focus on comprehensively assessing these parameters in appropriate animal models to further elucidate the therapeutic potential and translational applicability of the developed nanocarriers.
2. Another drawback of the current nanocarrier systems is that the cumulative drug release reached only slightly above 50%, indicating that a substantial fraction of the encapsulated drug remained trapped within the carrier matrix. Consequently, further optimization of the nanocarrier design is necessary to enhance drug release efficiency, thereby reducing the required drug dosage and improving therapeutic outcomes.
3. Future research should also aim to develop advanced nanocarriers with multi-stimuli-responsive controlled drug release and enhanced active targeting capabilities tailored to specific tumor types, enabling more precise and effective cancer therapy.

LIST OF PUBLICATIONS

1. **Nguyen-Dinh, Tien-Dung**, Ngoc Hoi Nguyen, Tan Phat Nguyen, Ngoc Thuy Trang Le, and Dai Hai Nguyen. "Enhancing Stability and Entrapment Efficiency of Liposome-Coated Mesoporous Silica Nanocarriers Using Polyoxyethylene Alkyl Ether as a Polyethylene Glycol Anchoring Agent." *ChemMedChem* 20, no. 22 (2025): e202500223. **(Q1, IF = 3.3)**
2. **Nguyen-Dinh, Tien-Dung**, Ngoc Hoi Nguyen, Pham Nguyen Dong Yen, Minh Hoang Vo Do, Dai Hai Nguyen, Ching Yern Chee, and Ngoc Thuy Trang Le. "Polymeric liposome-silica hybrid nanocarriers as stimuli-responsive systems for targeted drug delivery." *Journal of Polymers and the Environment* 33, no. 12 (2025): 5443-5459. **(Q1, IF = 6.1)**
3. **Nguyen-Dinh, Tien-Dung**, Ngoc Hoi Nguyen, Tan Phat Nguyen, Ching Yern Chee, and Dai Hai Nguyen. "Stealth Protocell System Based on Folated Polyoxyethylene Stearyl Ether-Modified Liposome-Encapsulated Mesoporous Silica for Active Tumor Targeting and pH-Responsive Releasing." *ACS Applied Engineering Materials* 4, no. 9 (2026): 5394-5406. **(Q2, IF = 3.5)**
4. **Nguyen-Dinh, Tien-Dung**, Ngoc Thuy Trang Le, Tan Phat Nguyen, Ching Yern Chee, and Dai Hai Nguyen. "Gelatin-Poly (Ethylene Glycol) Functionalized Lipid-Silica Hybrid Nanostructures With Enhanced Interfacial Properties and Colloidal Stability." *Journal of Applied Polymer Science* (2026): e71588. **(Q2, IF = 3.2)**

