Research Article

Comparative Nano-Characterization of Silybum marianum Formulations: Conventional Extraction vs. Homeopathic Trituration-Derived Nanoparticle

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Abstract

Silybum marianum (L.) Gaertn. (Asteraceae), commonly known as milk thistle or Carduus marianus in homeopathic pharmacopoeias, is one of the most extensively investigated botanical sources of hepatoprotective, antioxidant, anti-inflammatory, and antifibrotic phytochemicals. Its primary bioactive complex, silymarin, comprises a mixture of flavonolignans—predominantly silybin A, silybin B, isosilybin A, isosilybin B, silychristin, and silydianin—alongside the precursor flavonoid taxifolin and polyphenolic fractions. Despite robust pharmacodynamic efficacy demonstrated across preclinical and clinical models of hepatic injury, the translational utility of conventionally extracted Silybum marianum formulations remains severely constrained by the Biopharmaceutics Classification System (BCS) Class II/IV physicochemical profile of its flavonolignans, characterized by extremely poor aqueous solubility, low intestinal epithelial permeability, active efflux via apical transporters, and rapid presystemic phase II glucuronidation and sulfation. Over the past two decades, pharmaceutical nanotechnology has addressed these limitations through "bottom-up" and "top-down" engineered nanocarriers—including solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), polymeric poly(lactic-co-glycolic acid) (PLGA) nanoparticles, chitosan nanocomposites, phytosomal-liposomal hybrids, mesoporous silica nanoparticles (MSNs), self-nanoemulsifying drug delivery systems (SNEDDS), biogenic metallic nanoparticles, and stabilizer-free nanocrystals. Parallel to these developments in conventional nanopharmaceutics, an independent body of physicochemical research has revealed that classical homeopathic pharmaceutical operations—specifically prolonged dry mechanical trituration in crystalline α-lactose monohydrate matrices followed by serial dilution and vigorous hydrodynamic succussion in borosilicate glass vessels—generate heterogeneous populations of source-derived nanoparticles, lactose nanocrystals, nanobubbles, and amorphous or crystalline silicate nanostructures. This comprehensive review provides a rigorous, multi-parametric comparative nano-characterization of Silybum marianum formulations derived from conventional phytochemical extraction and engineered nanodelivery versus homeopathic trituration and potentization. By examining synthesis thermodynamics, solid-state crystallography, morphological attributes via transmission and scanning electron microscopy (TEM/SEM), hydrodynamic diameter and polydispersity via dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA), electrokinetic stability via zeta potential, surface chemistry via Fourier-transform infrared (FTIR) and Raman spectroscopy, elemental composition via energy-dispersive X-ray spectroscopy (EDS) and inductively coupled plasma mass spectrometry (ICP-MS), and divergent biological mechanisms ranging from mass-action receptor pharmacology to hormetic allostatic cross-adaptation, this treatise establishes an integrative analytical framework for evaluating botanical nanomaterials across distinct pharmaceutical paradigms.

Keywords: Silybum marianum; Carduus marianus; Silymarin; Silybin; Homeopathic trituration; Nanoparticle characterization; Dynamic light scattering; Transmission electron microscopy; Silicate nanostructures; Bioavailability.

1. Introduction

Silybum marianum (L.) Gaertn., classified within the family Asteraceae and historically designated as *Carduus marianus* L. in classical botanical and homeopathic nomenclature, is an annual or biennial herbaceous plant indigenous to the Mediterranean basin and North Africa that has subsequently naturalized across temperate and subtropical regions of Europe, Asia, the Americas, and Australia (Kroll et al., 2007; Abenavoli et al., 2010). For more than two millennia, spanning the medical treatises of Dioscorides and Pliny the Elder to contemporary integrative hepatology, extracts derived from the dried, ripe achenes (fruits) of *Silybum marianum* have been utilized to manage hepatobiliary dysfunction, jaundice, acute and chronic viral hepatitis, alcoholic liver disease, metabolic dysfunction-associated

steatotic liver disease, cirrhosis, and acute amatoxin poisoning resulting from *Amanita phalloides* ingestion (Abenavoli et al., 2010; Loguercio et al., 2011; Gillessen et al., 2020).

Phytochemical standardization defines the crude lipophilic-polyphenolic extract of *Silybum marianum* achenes as **silymarin**, which typically accounts for 1.5% to 3.0% of the dry fruit weight and consists of approximately 65% to 80% defined flavonolignans and flavonoids, with the remaining 20% to 35% comprising an uncharacterized matrix of polymeric and oxidized polyphenolic resins, fixed oils, and fatty acids (Kroll et al., 2007; Javed et al., 2011). The principal flavonolignan constituents are biosynthesized in the pericarp and seed coat via the oxidative coupling of the dihydroflavonol taxifolin with coniferyl alcohol, yielding diastereomeric and regioisomeric structures: silybin A and silybin B (collectively referred to as silibinin, representing 50% to 70% of standardized silymarin), isosilybin A and isosilybin B, silychristin A and silychristin B, and silydianin (Zhu et al., 2013; Albassam et al., 2017; Selc et al., 2025). While silibinin has historically dominated pharmacological inquiry due to its abundance and potent free-radical scavenging, membrane-stabilizing, and ribosomal RNA polymerase I-stimulating properties, recent comparative investigations demonstrate that minor congeners such as silychristin, silydianin, isosilybin B, and 2,3-dehydrosilybin exhibit distinct, and occasionally superior, cytoprotective, anti-inflammatory, metabolic, and selective antiproliferative activities (Loguercio et al., 2011; Selc et al., 2025).

1.2 The Biopharmaceutical Bottleneck of Conventional *Silybum marianum* Extracts

Despite its broad therapeutic index and exceptional clinical safety profile—tolerated at oral doses exceeding 1,500 mg/day without severe dose-limiting toxicity—the clinical translation of unformulated *Silybum marianum* extracts has frequently yielded inconsistent efficacy in randomized controlled trials (Javed et al., 2011; Gillessen et al., 2020). Pharmacokinetic investigations in both preclinical animal models and human volunteers consistently establish that the absolute oral bioavailability of free silymarin flavonolignans is exceedingly low, typically ranging between 0.45% and 2.0% in rodents and exhibiting systemic oral absorption fractions of only 23% to 47% in humans, accompanied by substantial inter-individual variability (Javed et al., 2011; Zhu et al., 2013; Xie et al., 2019).

This biopharmaceutical bottleneck arises from four converging physicochemical and physiological barriers:

1. **Thermodynamic Aqueous Insolubility:** Silymarin flavonolignans possess multi-ring, rigid polyphenolic structures with high crystal lattice energies and poor hydrogen-bonding capacity with bulk water. Aqueous solubility values for crude silymarin and pure silibinin at ambient physiological temperatures are reported between 6.2 μg/mLand 40 μg/mL(Choi et al., 2013; Javed et al., 2011). Under the Noyes–Whitney dissolution kinetics framework, this low saturation solubility (C_s) restricts the concentration gradient across the unstirred water layer adjacent to the intestinal mucosa, rendering dissolution the primary rate-limiting step in gastrointestinal absorption.
2. **Restricted Passive Transcellular Permeability:** With a molecular weight of 482.44 g/mol for silibinin and a partition coefficient ($\log P$) of approximately 1.4 to 2.4 depending on ionization state, flavonolignans exhibit suboptimal lipophilicity and high topological polar surface area (155.14 AA^2), impeding passive diffusion across the phospholipid bilayer of enterocytes (Piazzini et al., 2019a; Xie et al., 2019).
3. **Active Apical Efflux Transport:** Caco-2 monolayer and transporter-overexpressing Madin–Darby canine kidney (MDCKII) studies demonstrate that silybin A and silybin B are substrates for ATP-binding cassette (ABC) efflux transporters expressed on the apical brush-border membrane of enterocytes, specifically multidrug resistance-associated protein 2 (MRP2/ABCC2) and breast cancer resistance protein (BCRP/ABCG2), exhibiting efflux ratios of 5.05 and 4.61, respectively (Xie et al., 2019).
4. **Extensive Presystemic Phase II Biotransformation and Enterohepatic Recycling:** Upon entering enterocytes and hepatocytes, unconjugated flavonolignans undergo rapid stereoselective conjugation mediated by UDP-glucuronosyltransferases (UGT1A1, UGT1A6, UGT1A7, UGT1A9, UGT2B7, and UGT2B15) and sulfotransferases (SULTs), alongside minor cytochrome P450 2C8 (CYP2C8)-mediated *O*-demethylation (Albassam et al., 2017; Li et al., 2018; Xie et al., 2019). Consequently, greater than 80% of circulating systemic silybin exists as pharmacologically less active monoglucuronide (55%) and monosulfate (28%) conjugates, which are rapidly excreted into bile and urine with a terminal elimination half-life ($t_{1/2}$) of approximately 1 to 3 hours (Zhu et al., 2013; Xie et al., 2019).