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APPENDIX

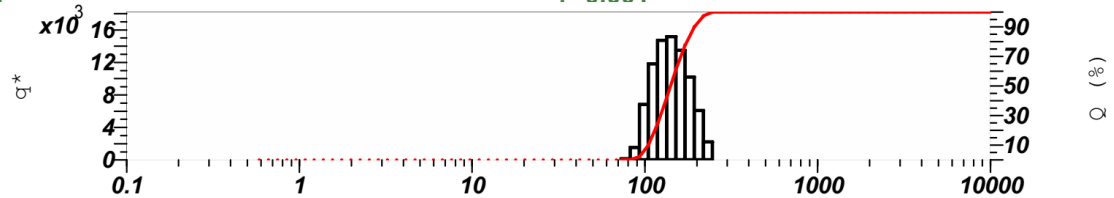
Cumulant Operations

Z-Average

: 139.9 nm

PI

: 0.001



Appendix 1. DLS size of MSN

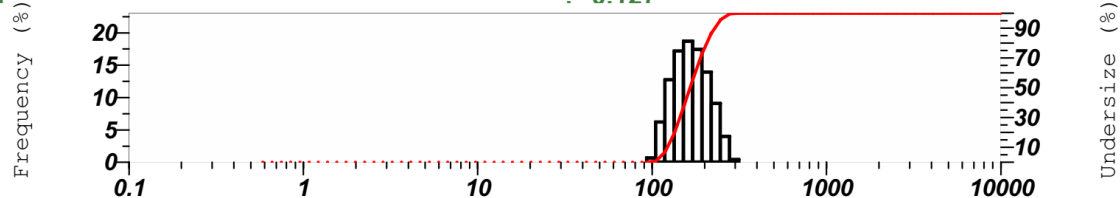
Cumulant Operations

Z-Average

: 157.1 nm

PI

: 0.127



Appendix 2. DLS size of MSN-NH2

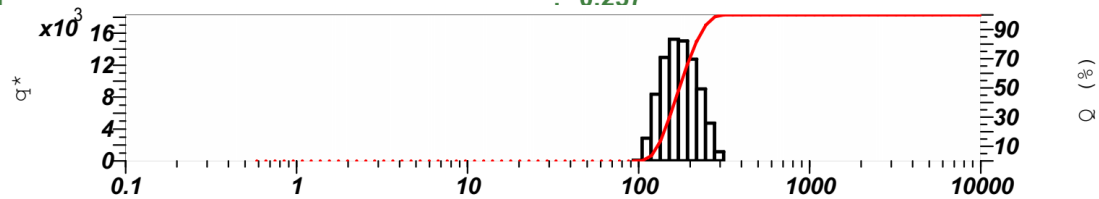
Cumulant Operations

Z-Average

: 164.3 nm

PI

: 0.257



Appendix 3. DLS size of ML

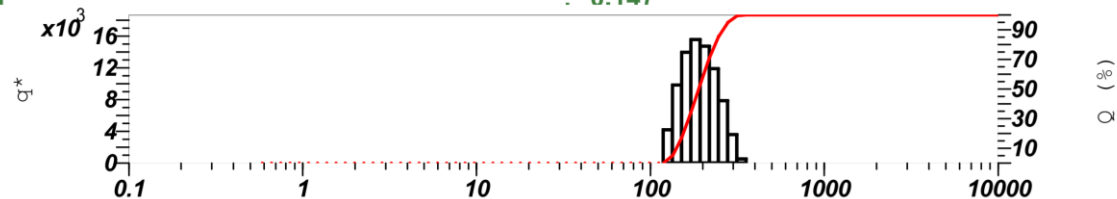
Cumulant Operations

Z-Average

: 184.4 nm

PI

: 0.147

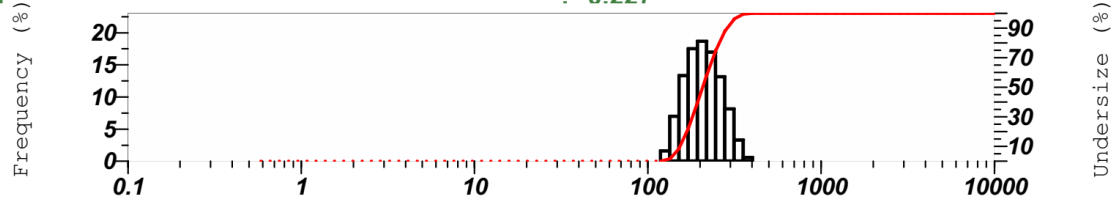
Appendix 4. DLS size of MLB₁₀

Cumulant Operations**Z-Average**

: 199.8 nm

PI

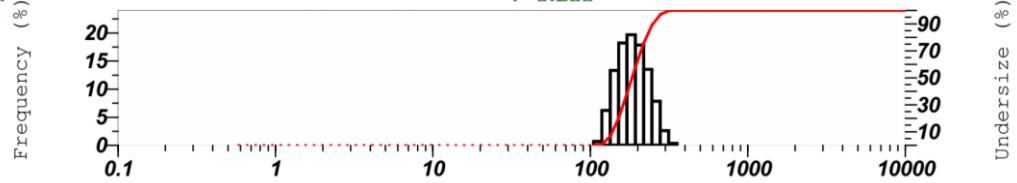
: 0.227

Appendix 5. DLS size of MLB₁₀₀-5**Cumulant Operations****Z-Average**

: 194.2 nm

PI

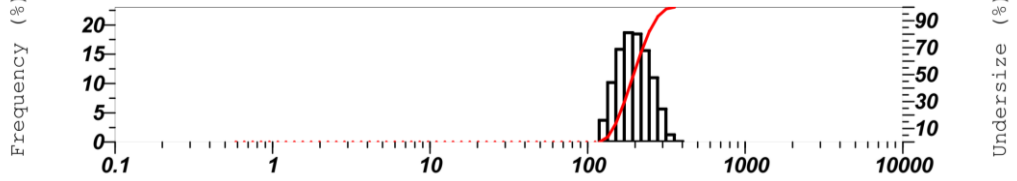
: 0.259

Appendix 6. DLS size of MLB₁₀₀-10**Cumulant Operations****Z-Average**

: 192.9 nm

PI

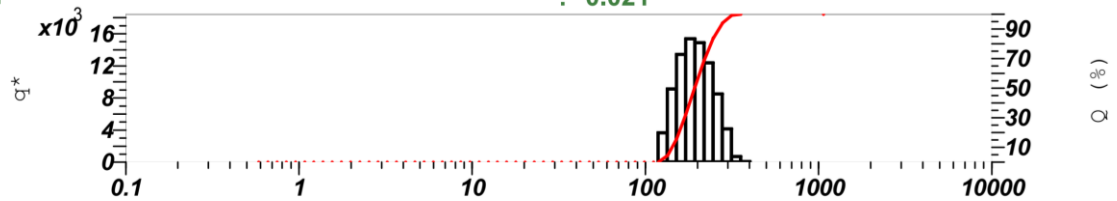
: 0.025

Appendix 7. DLS size of MLB₁₀₀-20**Cumulant Operations****Z-Average**

: 194.8 nm

PI

: 0.021

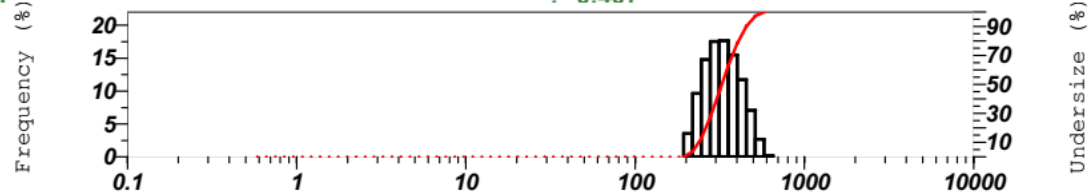
Appendix 8. DLS size of MLB₁₀₀-30

Cumulant Operations**Z-Average**

: 359.7 nm

PI

: 0.407



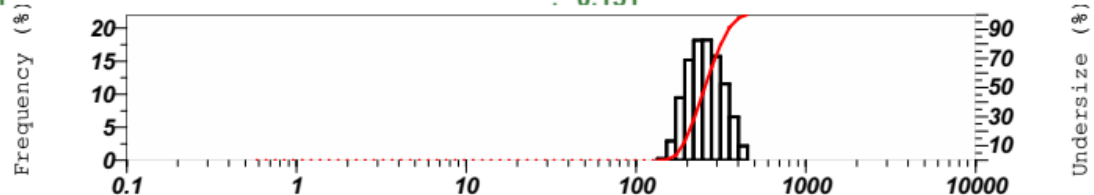
Appendix 9. DLS size of ML-GPF 1-2

Cumulant Operations**Z-Average**

: 250.6 nm

PI

: 0.151



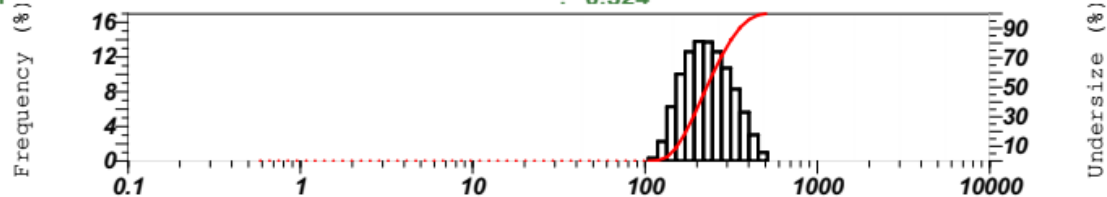
Appendix 10. DLS size of ML-GPF 1-3

Cumulant Operations**Z-Average**

: 252.3 nm

PI

: 0.324



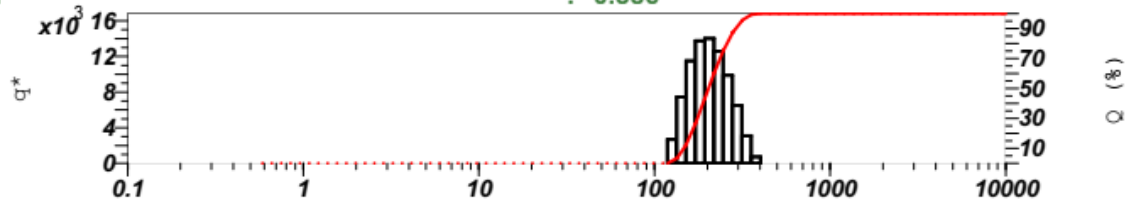
Appendix 11. DLS size of ML-GPF 1-4

Cumulant Operations**Z-Average**

: 857.8 nm

PI

: 0.386



Appendix 12. DLS size of ML-GPF 500

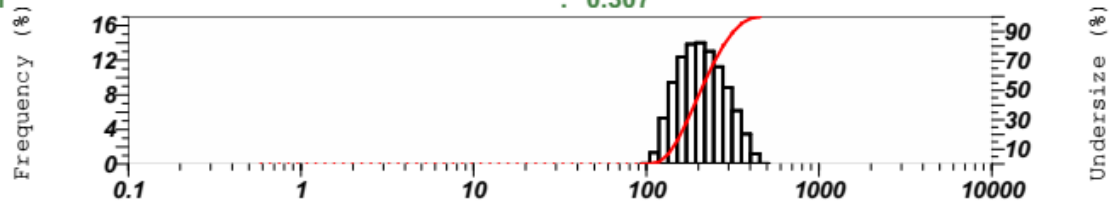
Cumulant Operations

Z-Average

: 218.9 nm

PI

: 0.307



Appendix 13. DLS size of ML-GPF 2000

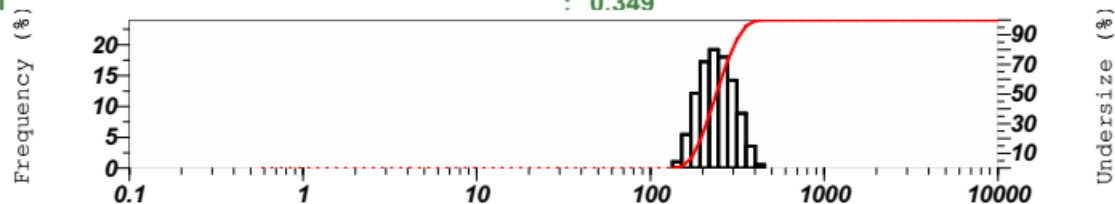
Cumulant Operations

Z-Average

: 256.4 nm

PI

: 0.349



Appendix 14. DLS size of ML-GPF 3000

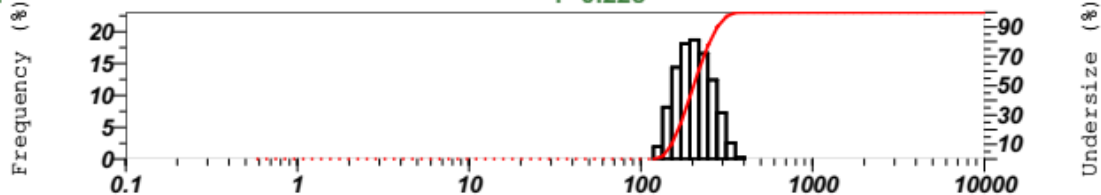
Cumulant Operations

Z-Average

: 201.3 nm

PI

: 0.225



Appendix 15. DLS size of ML-GPF 1-5

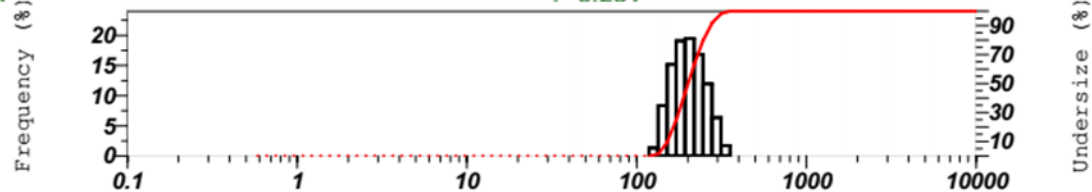
Cumulant Operations

Z-Average

: 204.6 nm

PI

: 0.261



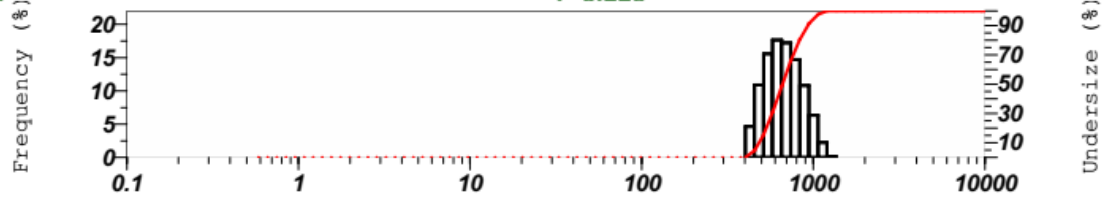
Appendix 16. DLS size of ML-GPF 1-10

Cumulant Operations**Z-Average**

: 687.8 nm

PI

: 0.220



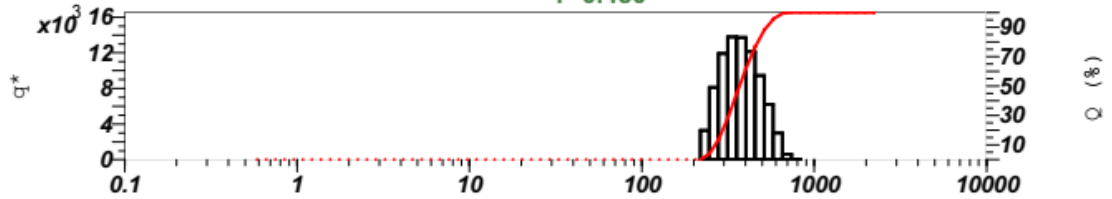
Appendix 17. DLS size of ML-GPF 1-30

Cumulant Operations**Z-Average**

: 812.3 nm

PI

: 0.450



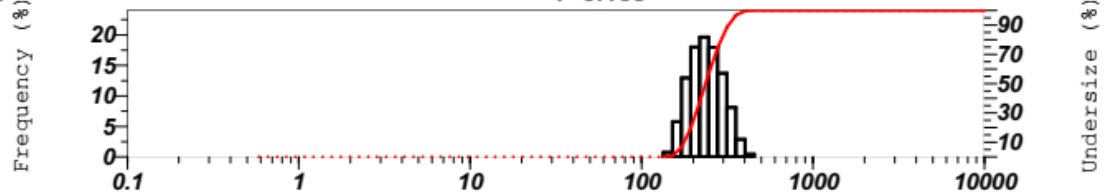
Appendix 18. DLS size of ML-GPF 1-40

Cumulant Operations**Z-Average**

: 241.2 nm

PI

: 0.163



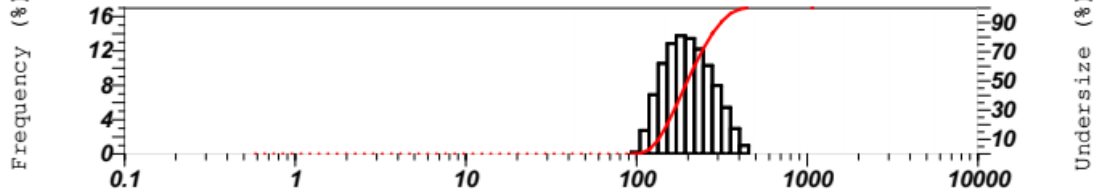
Appendix 19. DLS size of ML-CPF(1-5)-(1-2)

Cumulant Operations**Z-Average**

: 244.5 nm

PI

: 0.234



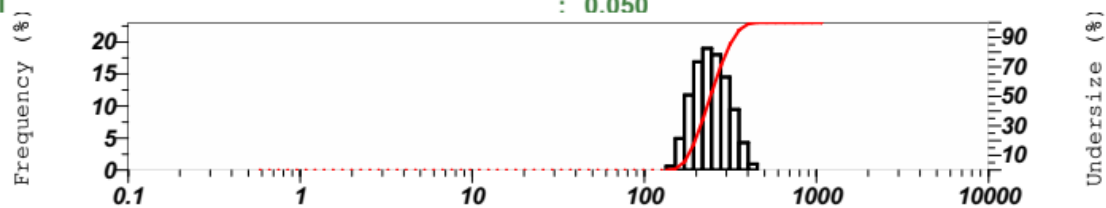
Appendix 20. DLS size of ML-CPF(1-5)-(1-1)

Cumulant Operations**Z-Average**

: 253.2 nm

PI

: 0.050



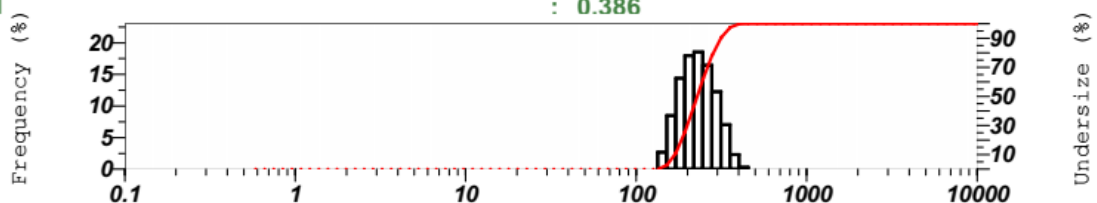
Appendix 21. DLS size of ML-CPF(1-5)-(2-1)

Cumulant Operations**Z-Average**

: 243.5 nm

PI

: 0.386



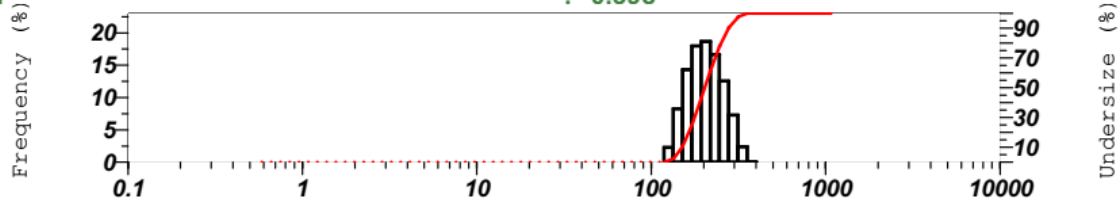
Appendix 22. DLS size of ML-CPF(1-10)-(1-2)

Cumulant Operations**Z-Average**

: 228.5 nm

PI

: 0.398



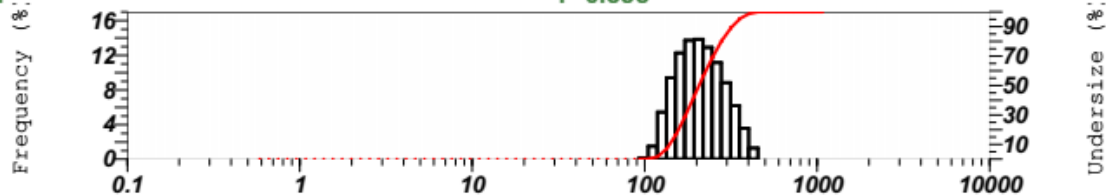
Appendix 23. DLS size of ML-CPF(1-10)-(1-1)

Cumulant Operations**Z-Average**

: 245.5 nm

PI

: 0.355



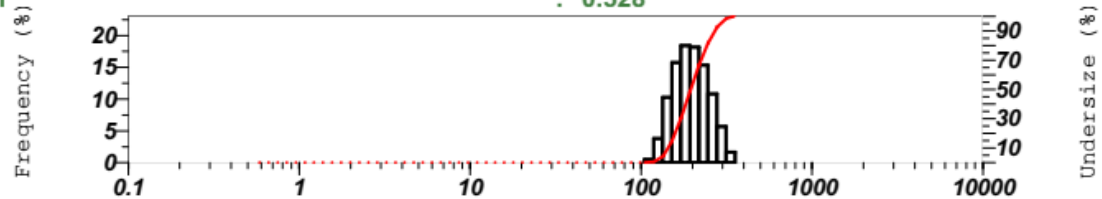
Appendix 24. DLS size of ML-CPF(1-10)-(2-1)

Cumulant Operations**Z-Average**

: 243.8 nm

PI

: 0.528



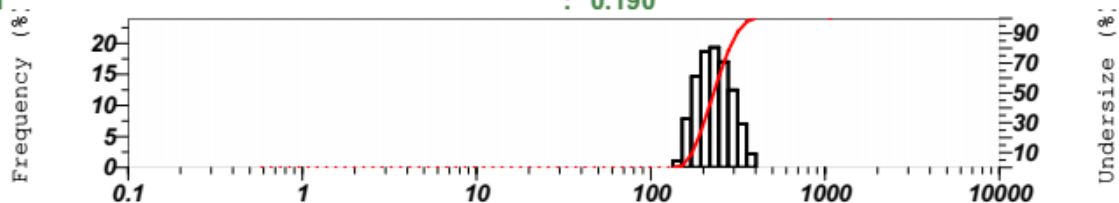
Appendix 25. DLS size of ML-CPF(1-20)-(1-2)

Cumulant Operations**Z-Average**

: 246.8 nm

PI

: 0.190



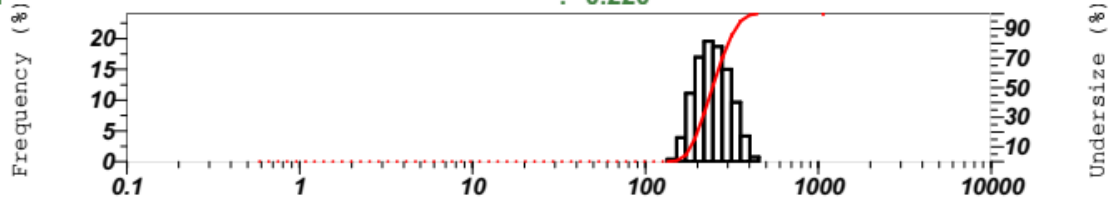
Appendix 26. DLS size of ML-CPF(1-20)-(1-1)

Cumulant Operations**Z-Average**

: 239.4 nm

PI

: 0.220



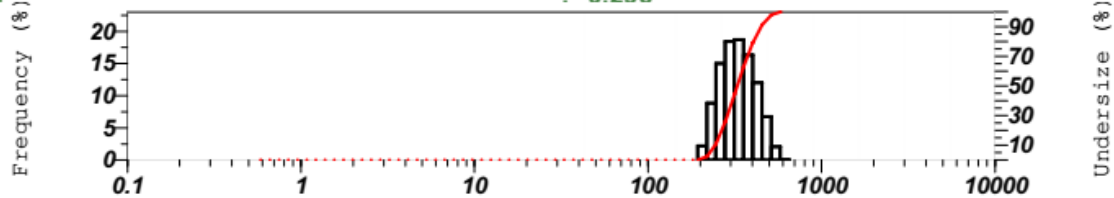
Appendix 27. DLS size of ML-CPF(1-20)-(2-1)

Cumulant Operations**Z-Average**

: 282.8 nm

PI

: 0.296



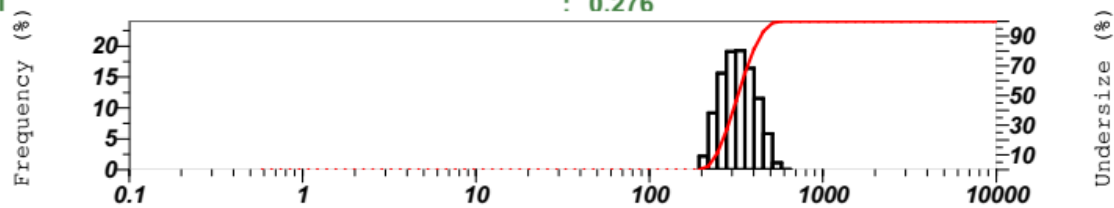
Appendix 28. DLS size of ML-CPF(1-30)-(1-2)

Cumulant Operations**Z-Average**

: 280.8 nm

PI

: 0.276



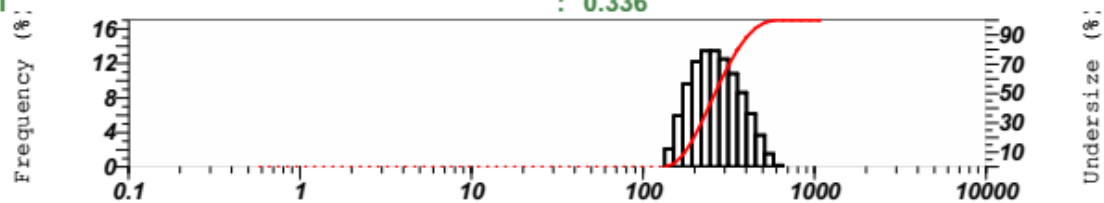
Appendix 29. DLS size of ML-CPF(1-30)-(1-1)

Cumulant Operations**Z-Average**

: 285.0 nm

PI

: 0.336



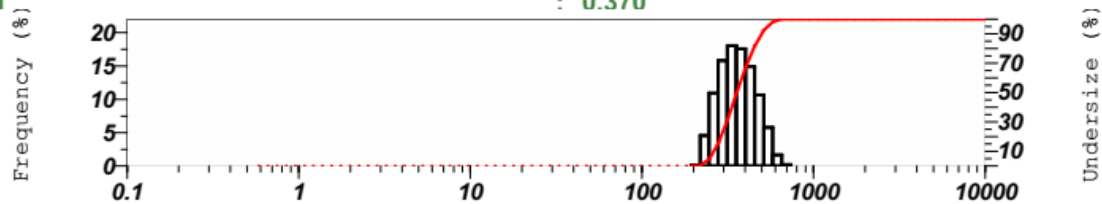
Appendix 30. DLS size of ML-CPF(1-30)-(2-1)

Cumulant Operations**Z-Average**

: 350.5 nm

PI

: 0.370



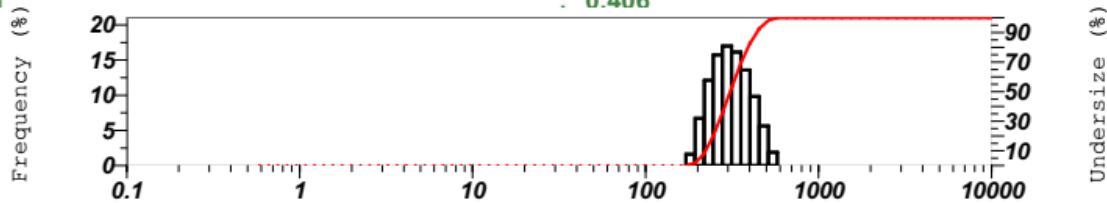
Appendix 31. DLS size of ML-CPF(1-40)-(1-2)

Cumulant Operations**Z-Average**

: 344.4 nm

PI

: 0.406



Appendix 32. DLS size of ML-CPF(1-40)-(1-1)