1.3 Convergence of Nanopharmaceutics and Homeopathic Pharmaceutical Processing

To overcome the dissolution and permeability deficits of *Silybum marianum*, conventional pharmaceutical engineering has systematically applied nanoscale formulation strategies. By reducing particle dimensions below 1,000 nm—and

ideally within the 10 nm to 200 nm colloidal window—engineered nanocarriers exponentially increase specific surface area ($A/V \propto 1/r$), elevate kinetic saturation solubility via the Ostwald–Freundlich effect, bypass apical efflux pumps via endocytic or M-cell uptake, and promote intestinal lymphatic transport (Javed et al., 2011; Shangguan et al., 2013; Tu et al., 2024).

Remarkably, an entirely distinct historical tradition of pharmaceutical preparation—classical homeopathy, codified by Samuel Hahnemann in the early nineteenth century—employs mechanical attrition and hydrodynamic agitation protocols that inadvertently operate within the nanoscale regime (Rao et al., 2007; Chikramane et al., 2010; Bell et al., 2012). In homeopathic pharmacy, *Carduus marianus* is prepared either as an ethanolic mother tincture (Ø or Q) via maceration/percolation of ripe achenes in 60%–70% v/v ethanol-water, or via dry **trituration** of the crude botanical matrix with crystalline α-lactose monohydrate in a porcelain mortar and pestle, followed by serial decimal (1:10, X/D) or centesimal (1:100, C) dilutions accompanied by vigorous **succussion** (impaction) in borosilicate glass vials (Bell et al., 2012; Bell et al., 2015a; Van Wassenhoven et al., 2018).

For decades, homeopathic preparations diluted beyond Avogadro's limit (10^{-23} M, corresponding to 12C or 24X potencies) were dismissed by mainstream physical chemistry as devoid of any starting material. However, landmark materials-science investigations utilizing high-resolution transmission electron microscopy (HR-TEM), selected area electron diffraction (SAED), energy-dispersive X-ray spectroscopy (EDS), dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), and inductively coupled plasma mass spectrometry (ICP-MS) have demonstrated that both low-potency triturations and ultra-high homeopathic dilutions retain measurable populations of source-derived nanoparticles, lactose nanocrystals, nanobubbles, and glass-leached silicate nanostructures stabilized at air–liquid interfaces via froth flotation (Chikramane et al., 2010; Upadhyay et al., 2011; Chikramane et al., 2012; Temgire et al., 2016; Kalliantas et al., 2021).

This article presents an exhaustive, comparative nano-characterization of *Silybum marianum* formulations across these two paradigms: conventional phytochemical extraction coupled with engineered nanodelivery versus homeopathic trituration and potentization. Through detailed analysis of manufacturing physics, solid-state and colloidal properties, multi-modal analytical characterization, and cellular pharmacodynamics, this work delineates the structural convergences and fundamental mechanistic divergences between engineered botanical nanomedicines and trituration-derived nanostructures.

2. Pharmaceutical Processing Paradigms: Conventional Extraction vs. Homeopathic Trituration

The physicochemical architecture of any *Silybum marianum* formulation is dictated by the thermodynamic and mechanical pathways utilized to liberate, disperse, and stabilize its bioactive constituents from the tough lignocellulosic pericarp of the achene.

2.1 Conventional Phytochemical Extraction and Engineered Nanoformulation

Conventional extraction of *Silybum marianum* aims to maximize the mass yield, chemical purity, and stoichiometric reproducibility of the silymarin flavonolignan complex while eliminating inert structural polymers and interfering triglycerides. Because milk thistle achenes contain 20% to 30% fixed lipids (predominantly linoleic and oleic acids), standard pharmacopoeial protocols—such as those prescribed by the European Pharmacopoeia—mandate a two-stage solvent extraction process (Wianowska et al., 2014).

2.1.1 Solvent-Based Mass Transfer Mechanisms

In classical Soxhlet extraction, pulverized *Silybum marianum* fruits are first defatted for 4 to 6 hours using nonpolar aliphatic hydrocarbons such as *n*-hexane or petroleum ether (boiling range 60–90 °C) to strip neutral lipids without solubilizing polar flavonolignans (Wianowska et al., 2014; Zhao et al., 2016). Subsequently, the defatted meal is extracted for 5 to 6 hours using polar protic or aprotic organic solvents—most commonly methanol, ethanol, ethyl acetate, or acetone—followed by vacuum rotary evaporation to yield a dry amorphous or microcrystalline silymarin powder (Wianowska et al., 2014; Aziz et al., 2020). To overcome the thermal degradation risks and prolonged solvent consumption of Soxhlet extraction, intensified green extraction modalities have been developed:

- **Pressurized Liquid Extraction (PLE):** Operating at elevated temperatures (100–125 °C) and pressures (10–15 MPa) with acetone or ethanol, PLE achieves complete flavonolignan recovery within 10 minutes without requiring a prior defatting step, yielding approximately 28% higher silymarin recovery than 5-hour Soxhlet extraction (Wianowska et al., 2014).
- **Ultrasound-Assisted Enzymatic Extraction (UAEE):** Utilizing acoustic cavitation (200 W at 35 °C) combined with cellulolytic enzymes (cellulase at pH 5.0), UAEE disrupts the cellulose-hemicellulose matrix of the seed shell, enhancing mass transfer of silymarin into aqueous-ethanolic media while preserving thermolabile diastereomers (Zhao et al., 2016).
- **Supercritical Fluid Extraction and Dispersion (SCFE/SEDS):** Supercritical carbon dioxide ($scCO_2$) modified with ethanol co-solvents extracts silymarin cleanly and can be directly coupled with Solution-Enhanced Dispersion by Supercritical Fluids (SEDS) to co-precipitate silymarin with phospholipids and bile salts into nanoscale liposomes (Yang et al., 2015).

2.1.2 Bottom-Up and Top-Down Engineered Nanocarriers

Because conventional solvent evaporation yields bulk silymarin aggregates with particle diameters ranging from 10 μmto 150 μm, secondary nano-engineering is required to produce functional nanomedicines (Choi et al., 2013; Tu et al., 2024). These engineered systems fall into two primary fabrication categories:

1. **Bottom-Up Self-Assembly and Precipitation:** Molecularly dissolved silymarin in an organic phase is nucleated into nanostructures via antisolvent precipitation, emulsion-solvent evaporation, ionic gelation, or self-assembly with amphiphilic excipients. Examples include:

- *Lipid-Based Nanocarriers:* Solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs) blending solid lipids (e.g., cetyl palmitate, glycerol distearate) with liquid lipids (e.g., Lauroglycol 90, oleic acid), phytosomal-liposomal bilayers (silybin-phosphatidylcholine complexes), and self-nanoemulsifying drug delivery systems (SNEDDS) composed of medium-chain glycerides, Capmul, Tween 80, and Transcutol HP (Kidd et al., 2005; Shangguan et al., 2013; Kumar et al., 2014; Ahmad et al., 2015; Fang et al., 2015; Piazzini et al., 2019a).
- *Polymeric and Hybrid Nanocarriers:* Poly(D,L-lactic-co-glycolic acid) (PLGA) nanoparticles fabricated via single/double emulsion-solvent evaporation, chitosan-tripolyphosphate (TPP) nanoparticles prepared via ionotropic gelation, chitosan-functionalized lipid-polymer hybrid nanoparticles (CS-LPNs), and Soluplus/TPGS polymeric nanomicelles (Gupta et al., 2014; Snima et al., 2014; Liang et al., 2018; Piazzini et al., 2019b; Rahimnia et al., 2024).
- *Inorganic and Biogenic Metallic Nanocomposites:* Loading of extracted silymarin into ordered mesoporous silica nanoparticles (MSNs, such as MCM-41) via solvent impregnation, or utilizing silymarin polyphenolic hydroxyl groups as both reducing and capping agents to synthesize biogenic gold nanoparticles (AuNPs) from $HAuCl_4$and zinc oxide or silver nanoparticles (ZnO-NPs, AgNPs) from metal salts (Nasr et al., 2019; Clichici et al., 2020; Ibrahim et al., 2020; Abdullah et al., 2021).

2. **Top-Down Mechanical Comminution:** Coarse silymarin suspensions are subjected to high-energy mechanical shear, cavitation, or media milling—such as wet ball milling with xanthan gum and gum ghatti stabilizers, high-pressure homogenization, or Shirasu porous glass (SPG) membrane emulsification—to fracture macroscopic crystals into drug nanocrystals (Choi et al., 2013; Seo et al., 2024; Tu et al., 2024).

2.2 Homeopathic Trituration and Potentization of *Carduus marianus*

In sharp contrast to conventional extraction, homeopathic pharmaceutical manufacturing does not isolate or purify individual flavonolignans from the botanical matrix. Instead, it preserves the whole phytochemical ensemble—comprising flavonolignans, flavonoids, lignans, fixed oils, proteins, polysaccharides, and endogenous trace minerals—and subjects it to sequential mechanochemical attrition and hydrodynamic potentization (Bell et al., 2012; Bell et al., 2015a).

2.2.1 Dry Mechanochemical Trituration in Lactose Monohydrate

According to standard homeopathic pharmacopoeias (e.g., German Homoeopathic Pharmacopoeia [GHP], Homoeopathic Pharmacopoeia of the United States [HPUS], and Homoeopathic Pharmacopoeia of India [HPI]), solid triturations of *Carduus marianus* (typically prepared from dried, ripe, unroasted achenes) are executed by grinding the coarse botanical material with crystalline α-lactose monohydrate ($C_{12}H_{22}O_{11} \cdot H_2O$) at a 1:9 weight ratio (decimal scale, 1X) or 1:99 weight ratio (centesimal scale, 1C) (Bell et al., 2012; Chikramane et al., 2012).

Each trituration stage follows a strict, time-standardized mechanical protocol lasting 60 minutes, divided into three 20-

minute cycles. Within each cycle, the lactose carrier is introduced in three geometric portions, and each portion undergoes 6 minutes of vigorous manual or automated pestle grinding followed by 4 minutes of scraping the compacted powder from the porcelain mortar walls (Bell et al., 2012; Chikramane et al., 2012). From a materials-science perspective, prolonged mortar-and-pestle grinding constitutes a high-stress **top-down mechanochemical ball-milling process**. Localized compressive and shear stresses at the contact points between the porcelain pestle, mortar, and crystal asperities generate transient micro-hotspots, lattice dislocations, and brittle fracture (Rao et al., 2007; Bell et al., 2012). As α-lactose monohydrate crystals fracture along cleavage planes, they undergo partial solid-state amorphization and shed water of crystallization, creating high-energy, defect-rich surfaces capable of mechanochemically embedding and stabilizing sub-micron and nanoscale fragments of the botanical matrix (Chikramane et al., 2012; Bell et al., 2013). This solid-state grinding is typically repeated serially up to the 3C (10^{-6}) or 6X (10^{-6}) potency, at which point the triturated powder becomes dispersible in aqueous-ethanolic media.

2.2.2 Liquid Serial Dilution, Succussion, and Froth Flotation Physics

Beyond the 3C or 6X trituration stage—or directly from a *Carduus marianus* ethanolic mother tincture (macerated for 3 to 5 weeks in 65% v/v ethanol)—the preparation transitions to liquid potentization (Bell et al., 2015a; Van Wassenhoven et al., 2018). One part of the preceding potency is combined with 9 parts (decimal) or 99 parts (centesimal) of hydro-alcoholic diluent (typically 43% to 90% ethanol in double-distilled or purified water) inside a borosilicate or soda-lime glass vial occupying approximately two-thirds of the container volume, followed by 10 to 100 forceful downward succussion strokes against an elastic surface (Chikramane et al., 2010; Bell et al., 2015a).

Thermodynamic and fluid-dynamic analyses reveal three critical nanoscale phenomena occurring during succussion:

1. **Cavitation and Acoustic Shockwaves:** Rapid acceleration and abrupt deceleration of the liquid column generate transient pressure drops below the vapor pressure of the ethanol-water mixture, nucleating microscopic vapor-gas cavities (nanobubbles). Asymmetric collapse of these cavitation bubbles produces localized shockwaves, micro-jets, and extreme shear gradients that fragment suspended botanical-lactose aggregates into discrete nanoparticles (Rao et al., 2007; Bell et al., 2012).
2. **Silica Leaching and Epitaxial Templating:** The mechanical impact of succussion and alkaline micro-environments at glass–liquid interfaces erode nanometer-scale silica (SiO_2) and polysilicic acid species from the borosilicate glass walls (Ives et al., 2010; Upadhyay et al., 2011; Temgire et al., 2016). These nascent silica oligomers polycondense around suspended botanical nanoparticles and nanobubbles, forming stable organo-silicate core–shell nanostructures that inherit structural motifs via epitaxy (Rao et al., 2007; Upadhyay et al., 2011; Temgire et al., 2016).
3. **Froth Flotation and Surface-Layer Partitioning:** As demonstrated by Chikramane et al. (2012), vigorous succussion aerates the hydro-alcoholic medium, generating rising micro- and nanobubbles. Hydrophobic and amphiphilic nanoparticles—such as silymarin flavonolignan-lipid complexes and silica-coated botanical nanocrystals—adsorb selectively onto the air–liquid interface of these rising bubbles via **froth flotation**, concentrating within a thin surface monolayer (top 1% of the liquid volume). Because routine pharmaceutical pipetting aspirates aliquots from the upper meniscus of the vial to seed the subsequent dilution step, this surface-enriched nanoparticle population is preferentially transferred across serial dilutions, causing the nanoparticle concentration to reach a non-zero asymptotic plateau (typically in the pg/mL to ng/mL range, corresponding to $10^6$to 10^9particles/mL) rather than decaying exponentially to zero beyond Avogadro's threshold (Chikramane et al., 2010; Chikramane et al., 2012).

Processing Dimension	Conventional Extraction & Engineered Nanoformulation	Homeopathic Trituration & Potentization (*Carduus marianus*)
Starting Botanical Material	Dried, ripe achenes of *Silybum marianum*; typically defatted with *n*-hexane or petroleum ether prior to polar solvent extraction (Wianowska et al., 2014).	Whole dried, ripe, unroasted achenes of *Carduus marianus* (*Silybum marianum*) containing full lipid, protein, and flavonolignan matrix (Bell et al., 2012).
Primary Extraction / Dispersion Medium	Organic solvents (methanol, ethanol, acetone, ethyl acetate), supercritical CO_2, or aqueous-enzymatic buffers (Zhao et al., 2016).	Dry crystalline α-lactose monohydrate matrix (1:9 or 1:99 w/w) followed by hydro-alcoholic solutions (43%–90% v/v ethanol in purified water) (Bell et al., 2015a).

Energy Input Mechanism	Thermal reflux (Soxhlet), high pressure (PLE), ultrasonic cavitation (UAEE), high-pressure homogenization, or wet media milling (Choi et al., 2013; Seo et al., 2024).	Prolonged manual/mechanical mortar-and-pestle shear grinding (60 min/stage) followed by high-velocity percussive succussion in glass vials (Chikramane et al., 2012).
Nanoparticle Formation Pathway	Controlled "bottom-up" self-assembly (liposomes, NLCs, PLGA, micelles, MSNs) or stabilized "top-down" wet milling (nanocrystals) (Piazzini et al., 2019a; Tu et al., 2024).	"Top-down" mechanochemical attrition of botanicals + lactose, cavitation-induced fragmentation, glass silica leaching, and froth flotation enrichment (Chikramane et al., 2012; Temgire et al., 2016).
Excipients & Stabilizing Matrix	Synthetic or semi-synthetic surfactants (Tween 80, Poloxamer, Brij S20), phospholipids, PLGA, chitosan, or mesoporous silica (Kumar et al., 2014; Nasr et al., 2019).	Crystalline/amorphous α-lactose monohydrate, endogenous plant lipids/proteins, ethanol-water clathrates, and vessel-derived silicate shells (Upadhyay et al., 2011; Temgire et al., 2016).
Active Payload Concentration	High macroscopic mass loading (typically 10 mg/mL to 200 mg/mL; encapsulation efficiency 55%–99%) (Mombeini et al., 2018; Piazzini et al., 2019a).	Ultra-low, asymptotic trace concentrations (ng/mL in low potencies 1X–6X; pg/mL or 10^6–10^9 particles/mL in 30C–200C) (Chikramane et al., 2010; Bell et al., 2015b).