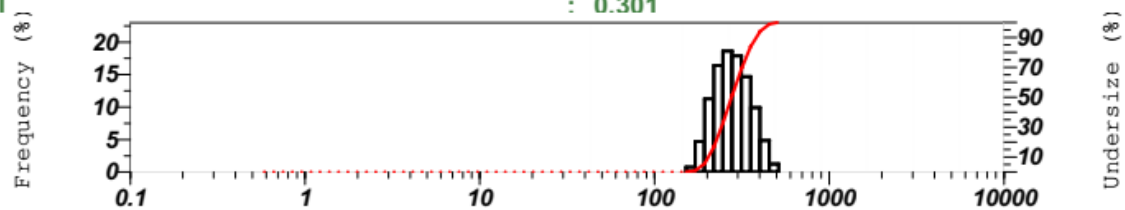
Cumulant Operations

Z-Average

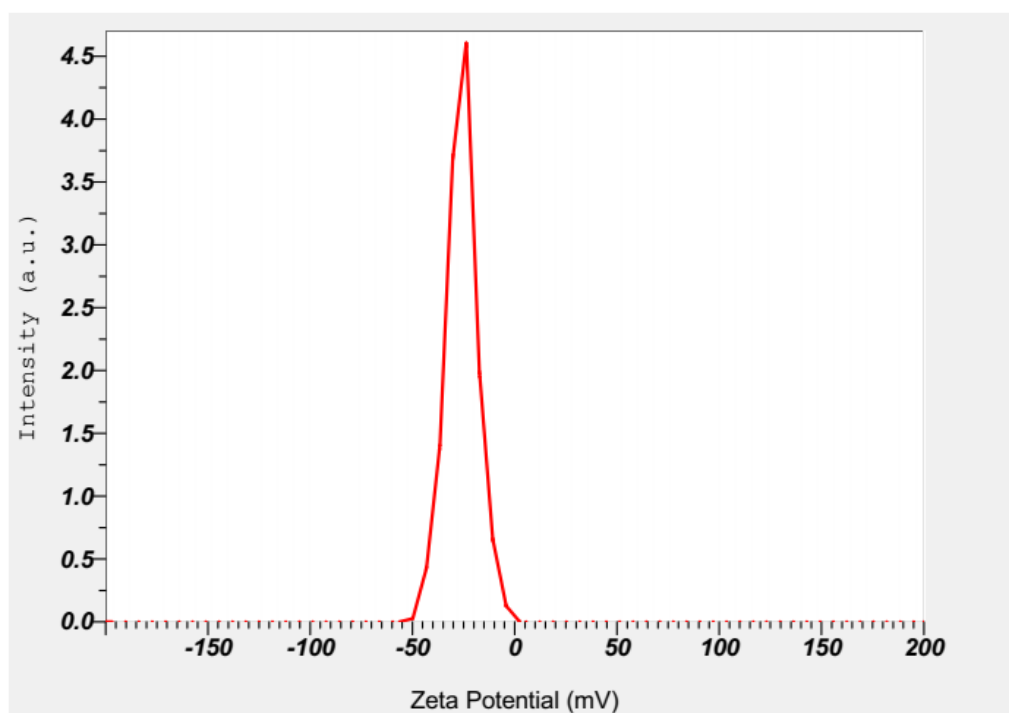
: 283.8 nm

PI

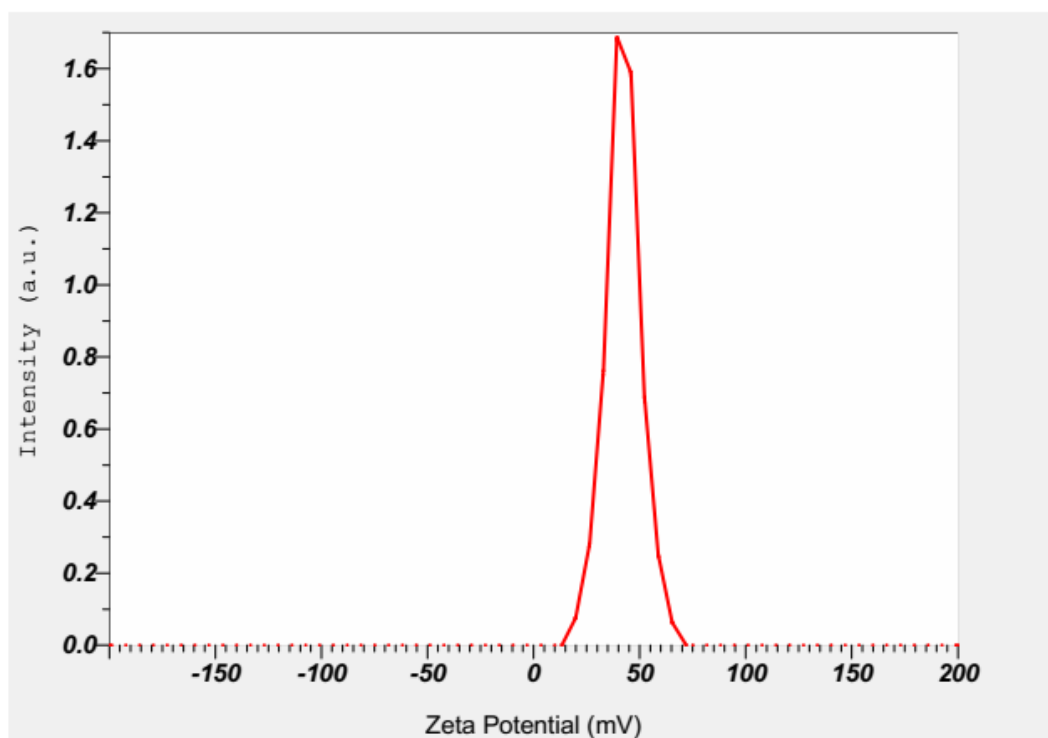
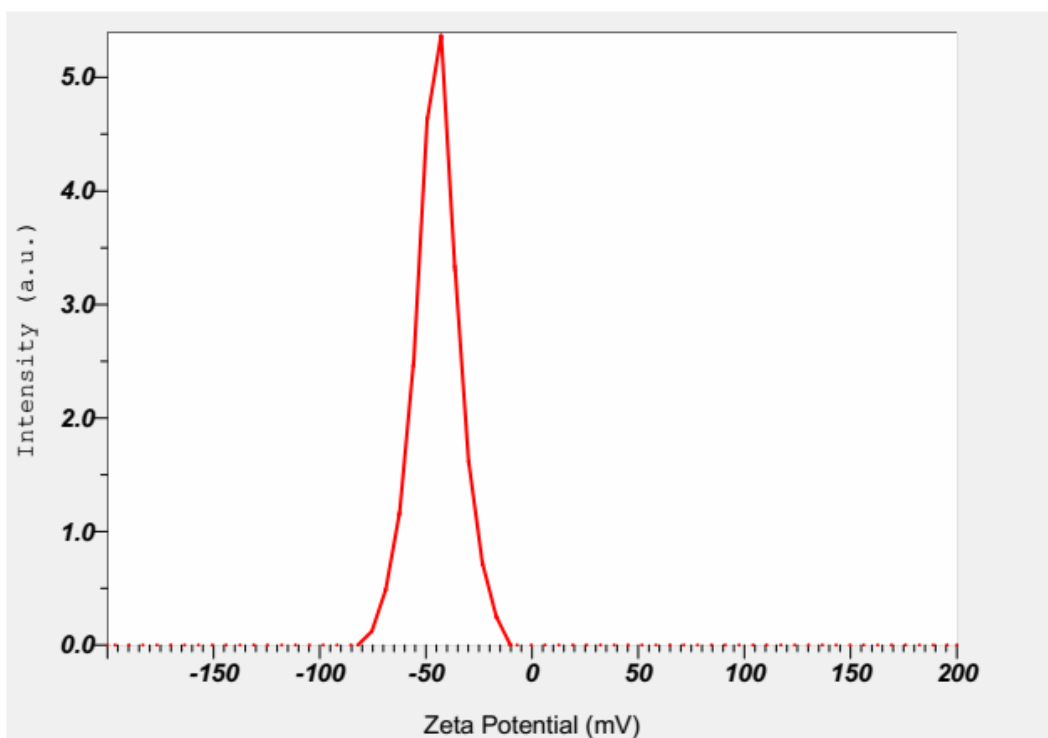
: 0.301

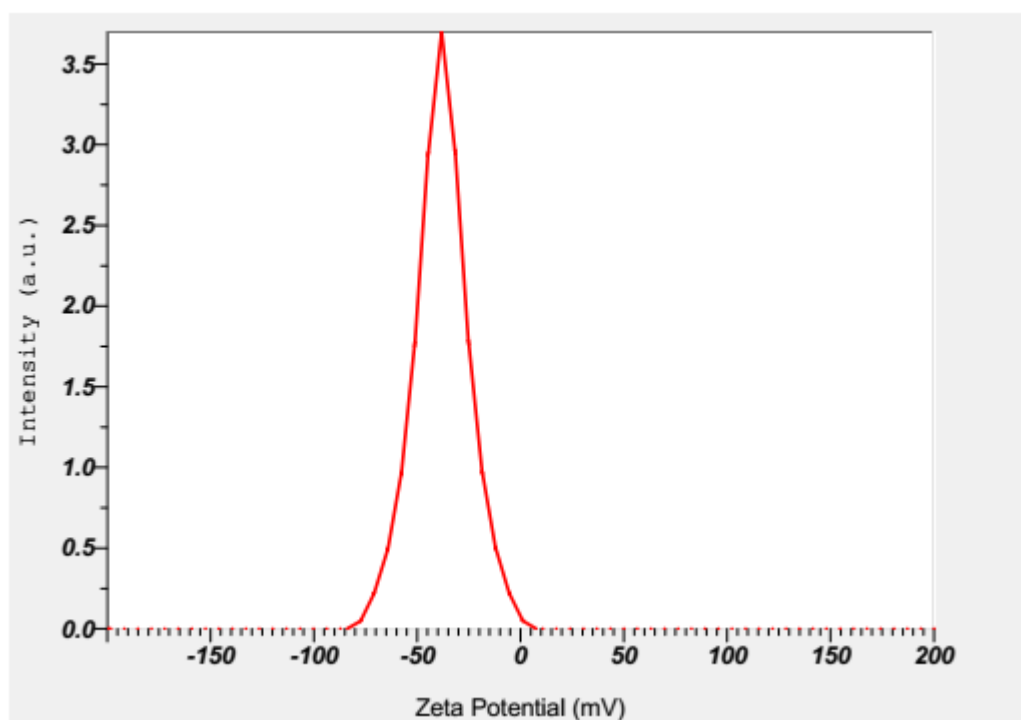


Appendix 33. DLS size of ML-CPF(1-40)-(2-1)