3. Multi-Parametric Comparative Nano-Characterization

Rigorous comparison between conventionally engineered *Silybum marianum* nanoformulations and homeopathic trituration-derived nanoparticles requires evaluating their physicochemical signatures across complementary analytical modalities: particle size distribution, morphology, electrokinetic surface charge, solid-state crystallinity, surface functional group chemistry, and elemental composition.

3.1 Particle Size Distribution and Polydispersity (DLS and NTA)

Dynamic Light Scattering (DLS) and Nanoparticle Tracking Analysis (NTA) serve as the primary hydrodynamic characterization tools for colloidal dispersions, though they probe distinct physical regimes. DLS measures time-dependent fluctuations in scattered laser intensity caused by Brownian motion to derive an intensity-weighted hydrodynamic diameter (Z-average) and Polydispersity Index (PDI), which is inherently biased toward larger scatterers ($I \propto d^6$). Conversely, NTA tracks the individual Brownian trajectories of single nanoparticles via ultramicroscopy, yielding number-weighted size distributions and absolute particle concentrations (particles/mL) with superior resolution for polydisperse systems (Bell et al., 2015a; Van Wassenhoven et al., 2018).

3.1.1 Hydrodynamic Profiles of Conventional Silymarin Nanoformulations

Engineered *Silybum marianum* nanocarriers are explicitly optimized through response-surface methodologies (such as Box–Behnken or central composite designs) to achieve narrow, unimodal hydrodynamic distributions tailored for mucosal penetration and hepatic passive targeting (Gupta et al., 2014; Rahimnia et al., 2024):

- **Polymeric Nanomicelles and Microemulsions:** Self-assembled silymarin polymeric nanomicelles utilizing Soluplus and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) exhibit ultra-small hydrodynamic diameters of 26.5 $\pm$4.3 nm to 62.4 nm with PDI values ranging from 0.16 to 0.53, permitting direct paracellular and transcellular passage across enterocytes (Mombeini et al., 2018; Piazzini et al., 2019b). Similarly, oil-in-water silymarin nanoemulsions stabilized with Tween 80 and Sefsol 218 achieve mean droplet diameters of 41.22 $\pm$0.003 nm and a narrow PDI of 0.165, maintaining physical stability over 3 months at 5 °C and 25 °C (Ahmad et al., 2015).
- **Lipid Nanocarriers (SLNs, NLCs, and Liposomes):** Binary lipid NLCs composed of glycerol distearate and oleic acid or cetyl palmitate and Lauroglycol 90 demonstrate mean diameters between 78.5 nm and 165.4 nm with PDI values consistently below 0.25 (Shangguan et al., 2013; Piazzini et al., 2019a). Supercritical fluid-prepared bile-salt liposomes exhibit uniform vesicular diameters of 112.6 $\pm$8.4 nm (PDI 0.19), whereas film-hydrated phytosomal-liposomal hybrids range from 140 nm to 210 nm (Kumar et al., 2014; Yang et al., 2015).

- **Polymeric and Inorganic Nanocomposites:** Silymarin-encapsulated PLGA nanoparticles fabricated via emulsion-solvent evaporation display mean diameters of 135 nm to 220 nm (PDI 0.12–0.21), while ionic-gelated chitosan-TPP nanoparticles measure 110 nm to 185 nm (Gupta et al., 2014; Snima et al., 2014; Rahimnia et al., 2024). Biogenic silymarin-coated gold nanoparticles (SGNPs) exhibit remarkably tight hydrodynamic sizes of 18 nm to 45 nm (PDI < 0.25), whereas wet-milled silymarin nanocrystals stabilized with xanthan gum and gum ghatti (HM40) or stabilizer-free nanocrystals measure between 240 nm and 450 nm (PDI < 0.30) (Clichici et al., 2020; Abdullah et al., 2021; Seo et al., 2024; Tu et al., 2024).

3.1.2 Hydrodynamic Profiles of Homeopathic Trituration-Derived Formulations

In contrast to the narrow, unimodal distributions engineered in conventional nanopharmaceutics, homeopathic triturations and potentized dispersions of *Carduus marianus* and analogous botanical/mineral stocks exhibit **broad, multimodal, and highly polydisperse** size distributions across both DLS and NTA (Bell et al., 2015a; Bell et al., 2015b; Van Wassenhoven et al., 2018).

- **Low-Potency Trituration Dispersions (1X–6X / 1C–3C):** When triturated *Carduus marianus* lactose powders are dispersed in aqueous or hydro-alcoholic media, DLS reveals a tri-modal distribution: a primary nanoscale fraction centered between **15 nm and 85 nm** (representing fractured flavonolignan-lipid complexes and primary lactose nanocrystallites), an intermediate sub-micron fraction spanning **150 nm to 450 nm** (comprising agglomerated botanical-silicate-lactose clusters and nanobubbles), and a micron-scale tail (> 1,000 nm) arising from partially undissolved lactose micro-fragments (Chikramane et al., 2012; Bell et al., 2013). Consequently, PDI values in trituration-derived dispersions routinely exceed 0.45 to 0.85, reflecting the stochastic nature of top-down mechanical comminution in the absence of synthetic steric stabilizers.

- **Ultra-High Potency Dispersions (12C, 30C, 200C):** NTA and DLS analyses of potentized homeopathic preparations demonstrate persistent nanoparticle populations ranging from **10 nm to 300 nm**, with modal diameters frequently clustering between **30 nm and 120 nm** across potencies (Bell et al., 2015a; Bell et al., 2015b; Kalliantas et al., 2021). Notably, particle size in potentized remedies does not decrease monotonically with increasing dilution factor; rather, successive succussion cycles induce continuous cycles of aggregate breakup and secondary coalescence, maintaining mean hydrodynamic sizes in the 40–150 nm range at concentrations of $10^7$to 10^9particles/mL (Bell et al., 2015a; Van Wassenhoven et al., 2018). Crucially, blinded controlled studies comparing verum botanical potencies (*Gelsemium sempervirens*, *Hypericum perforatum*, *Carduus marianus*) against succussed ethanol-water controls confirm that verum formulations exhibit statistically significant shifts in particle size distribution, higher total nanoparticle counts, and distinct scattering intensity profiles attributable to the presence of organic phytochemical templates (Bell et al., 2015a; Kalliantas et al., 2021).

3.2 Morphological Architecture (TEM, HR-TEM, SEM, and AFM)

Electron microscopy and atomic force microscopy (AFM) provide direct real-space visualization of nanoparticle geometry, surface topography, core–shell architecture, and aggregation state, circumventing the spherical-assumption artifacts of DLS.

3.2.1 Morphology of Conventional Engineered Silymarin Nanoparticles

- **Spherical Symmetry and Core–Shell Uniformity:** Transmission electron microscopy (TEM) and field-emission scanning electron microscopy (FE-SEM) of conventionally engineered silymarin nanocarriers predominantly reveal **smooth, discrete, spherical or quasi-spherical particles** with well-defined boundaries (Choi et al., 2013; Mombeini et al., 2018; Piazzini et al., 2019a). In polymeric nanomicelles stained with phosphotungstic acid, TEM micrographs demonstrate a distinct core–shell contrast consisting of an electron-dense amphiphilic corona surrounding a hydrophobic silymarin-loaded core of 26.1 $\pm$4.3 nm (Mombeini et al., 2018).

- **Liposomal Bilayers and Mesoporous Channels:** Cryo-TEM and negative-stain TEM of bile-salt and phytosomal silymarin liposomes reveal unilamellar or oligolamellar spherical vesicles with bilayer membrane thicknesses of 4 to 6 nm (Kumar et al., 2014; Yang et al., 2015). Conversely, HR-TEM of silymarin-loaded MCM-41 mesoporous silica nanoparticles displays highly ordered hexagonal arrays of cylindrical nanopores (pore diameter ~2.5–3.5 nm) in which silymarin molecules are molecularly confined, preventing external crystal growth (Nasr et al., 2019; Ibrahim et al., 2020).

- **Crystalline Facets in Nanocrystals and Biogenic AuNPs:** Stabilizer-free silymarin nanocrystals prepared via antisolvent precipitation exhibit elongated, rod-like or prismatic orthorhombic morphologies (length 350–450 nm, width 80–120

nm) with sharp crystallographic edges (Tu et al., 2024). Biogenic silymarin-reduced gold nanoparticles display predominantly spherical, triangular, and hexagonal polyhedral morphologies (15–35 nm) encapsulated within a thin, low-contrast organic halo (2–4 nm) composed of oxidized silymarin flavonolignans (Clichici et al., 2020; Abdullah et al., 2021).

3.2.2 Morphology of Homeopathic Trituration-Derived Nanoparticles

HR-TEM, FE-SEM, and AFM investigations of homeopathic triturations and potentized botanical/mineral preparations reveal a strikingly different morphological landscape characterized by **heterogeneous, irregular, faceted, and composite fractal aggregates** (Chikramane et al., 2010; Upadhyay et al., 2011; Temgire et al., 2016; Kalliantas et al., 2021):

- **Irregular Fracture-Derived Nanocrystals:** In dry-triturated *Carduus marianus* powders and their aqueous dispersions, SEM and TEM reveal angular, irregular, and plate-like nanocrystallites (5–50 nm) resulting from brittle mechanical cleavage of α-lactose monohydrate and plant cell-wall components during mortar-and-pestle attrition (Chikramane et al., 2012; Bell et al., 2013). Unlike the smooth spheres produced by interfacial tension in emulsion-evaporation, mechanically triturated nanoparticles possess high aspect ratios, stepped terraces, and acute fracture edges.

- **Core–Shell Organo-Silicate Encapsulation:** HR-TEM imaging of succussed homeopathic potencies demonstrates individual nanocrystals (5–20 nm) and primary nanoclusters embedded within or coated by an **amorphous, translucent silicate shell** (2–10 nm thick) derived from borosilicate glass dissolution during succussion (Upadhyay et al., 2011; Temgire et al., 2016). Selected Area Electron Diffraction (SAED) patterns obtained from these nanostructures frequently exhibit discrete concentric diffraction rings or bright Bragg spots superimposed on diffuse amorphous halos, confirming the coexistence of crystalline source/lactose nanodomains encased within amorphous polysilicic acid matrices (Chikramane et al., 2010; Upadhyay et al., 2011; Temgire et al., 2016).

- **Dendritic and Chain-Like Mesoscale Agglomerates:** Upon drying on carbon-coated copper grids or mica substrates for TEM and AFM analysis, homeopathic nanoparticles assemble into loose, fractal, chain-like, or dendritic superstructures spanning 100 nm to 500 nm, driven by capillary forces, hydrogen-bonded interfacial water bridges, and dipole-dipole interactions between silicate-coated particles (Upadhyay et al., 2011; Kalliantas et al., 2021).

3.3 Electrokinetic Surface Charge and Colloidal Stability (Zeta Potential)

Zeta potential (ζ), derived from electrophoretic light scattering via the Smoluchowski or Hückel approximations, quantifies the electrostatic potential at the hydrodynamic slipping plane of a dispersed particle and governs colloidal stability against van der Waals-induced flocculation.

- **Conventional Engineered Formulations:** Engineered *Silybum marianum* nanoformulations exhibit predictable, excipient-dictated zeta potentials. Anionic lipid nanocarriers (SLNs, NLCs, SNEDDS) and PLGA nanoparticles typically display moderately to strongly negative zeta potentials ranging from -18.5 mV**to** -38.4 mV, attributable to ionized carboxylic acid groups of free fatty acids (oleic acid), terminal carboxylates of PLGA, or phosphate headgroups of phosphatidylcholine (Shangguan et al., 2013; Snima et al., 2014; Fang et al., 2015; Piazzini et al., 2019a). Biogenic silymarin-capped gold nanoparticles exhibit zeta potentials around -22.0 mV**to** -31.5 mVdue to deprotonated phenolic hydroxyl groups on the surface-bound flavonolignan corona (Clichici et al., 2020; Abdullah et al., 2021). Conversely, chitosan-coated nanoparticles and CS-LPNs exhibit strongly positive zeta potentials ($+24.0$ mV **to** $+42.5$ mV) arising from protonated $-NH_3^+$glucosamine residues in mildly acidic media, which promotes electrostatic mucoadhesion to negatively charged intestinal sialic acid residues (Gupta et al., 2014; Liang et al., 2018). When combined with non-ionic steric stabilizers (Tween 80, Poloxamer 188, TPGS), $|\zeta| > 25$ mVensures multi-month thermodynamic or kinetic shelf stability (Ahmad et al., 2015; Piazzini et al., 2019b).

- **Homeopathic Trituration-Derived Formulations:** Homeopathic dispersions of *Carduus marianus* and related botanicals consistently exhibit **negative zeta potentials ranging from** -8.0 mV**to** -28.5 mVin purified water and hydro-alcoholic vehicles (Bell et al., 2015a; Bell et al., 2015b; Goyal et al., 2025). This negative surface charge originates from two synergistic sources: (1) deprotonated silanol groups (≡ Si–O$^-$) on the surface of glass-leached silica nanoparticles at near-neutral pH (above the isoelectric point of silica at pH ~2.0), and (2) dissociated phenolic hydroxyl and carboxylic acid moieties of adsorbed silymarin flavonolignans and organic acids (Bell et al., 2015a; Temgire et al., 2016). Notably, Bell et al. (2015a, 2015b) demonstrated that verum homeopathic potencies exhibit statistically more negative zeta potentials compared to unsuccussed solvent controls, indicating that mechanical succussion and source-templated silica assembly enhance interfacial charge density. Although $|\zeta|$values of 10–20 mV lie near the classical DLVO threshold for

incipient aggregation, colloidal persistence in homeopathic potencies is maintained by ultra-low particle volume fractions (minimizing collision frequency) and structured hydration shells around hydrophilic silanol and lactose-derived hydroxyl surfaces (Upadhyay et al., 2011; Bell et al., 2015a).

3.4 Solid-State Crystallography and Thermal Behavior (XRD and DSC)

X-Ray Diffraction (XRD) and Differential Scanning Calorimetry (DSC) elucidate the internal lattice order, polymorphic state, and drug–excipient molecular interactions within solid-state formulations.

- **Conventional Engineered Nanoformulations:** Crude extracted silymarin exhibits sharp, intense Bragg diffraction peaks at 2θangles of approximately $13.2°$, $14.6°$, $16.1°$, $19.8°$, $22.4°$, and $24.5°$, accompanied by a broad endothermic melting peak in DSC thermograms spanning $140°$Cto $168°$C(Choi et al., 2013; Yang et al., 2015; Ibrahim et al., 2020). When silymarin is encapsulated within NLCs, PLGA nanoparticles, supercritical fluid liposomes, or mesoporous silica pores, these characteristic crystalline Bragg reflections and melting endotherms **disappear completely** (Yang et al., 2015; Piazzini et al., 2019a; Ibrahim et al., 2020). This confirms that conventional bottom-up nanocarriers convert silymarin into a **high-energy molecularly dispersed or amorphous solid-state**, eliminating crystal lattice energy barriers to dissolution. In contrast, top-down wet-milled silymarin nanocrystals retain their native crystalline Bragg peaks with slightly reduced peak intensity and Scherrer broadening due to crystallite size reduction, demonstrating that their enhanced dissolution derives purely from increased specific surface area rather than amorphization (Choi et al., 2013; Seo et al., 2024; Tu et al., 2024).
- **Homeopathic Trituration-Derived Formulations:** Solid-state XRD and DSC analyses of triturated *Carduus marianus* preparations are dominated by the crystallographic signature of the α-lactose monohydrate excipient matrix (sharp peaks at $2\theta = 12.5°, 16.4°, 19.1°, 19.9°, 20.0°, 21.2°$) and a characteristic dehydration endotherm at ~$145°$C followed by melting at ~$215°$C (Chikramane et al., 2012; Bell et al., 2013). However, prolonged 60-minute mechanical trituration induces **mechanochemical lattice distortion, peak broadening, and partial solid-state amorphization** of the lactose carrier, generating disordered amorphous surface layers alongside intact crystalline nanodomains (Chikramane et al., 2012). Within evaporated residues of high-potency liquid dilutions, SAED and micro-XRD reveal nanoscale crystalline reflections attributable to retained source-derived mineral/organic nanocrystals and crystalline quartz/cristobalite-like silica polymorphs nucleated under high-pressure succussion cavitation (Chikramane et al., 2010; Upadhyay et al., 2011).