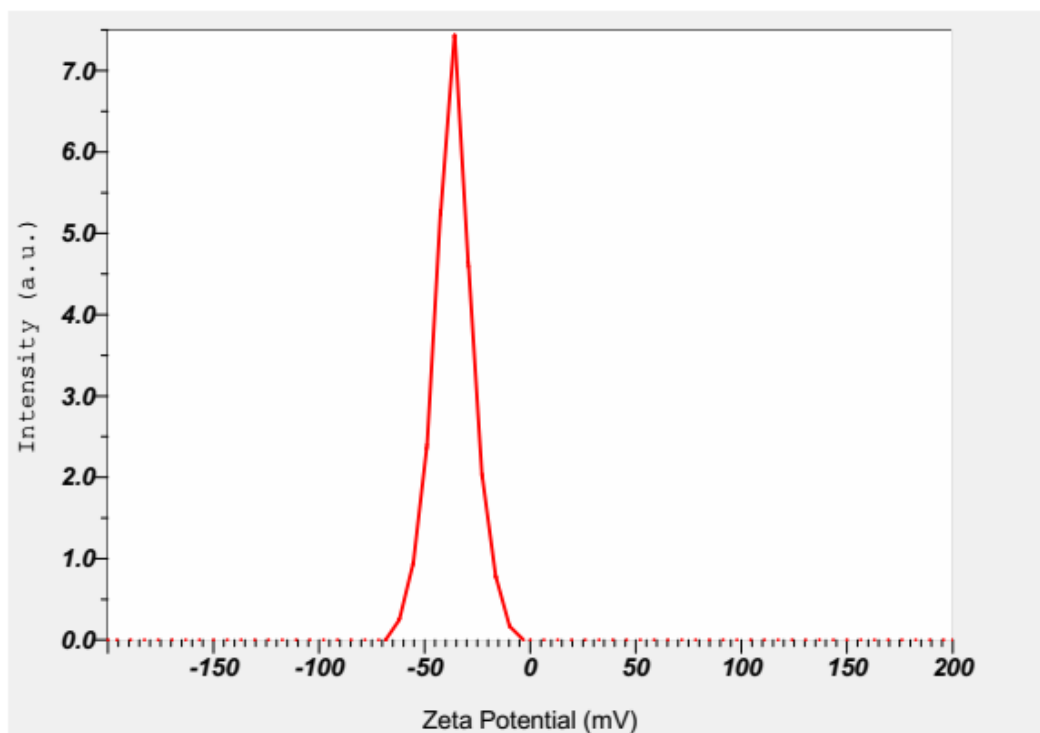


Appendix 34. Zeta potential of MSN

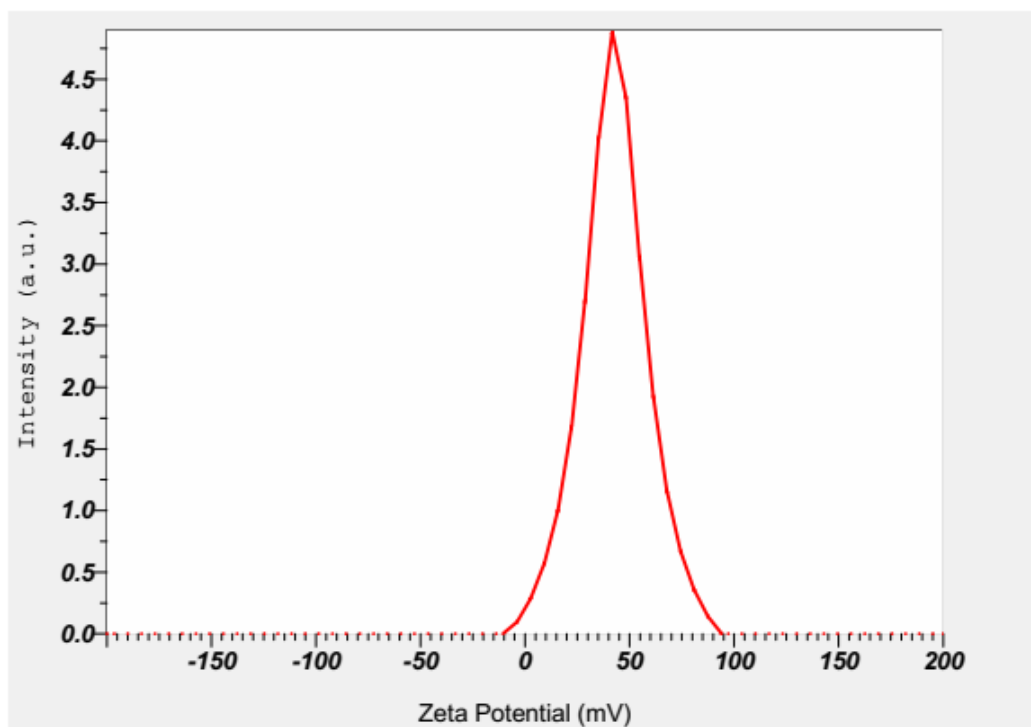
Appendix 35. Zeta potential of MSN-NH₂Appendix 36. Zeta potential of MLB₁₀



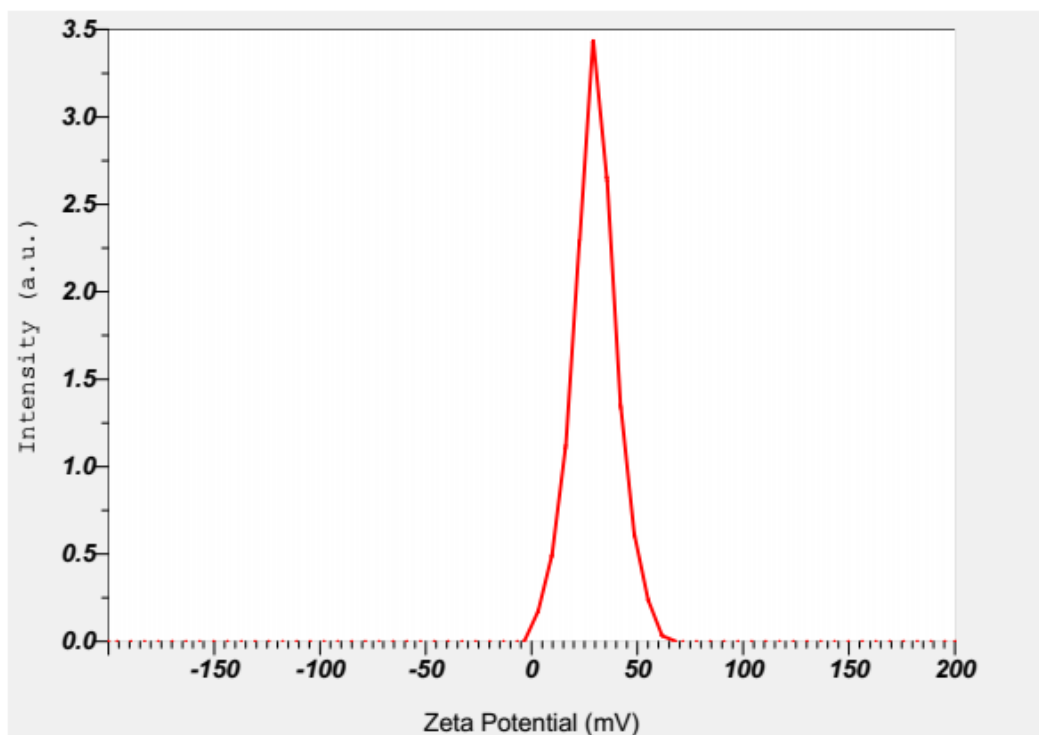
Appendix 37. Zeta potential of MLB₂₀



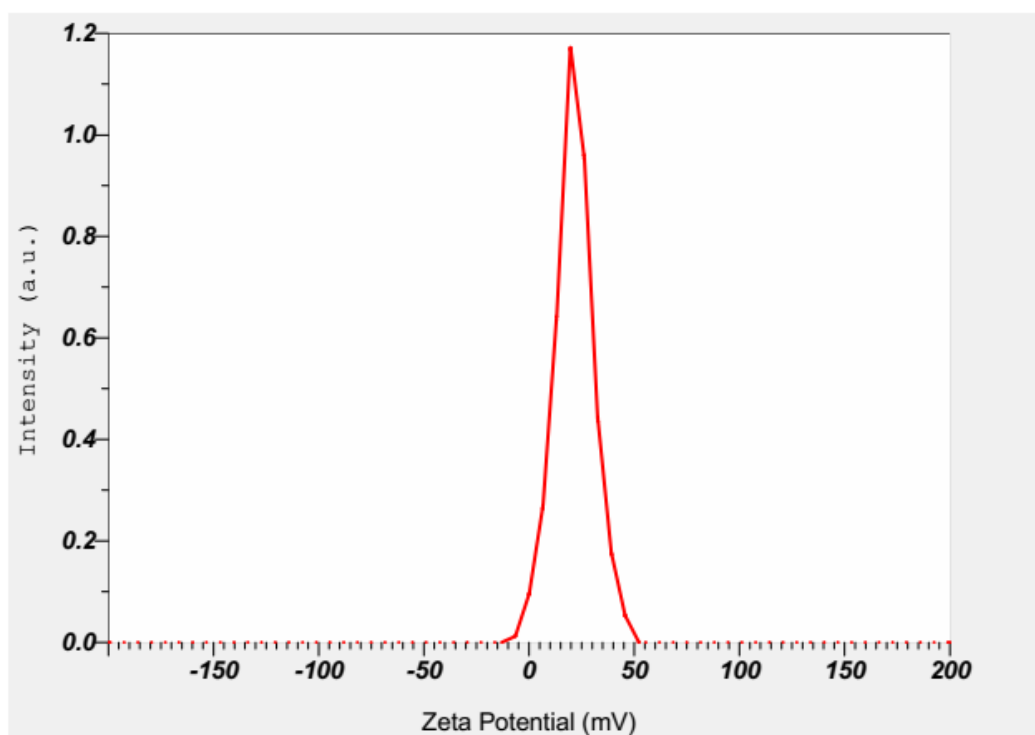
Appendix 38. Zeta potential of MLB₁₀₀



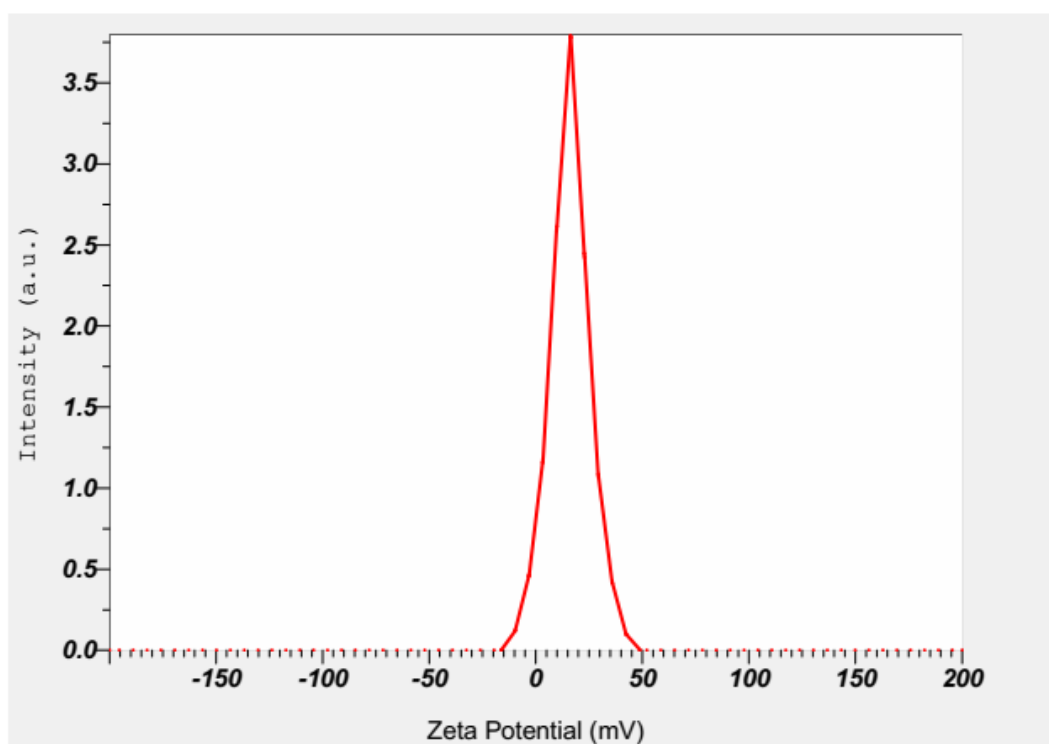
Appendix 39. Zeta potential of ML-CPF(1-5)-(2-1)