3.5 Surface Chemistry and Molecular Vibrational Fingerprints (FTIR and Raman)

Fourier-Transform Infrared (FTIR) and Raman spectroscopy probe covalent bond vibrations, hydrogen-bonding networks, and interfacial ligand coordination.

- **Conventional Extracted and Engineered Silymarin:** The FTIR spectrum of pure extracted silymarin displays a broad, intense absorption band centered at $3420 - 3450$ cm^{-1}(phenolic and alcoholic O–Hstretching), aliphatic C–Hstretching at $2924 - 2940$ cm^{-1}, a diagnostic benzopyranone conjugated ketone carbonyl (C=O) stretching band at $1635 - 1647$ cm^{-1}, aromatic ring skeletal vibrations (C=C) at 1510 cm^{-1}**and** 1465 cm^{-1}, and aryl ether (C–O–C) stretching bands at 1270 cm^{-1}**and** 1085 cm^{-1}(Saleh et al., 2026; Clichici et al., 2020; Ibrahim et al., 2020). In engineered nanocarriers (PLGA, NLCs, MSNs), the retention of the 1635 cm^{-1}carbonyl band without covalent shift confirms non-covalent physical entrapment, whereas broadening and red-shifting of the 3440 cm^{-1}O–Hband indicates intermolecular hydrogen bonding between silybin hydroxyls and phospholipid phosphate groups, chitosan amines, or surface silanols (Si–OH) of mesoporous silica (Kumar et al., 2014; Ibrahim et al., 2020; El-Demerdash et al., 2024). In biogenic silymarin-gold nanoparticles, attenuation of the phenolic O–Hband and emergence of a new carbonyl band near 1710 cm^{-1}demonstrate oxidation of catechol/flavonolignan hydroxyls to quinone moieties during $Au^{3+} \rightarrow Au^{0}$reduction, accompanied by metal–oxygen coordination bands in the $400 - 600$ cm^{-1}region (Clichici et al., 2020; Abdullah et al., 2021).
- **Homeopathic Trituration and Potentized *Carduus marianus*:** In low-potency *Carduus marianus* triturations (1X–3X), FTIR spectra exhibit an overlapping composite of α-lactose monohydrate bands (sharp crystalline O–Hstretch at 3525 cm^{-1}and C–O–Cglycosidic bands at $1030 - 1150$ cm^{-1}) and diagnostic botanical flavonolignan/lipid bands at 2924 cm^{-1}(aliphatic lipid CH_2stretching from seed fixed oils), 1740 cm^{-1}(triglyceride ester C=O), and 1635 cm^{-1}(silymarin flavone C=O). As preparations undergo serial dilution and succussion into higher potencies (6C–200C), organic flavonolignan vibrational bands fall below the bulk detection threshold of conventional transmission FTIR, and spectra become governed by structured hydro-alcoholic O–Hstretching envelopes ($3200 - 3600$ cm^{-1}) and characteristic **silicate vibrational modes** at $1080 - 1110$ cm^{-1}(asymmetric Si–O–Sistretching), 950 cm^{-1}(Si–OH silanol

stretching), and 795 cm^{-1}(symmetric Si–O–Sibending) (Rao et al., 2007; Upadhyay et al., 2011; Temgire et al., 2016). Subtle frequency shifts and bandwidth alterations in the O–Hstretching envelope of succussed verum potencies compared to succussed solvent controls reflect epitaxial structuring of interfacial water shells around source-templated organo-silicate nanoparticles (Rao et al., 2007; Bell et al., 2015a).

3.6 Elemental Composition and Trace Quantitation (EDS, ICP-MS, and ICP-AES)

Elemental profiling via Energy-Dispersive X-Ray Spectroscopy (EDS) coupled to TEM/SEM and Inductively Coupled Plasma Mass Spectrometry or Atomic Emission Spectroscopy (ICP-MS/ICP-AES) exposes the fundamental compositional divergence between the two formulation classes:

- **Conventional Formulations:** EDS spectra of organic silymarin nanocarriers (liposomes, PLGA, chitosan, nanocrystals) show exclusively carbon (C) and oxygen (O) (plus nitrogen Nin chitosan/phospholipids or phosphorus Pin phospholipids/TPP), with trace heavy metals strictly limited below ICH Q3D pharmacopoeial thresholds (< 1 ppm). In biogenic metallic or mesoporous silica nanocarriers, EDS and ICP-MS quantify stoichiometric elemental ratios of gold (Au), zinc (Zn), silver (Ag), or silicon (Si) deliberately introduced as carrier cores (Nasr et al., 2019; Clichici et al., 2020).
- **Homeopathic Trituration-Derived Formulations:** EDS microanalysis of individual nanoparticles isolated from homeopathic potencies consistently reveals a **multi-elemental signature** comprising carbon (C) and oxygen (O) from retained organic botanical/lactose residues, **silicon (**Si**)** (frequently accounting for 15% to 60% of inorganic atomic weight) leached from borosilicate glass vessels, and trace alkali/alkaline-earth and transition metals—including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), boron (B), aluminum (Al), and iron (Fe)—originating from both the mineral ash of the botanical achene and the glass container matrix (Chikramane et al., 2010; Upadhyay et al., 2011; Temgire et al., 2016; Van Wassenhoven et al., 2018). ICP-AES and ICP-MS analyses confirm that while bulk elemental concentrations in ultra-high potencies reside in the picogram-per-milliliter (pg/mL) to low nanogram-per-milliliter (ng/mL) regime, they remain significantly above theoretical Avogadro dilution predictions due to froth flotation enrichment and silicate surface adsorption (Chikramane et al., 2010; Chikramane et al., 2012; Bell et al., 2015b).

Characterization Parameter	Conventional Engineered *Silybum marianum* Nanoformulations	Homeopathic Trituration-Derived *Carduus marianus* Formulations
Hydrodynamic Size (Z-avg / NTA)	Unimodal, narrow: 25–65 nm (micelles/nanoemulsions/AuNPs); 80–220 nm (NLCs/liposomes/PLGA); 240–450 nm (nanocrystals) (Ahmad et al., 2015; Piazzini et al., 2019a; Tu et al., 2024).	Broad, multimodal: 15–85 nm primary fraction, 150–450 nm secondary clusters, and >1,000 nm lactose remnants in low potencies; 30–150 nm in 30C–200C (Bell et al., 2015a; Van Wassenhoven et al., 2018).
Polydispersity Index (PDI)	Low and controlled: typically 0.12 to 0.30 (Mombeini et al., 2018; Piazzini et al., 2019a).	High and heterogeneous: typically 0.45 to 0.85 due to unstabilized top-down attrition (Bell et al., 2015a).
Morphology (TEM / SEM)	Smooth, discrete spheres (liposomes, NLCs, PLGA), ordered hexagonal pores (MSNs), or faceted rods (nanocrystals) (Nasr et al., 2019; Tu et al., 2024).	Irregular, angular fracture nanocrystallites encased in amorphous silicate shells; dendritic/fractal agglomerates upon drying (Upadhyay et al., 2011; Temgire et al., 2016).
Zeta Potential (ζ)	Tunable by excipient: $-18$to -38 mV(anionic lipids/PLGA/AuNPs) or $+24$to $+42$ mV(chitosan-coated) (Liang et al., 2018; Clichici et al., 2020).	Consistently negative: $-8$to -28.5 mV, driven by ionized silanol ($\equiv$ Si–O^-) and phenolic/carboxylic groups (Bell et al., 2015a; Bell et al., 2015b).
Solid-State Crystallinity (XRD / DSC)	Fully amorphous molecular dispersion in lipid/polymer/MSN matrices; preserved native orthorhombic crystallinity in wet-milled nanocrystals (Choi et al., 2013; Ibrahim et al., 2020).	Dominated by crystalline α-lactose monohydrate with mechanochemical amorphization zones; nanocrystalline source/silica inclusions in SAED (Chikramane et al., 2012; Temgire et al., 2016).
Surface Chemistry (FTIR / Raman)	Intense flavonolignan bands (3440 cm^{-1} O–H, 1635 cm^{-1}C=O, 1510 cm^{-1}aromatic C=C) + carrier bands; H-bonding shifts (Ibrahim et al., 2020).	Lactose + lipid/flavonolignan bands in 1X–3X; dominated by silicate (Si–O–Si at 1090 cm^{-1}, Si–OHat 950 cm^{-1}) and structured O–Hbands in higher potencies (Rao et al., 2007; Temgire et al., 2016).
Elemental Profile (EDS / ICP-MS)	Purely organic (C, H, O, N, P) or defined synthetic inorganic cores (Au, Zn, Ag, Si) at mg/mL concentrations (Abdullah et al., 2021).	Composite C, O, Si(15%–60% inorganic fraction) + trace Na, K, Ca, Mg, B, Al, Feat pg/mL to ng/mL asymptotic levels

		(Chikramane et al., 2010; Temgire et al., 2016).

4. Biopharmaceutical, Pharmacokinetic, and Biological Mechanisms

The structural differences documented between conventionally engineered *Silybum marianum* nanoformulations and homeopathic trituration-derived nanoparticles translate into fundamentally distinct biopharmaceutical fates and biological mechanisms of action.

4.1 Mass-Action Pharmacokinetics and Receptor Pharmacology of Conventional Silymarin Nanoformulations

Conventional engineered *Silybum marianum* nanomedicines operate strictly within the paradigm of **stoichiometric mass-action pharmacology**, wherein therapeutic efficacy is proportional to the molar concentration of free flavonolignans delivered to target intracellular and membrane receptors in hepatocytes, hepatic stellate cells (HSCs), Kupffer cells, and neoplastic cells (Loguercio et al., 2011; Gillessen et al., 2020).

4.1.1 Enhancement of Dissolution, Permeability, and Oral Bioavailability

Across preclinical pharmacokinetic evaluations, nanoscale encapsulation dramatically elevates the peak plasma concentration ($C_{\max}$), accelerates time to peak concentration ($T_{\max}$), and expands the area under the plasma concentration–time curve ($AUC_{0-\infty}$) compared to unformulated silymarin suspensions:

- **Solid Nanoparticles and Nanocrystals:** SPG membrane-emulsified silymarin solid nanoparticles (mean diameter ~180 nm) increased aqueous solubility by more than 100-fold, yielding a 1.44-fold higher $C_{\max}$(22.07 μg/mL vs. 15.36 μg/mL), a halving of $T_{\max}$(0.90 h vs. 1.82 h), and a significant increase in AUC(185.34 h $\cdot$ μg/mL vs. 145.18 h $\cdot$ μg/mL) in rats, which translated into superior protection against CCl_4-induced centrilobular hepatic necrosis (Choi et al., 2013). Similarly, wet-milled milk thistle nanocrystals (HM40) significantly enhanced oral silybin AUCand $C_{\max}$in both rats and healthy human subjects while maintaining 24-month solid-state stability (Seo et al., 2024), whereas stabilizer-free silymarin nanocrystals demonstrated 1.41-fold higher saturation solubility and rapid mucosal uptake (Tu et al., 2024).
- **Lipid-Based and Polymeric Hybrid Nanocarriers:** Binary lipid NLCs (glycerol distearate/oleic acid) achieved a 2.54-fold increase in oral bioavailability in beagle dogs by combining rapid micellar solubilization in intestinal bile salts with chylomicron-mediated **intestinal lymphatic transport**, thereby bypassing hepatic first-pass UGT/SULT conjugation (Shangguan et al., 2013). Phytosomal-liposomal silymarin hybrids yielded a 3.5-fold increase in oral bioavailability in Wistar rats and a 10-fold greater suppression of lipopolysaccharide (LPS)-induced reactive oxygen species (ROS) in RAW 264.7 macrophages compared to plain silymarin suspension (Kumar et al., 2014). Most remarkably, chitosan-functionalized lipid-polymer hybrid nanoparticles (CS-LPNs) achieved a **14.38-fold enhancement in oral silymarin bioavailability** in rats and a 1.92-fold increase in hepatocellular uptake in fatty liver cells, markedly reducing hepatic triglycerides, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and macrovesicular steatosis in transgenic models of non-alcoholic fatty liver disease (Liang et al., 2018).
- **Self-Nanoemulsifying Systems and Nanomicelles:** Silymarin-loaded SNEDDS restored oral absorption even in rats subjected to Roux-en-Y gastric bypass surgery, where reduced gastric volume and altered bile flow severely impair conventional dissolution (Fang et al., 2015). Polymeric Soluplus/TPGS nanomicelles increased apparent permeability (P_{app}) across Caco-2 monolayers via energy-dependent clathrin- and caveolae-mediated endocytosis while inhibiting MRP2/BCRP efflux (Piazzini et al., 2019b).

4.1.2 Molecular Targets and Cellular Signaling

Once delivered intracellularly at micromolar concentrations, conventionally nanoformulated silymarin flavonolignans directly engage multiple molecular pathways:

- **Antioxidant Defense and Nrf2 Activation:** Direct scavenging of hydroxyl, peroxyl, and superoxide radicals, inhibition of lipid peroxidation (reducing malondialdehyde [MDA] and thiobarbituric acid reactive substances [TBARS]), restoration of reduced glutathione (GSH), and upregulation of nuclear factor erythroid 2-related factor 2 (Nrf2)-driven endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Loguercio et al., 2011; Nasr et al., 2019; El-Demerdash et al., 2024).
- **Anti-Inflammatory and Antifibrotic Modulation:** Suppression of nuclear factor-kappa B (NF-κB) nuclear translocation, downregulation of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), inhibition of transforming growth factor-beta 1

(TGF-$\beta 1$)/Smad signaling, and attenuation of hepatic stellate cell activation and α-smooth muscle actin (α-SMA) expression (Loguercio et al., 2011; Gillessen et al., 2020). In CCl_4-induced hepatic fibrosis, silymarin-conjugated gold nanoparticles (SGNPs) modulated profibrotic and antifibrotic microRNAs (downregulating miR-21 and upregulating miR-29b), outperforming free silymarin in reversing collagen deposition (Abdullah et al., 2021).

- **Antineoplastic and Apoptotic Pathways:** Silymarin PLGA nanoparticles, 5TR1 aptamer-decorated silybin PLGA nanoparticles, and silymarin nanomicelles induce G_1or G_2/Mcell-cycle arrest, mitochondrial membrane depolarization, Bax/Bcl-2 ratio elevation, and caspase-3/9-mediated apoptosis in prostate (PC-3) and colorectal (HT-29) carcinoma cells while sparing non-malignant fibroblasts (Snima et al., 2014; Mombeini et al., 2018; Rahimnia et al., 2024).

4.2 Non-Linear Hormesis and Allostatic Cross-Adaptation of Homeopathic Trituration-Derived Nanoparticles

Homeopathic preparations of *Carduus marianus*—particularly those triturated and potentized beyond 6X or 12C—contain nanoparticle masses (10^{-12} to 10^{-9} g/mL) that are six to nine orders of magnitude lower than the micromolar concentrations required to saturate enzymatic active sites or scavenge stoichiometric equivalents of free radicals (Bell et al., 2012; Bell et al., 2013). Consequently, their biological activity cannot be explained by classical mass-action receptor occupancy. Instead, interdisciplinary research led by Bell, Koithan, Chikramane, Khuda-Bukhsh, and colleagues has established the **Nanoparticle–Cross-Adaptation–Sensitization (NPCAS)** and **hormetic signaling** framework (Bell et al., 2012; Bell et al., 2013; Bhattacharyya et al., 2012).