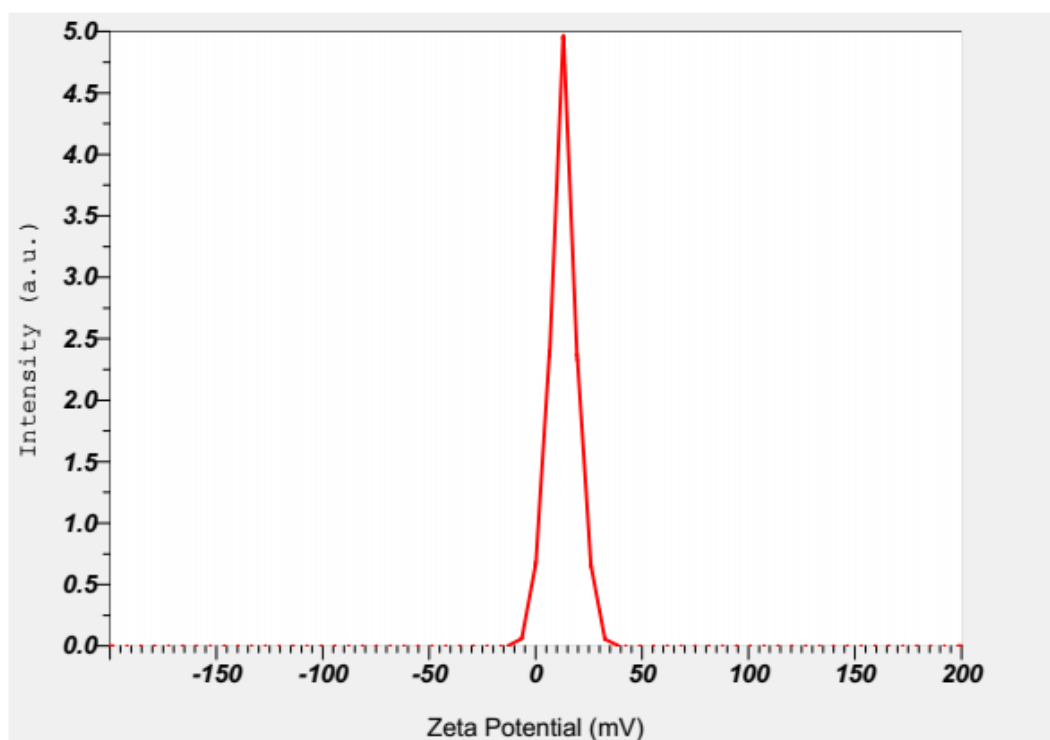
Appendix 40. Zeta potential of ML-CPF(1-10)-(2-1)



Appendix 41. Zeta potential of ML-CPF(1-20)-(2-1)

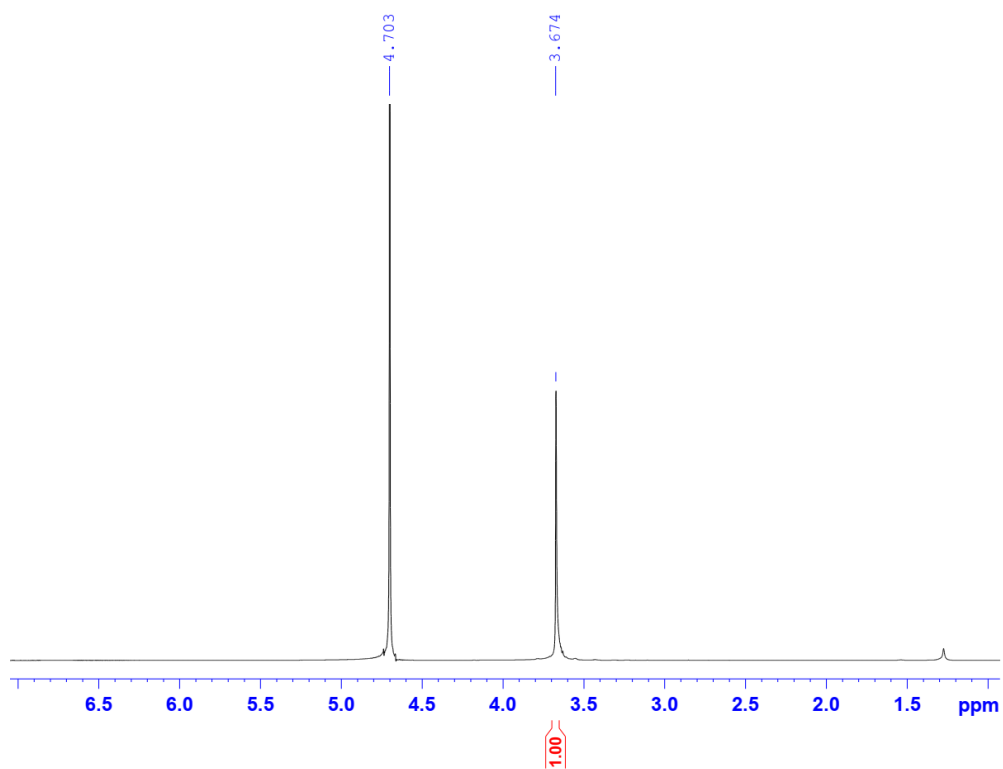


Appendix 42. Zeta potential of ML-CPF(1-30)-(2-1)

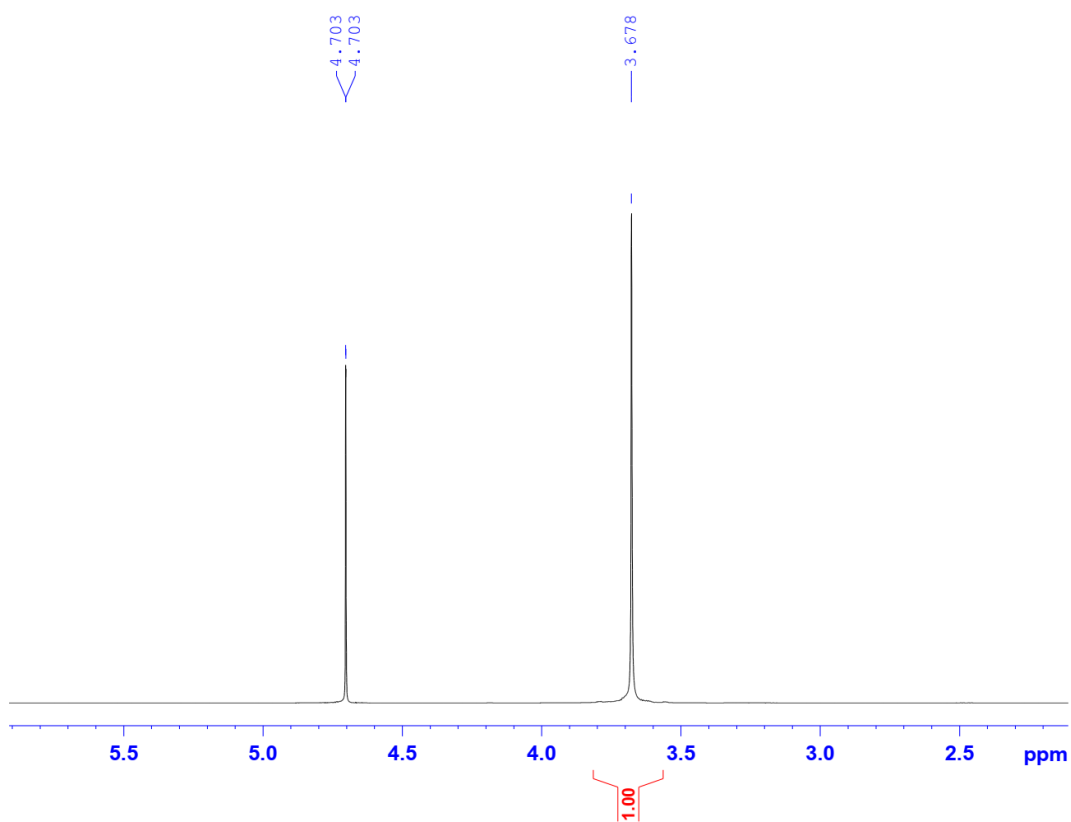


Appendix 43. Zeta potential of ML-CPF(1-40)-(2-1)

PL-15

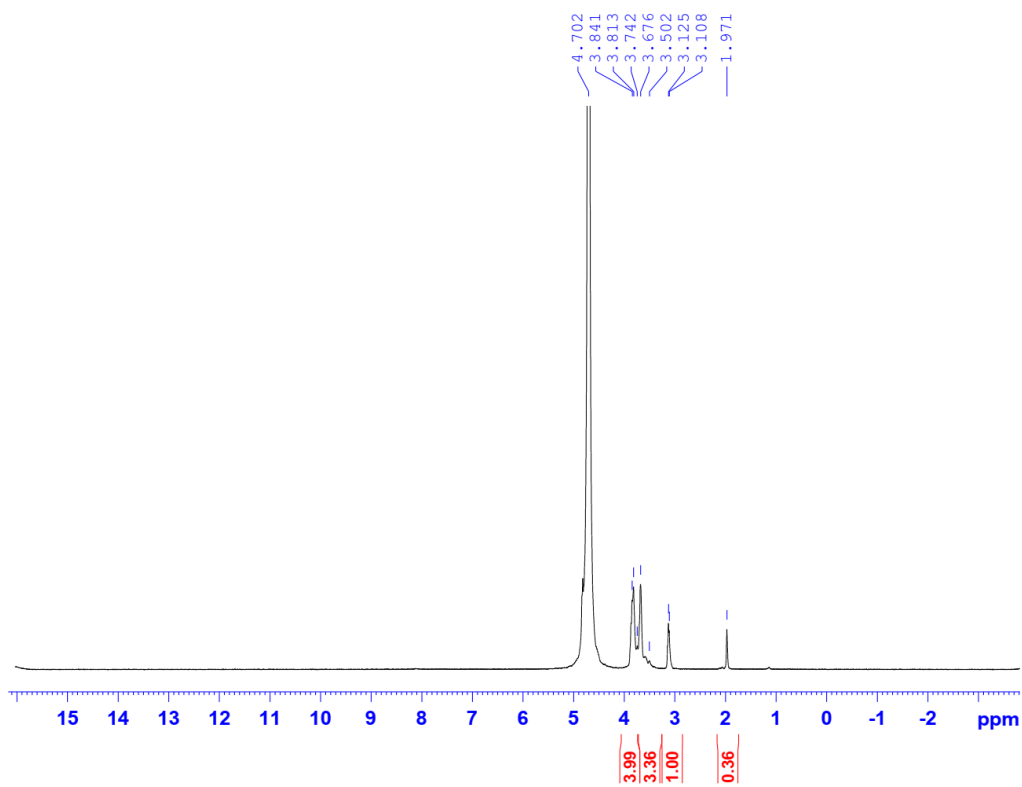


Appendix 44. ¹H-NMR of Brij-FA

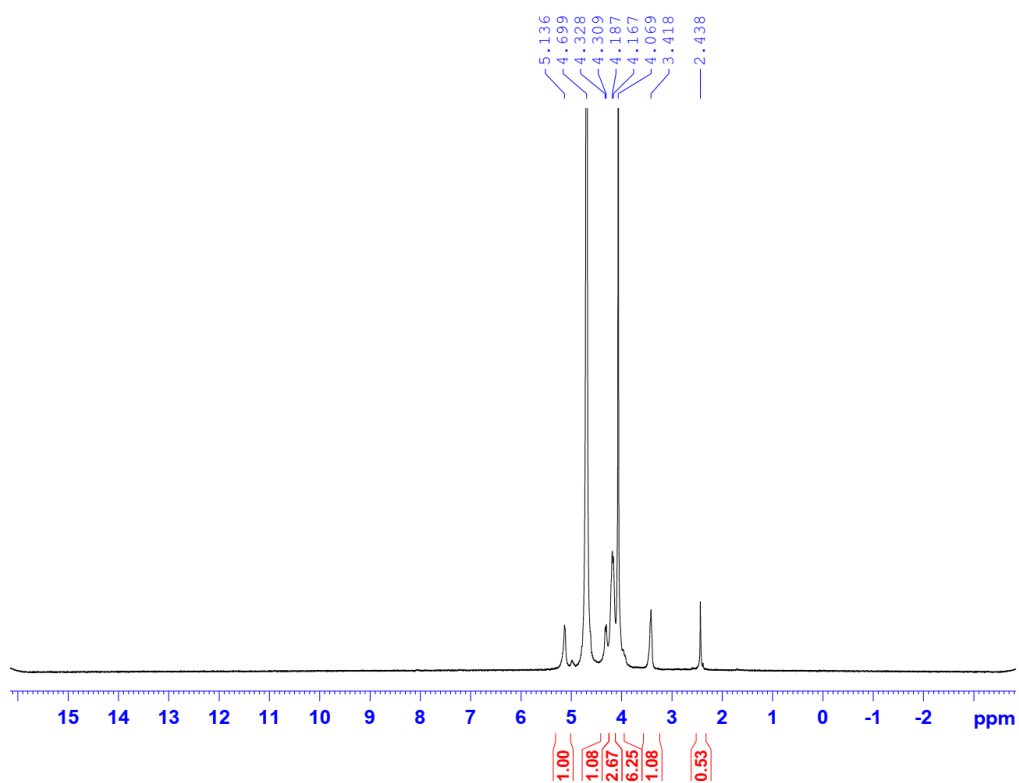


Appendix 45. ¹H-NMR of HOOC-PEG-FA

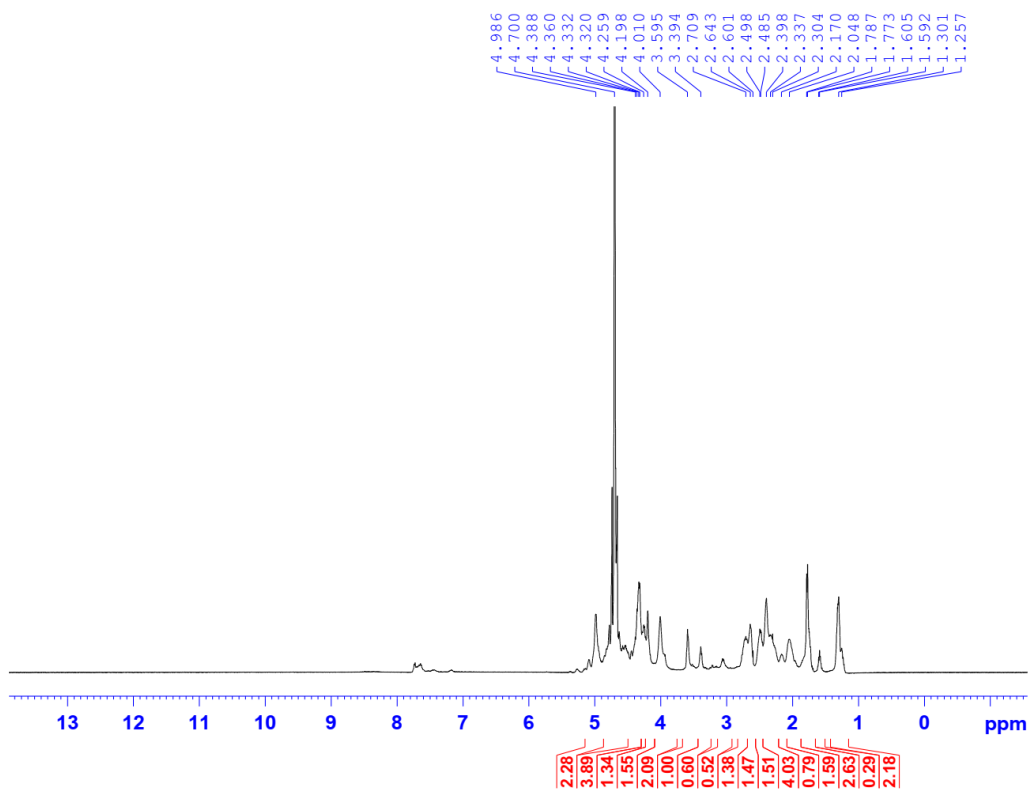
PL-16



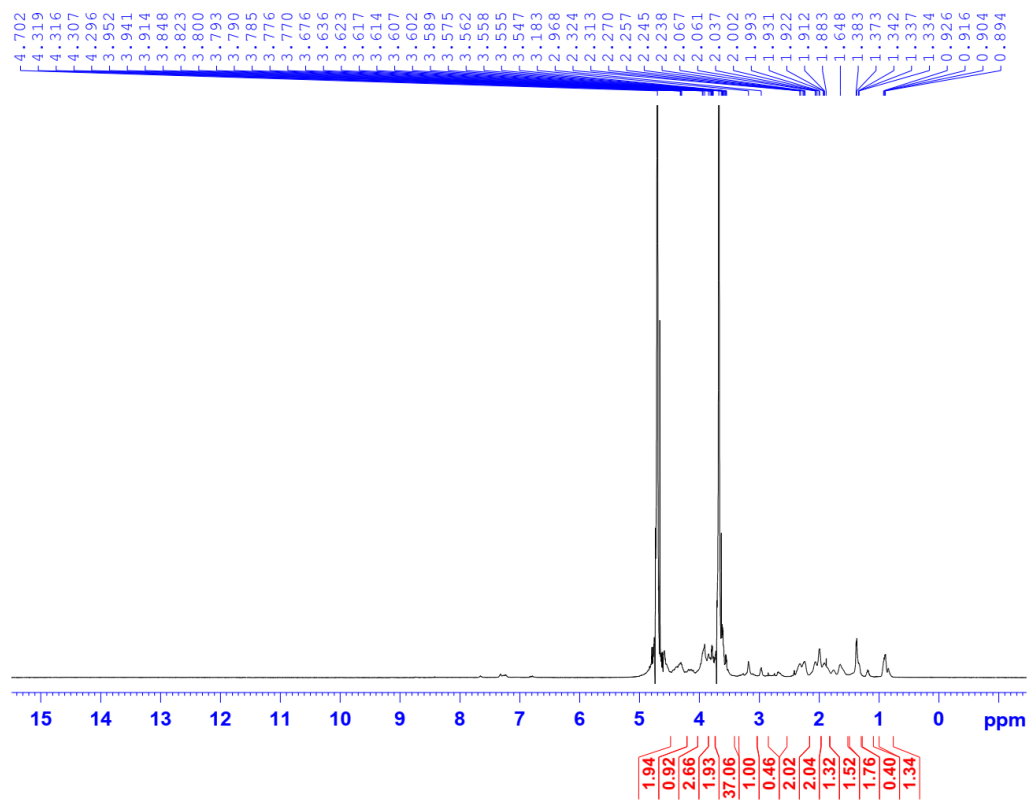
Appendix 46. ¹H-NMR of chitosan



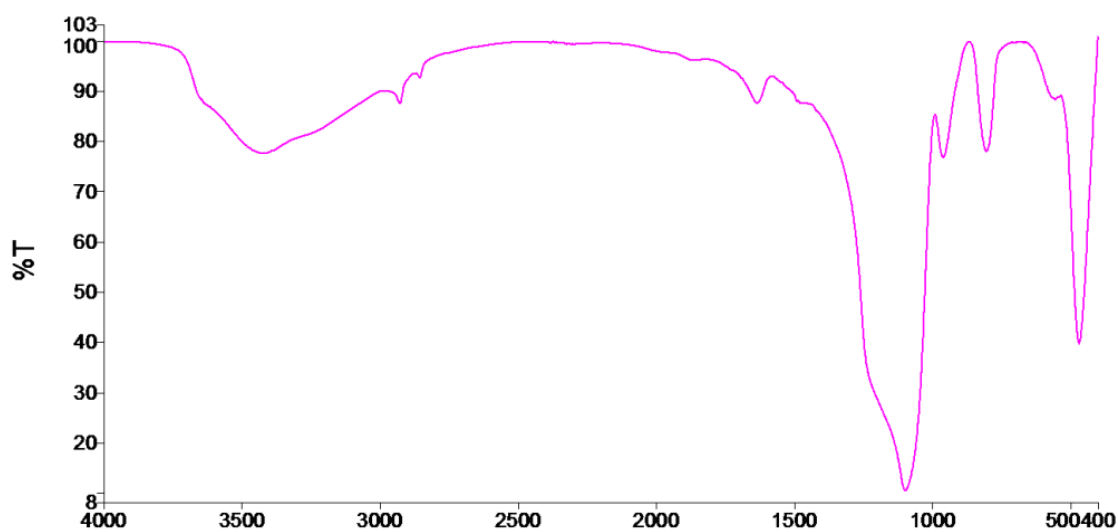
Appendix 47. ¹H-NMR of CPF



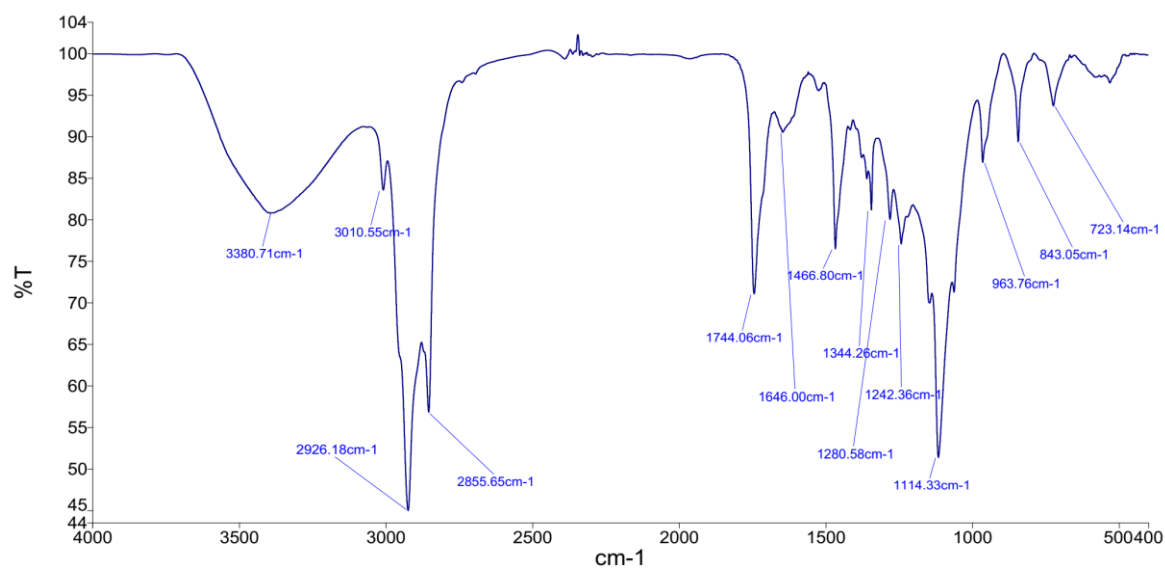
Appendix 48. ¹H-NMR of gelatin



Appendix 49. ¹H-NMR of GPF

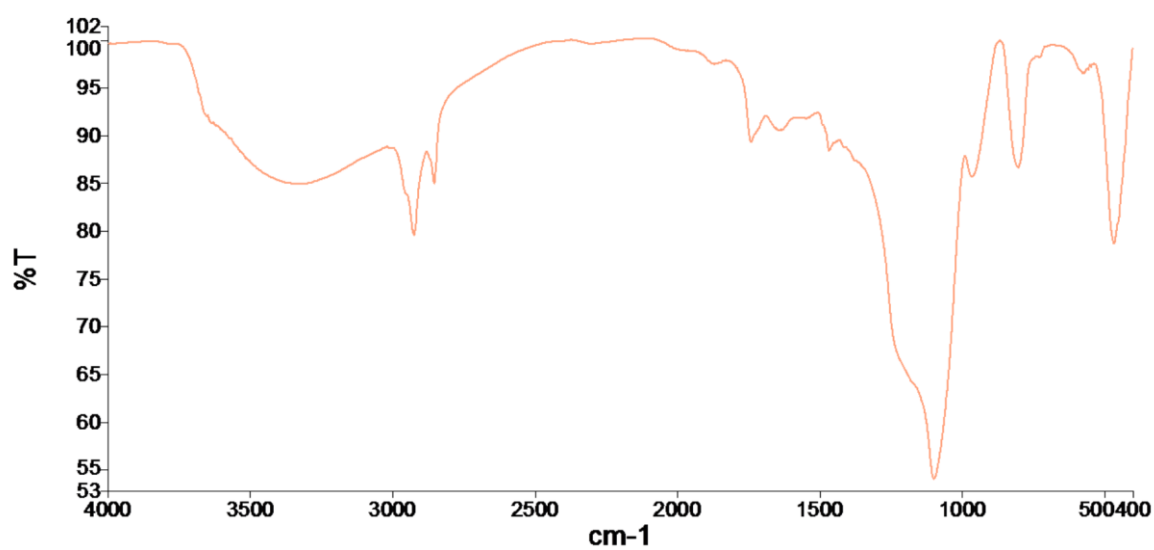


Appendix 50. IR spectra of MSN-NH₂

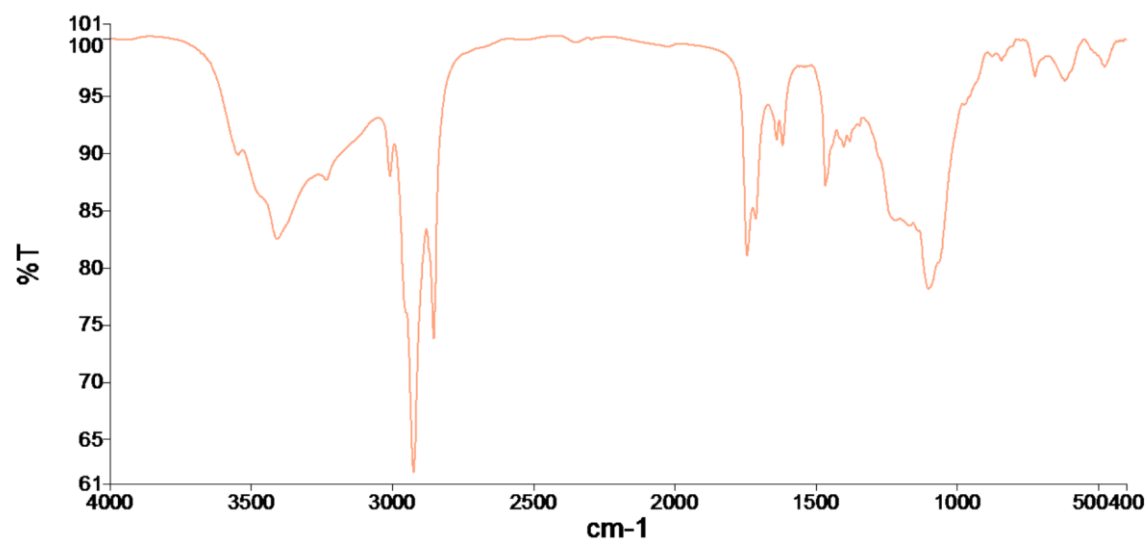


Appendix 51. IR spectra of LB

PL-19



Appendix 52. IR spectra of MLB

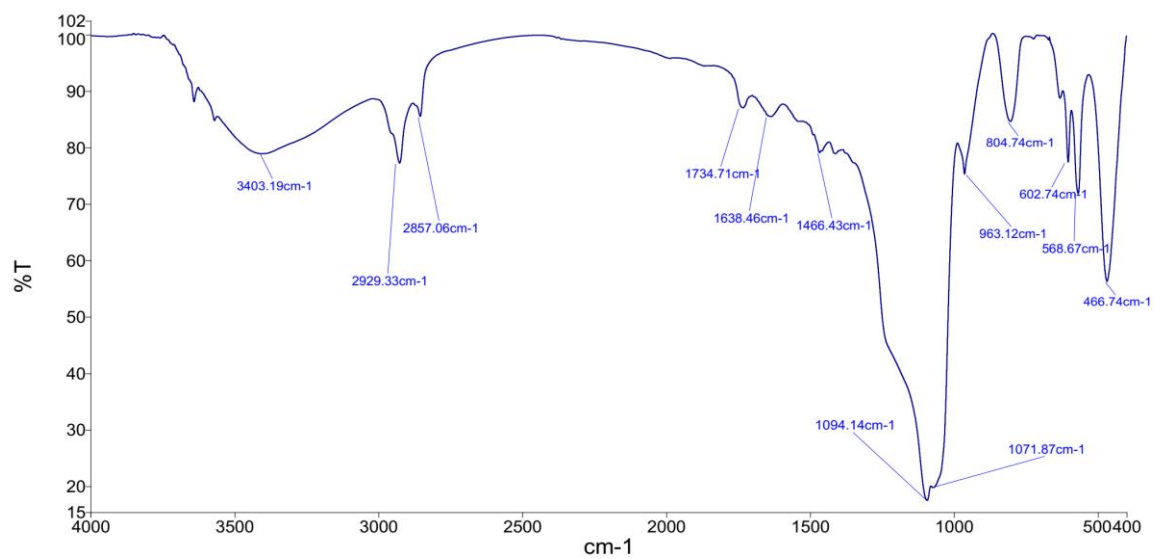


Appendix 53. IR spectra of LP

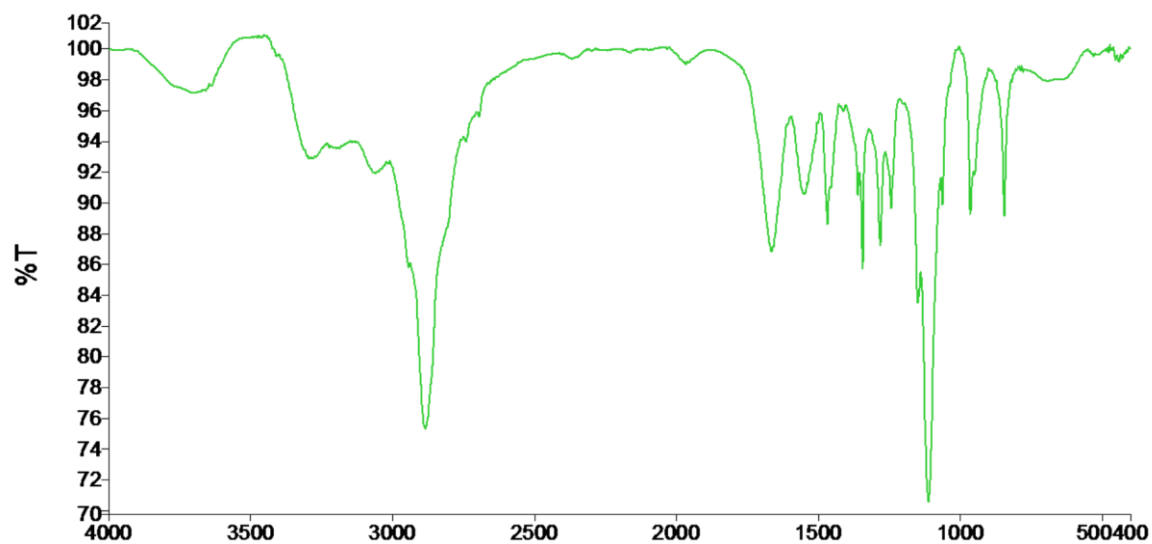


Appendix 54. IR spectra of CPF

PL-20



Appendix 55. IR spectra of MLCPF



Appendix 56. IR spectra of GPF

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Instructor Name	Nguyen Dinh Tien Dung	Expected Presentation Date	2026-08-21

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Title, Description or Numeric Reference of the Portion(s)	Figure 7	Title of the Article / Chapter the Portion Is From	Mechanism of formation and nanostructure of Stöber silica particles
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Article Title	Silica nanoparticle supported lipid bilayers for gene delivery.	Publication Type	e-Journal
Author / Editor	Royal Society of Chemistry (Great Britain)	Start Page	5100
Date	01/01/1996	Issue	34
Language	English	Volume	0
Country	United Kingdom of Great Britain and Northern Ireland	URL	http://pubs.rsc.org/en/journals/journalissues/cc

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Editor of Portion(s)	LiuCurrent address: Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada N2L 3G1., Juewen; Stace-Naughton, Alison; Brinker, C. Jeffrey	Author of Portion(s)	LiuCurrent address: Department of Chemistry, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada N2L 3G1., Juewen; Stace-Naughton, Alison; Brinker, C. Jeffrey
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Article Title	Formation of supported lipid bilayers on silica: relation to lipid phase transition temperature and liposome size.	Start Page	187
		End Page	195
		Issue	1
		Volume	10
Author / Editor	Royal Society of Chemistry (Great Britain)	URL	http://www.rsc.org/Publishing/Journals/sm/index.asp
Date	06/01/2005		
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