4.2.1 Nanoparticles as Salient Cellular Stressors and Hormetic Triggers

Biological systems possess evolutionarily conserved danger-recognition mechanisms—including pattern recognition receptors (Toll-like receptors, scavenger receptors), mechanosensitive ion channels, and redox-sensitive transcription factors—that detect nanoscale particulate matter as a salient, low-intensity environmental stressor or "cell danger signal" (Bell et al., 2012; Bell et al., 2013). When administered at ultra-low, intermittent doses, source-templated organo-silicate and lactose-botanical nanoparticles trigger **hormesis**: a biphasic adaptive dose–response phenomenon characterized by low-dose stimulation of endogenous cellular defense, repair, and homeostatic recovery pathways in contrast to high-dose toxicity (Bell et al., 2012).

Crucially, the amorphous and nanocrystalline **silica shell** acquired during succussion in borosilicate glass is not biologically inert; biogenic and synthetic nanosilica acts as a potent biological adjuvant that adsorbs serum proteins (forming a dynamic protein corona), facilitates caveolae- or scavenger-receptor-mediated cellular uptake, stabilizes intracellular enzymes against thermal and oxidative denaturation, and amplifies immune and neuroendocrine signaling at picogram concentrations (Ives et al., 2010; Upadhyay et al., 2011; Bell et al., 2012).

4.2.2 Allostatic Cross-Adaptation and Time-Dependent Sensitization (TDS)

Under the NPCAS model, the specificity of a homeopathic *Carduus marianus* preparation resides in the structural and epitaxial information encrypted onto the nanoparticle surface by the botanical phytochemical template during trituration and succussion (Rao et al., 2007; Upadhyay et al., 2011; Bell et al., 2012). In a diseased organism whose allostatic stress-response network (comprising interconnected hepatic, immune, autonomic, and neuroendocrine axes) is locked into a maladaptive physiological attractor state (e.g., chronic hepatobiliary inflammation or metabolic oxidative stress), exposure to low-dose *Carduus marianus* nanoparticles initiates **heterologous postconditioning hormesis** and **allostatic cross-adaptation** (Bell et al., 2012; Bell et al., 2013).

Furthermore, intermittent administration of these nanostructures engages **time-dependent sensitization (TDS)**—a non-linear metaplastic amplification mechanism wherein repeated, spaced exposure to a low-intensity stimulus progressively amplifies the host organism's compensatory physiological response over time without requiring an increase in administered mass (Bell et al., 2012). Experimental investigations using ethanolic mother tinctures and potentized homeopathic preparations (*Carduus marianus*, *Phytolacca decandra*, *Gelsemium sempervirens*) have demonstrated differential modulation of antioxidant enzyme gene expression (SOD, CAT, GSH), attenuation of chromosomal aberrations, regulation of matrix metalloproteinase-9 (MMP-9), and induction of caspase-3-dependent apoptosis in hepatocarcinogenesis and melanoma models, mediated via epigenetic and transcriptional remodeling rather than direct stoichiometric antioxidant scavenging (Bhattacharyya et al., 2012; Das et al., 2013; Bell et al., 2013).

Mechanistic Dimension	Conventional Engineered *Silybum marianum* Nanoformulations	Homeopathic Trituration-Derived *Carduus marianus* Formulations

Governing Pharmacological Paradigm	Linear/saturable **mass-action pharmacology**; efficacy scales with molar drug concentration at target receptors (Loguercio et al., 2011).	Non-linear **hormesis**, **allostatic cross-adaptation**, and **time-dependent sensitization (TDS)** (Bell et al., 2012).
Primary Site of Bio-Interaction	Direct intracellular enzyme modulation (Nrf2, NF-κB, UGT, CYP2C8), free-radical scavenging, and membrane stabilization (Xie et al., 2019; Gillessen et al., 2020).	Membrane danger-signal sensors, scavenger/Toll-like receptors, protein corona interfaces, and systemic neuro-immuno-hepatic signaling networks (Bell et al., 2012; Bell et al., 2013).
Role of Carrier Matrix	Passive/active pharmacokinetic vehicle: solubilizes lipophilic silymarin, inhibits P-gp/MRP2/BCRP efflux, and promotes lymphatic uptake (Shangguan et al., 2013; Piazzini et al., 2019b).	Epitaxial structural template and biological adjuvant: glass-leached silica and mechanochemically altered lactose stabilize and amplify low-dose signaling (Ives et al., 2010; Upadhyay et al., 2011).
Dose–Response Relationship	Monotonic sigmoid dose–response curve up to receptor saturation ($E_{\max}$) (Javed et al., 2011; Zhu et al., 2013).	Biphasic (inverted U-shaped / J-shaped) hormetic curve; ultra-low doses stimulate adaptive recovery whereas bulk doses exert direct pharmacological effects (Bell et al., 2012).
Pharmacokinetic Profile	Measurable plasma $C_{\max}$(μg/mL), $T_{\max}$(0.5–2 h), AUC, and biliary enterohepatic recirculation (Choi et al., 2013; Seo et al., 2024).	Below conventional LC-MS/MS plasma quantification limits; biological signal propagation via systemic cellular and neuroendocrine cascades (Bell et al., 2013).

5. Analytical Challenges, Artifacts, and Methodological Standardization

Comparative nano-characterization across conventional and homeopathic *Silybum marianum* formulations is fraught with analytical pitfalls that can generate false-positive or false-negative conclusions if rigorous controls and orthogonal techniques are omitted (Klein et al., 2018; Krishnan et al., 2025).

5.1 Instrumental Sensitivity, Limit of Detection, and Sampling Bias

In conventional silymarin nanoformulations containing 1 to 100 mg/mLof active flavonolignans, routine DLS, UV-Vis spectrophotometry ($\lambda_{\max} = 286$–288 nm), and HPLC-DAD/LC-MS/MS operate well within their linear dynamic ranges (Ahmad et al., 2015; Li et al., 2018). Conversely, in homeopathic triturations diluted beyond 6X or 12C, particle concentrations drop to 10^6–10^9 particles/mLand elemental mass concentrations fall to pg/mLlevels, pushing analytical instrumentation to its physical limit of detection (LOD) (Bell et al., 2015b; Klein et al., 2018).

- **DLS vs. NTA Artefacts:** In dilute, polydisperse homeopathic dispersions, stray dust particles or a small fraction of micron-sized lactose or silica aggregates can scatter orders of magnitude more light than 20 nm source nanoparticles, skewing DLS Z-average diameters upward and producing unstable autocorrelation functions. Consequently, DLS must be paired with number-weighted NTA and high-resolution electron microscopy (Bell et al., 2015a; Krishnan et al., 2025).
- **TEM Drying and Pre-Concentration Artefacts:** Because TEM requires high vacuum, liquid dispersions must be evaporated onto carbon-coated grids. During solvent evaporation, dissolved solutes—including residual lactose, ethanol-soluble extractives, and monomeric silicic acid—undergo supersaturation and precipitate *de novo* as crystalline or amorphous nanostructures on the grid surface (Van Wassenhoven et al., 2018; Krishnan et al., 2025). Furthermore, pre-concentration steps (such as ultracentrifugation or rotary evaporation prior to TEM/ICP-MS) can induce artificial nanoparticle coalescence or concentrate background trace contaminants from glassware and reagents (Bell et al., 2015b). Cryogenic TEM (Cryo-TEM) and liquid-cell TEM, which vitrify aqueous-ethanolic suspensions in their native hydrated state without drying-induced crystallization, represent the gold standard for resolving true in-solution nanoparticle morphology (Krishnan et al., 2025).

5.2 Container Leaching, Stopper Contamination, and Essential Control Arms

A critical methodological lesson emerging from homeopathic nano-characterization studies is the substantial contribution of container and closure materials to the observed nanoparticle population (Ives et al., 2010; Bell et al.,

2015a; Bell et al., 2015b). Borosilicate glass vials shed silica and boron-containing nanoflakes during vigorous mechanical succussion, while traditional natural cork stoppers (*Quercus suber*) release suberin, tannins, and organic particulates into ethanolic solvents that produce confounding UV-Vis absorbance bands around 260–280 nm (overlapping with the UV absorption maximum of silymarin at 286 nm) (Bell et al., 2015a; Bell et al., 2015b).

Therefore, any scientifically defensible comparative nano-characterization protocol for *Silybum marianum* must incorporate **four mandatory parallel control arms** processed in identical pharmaceutical-grade Type I borosilicate glassware with inert polytetrafluoroethylene (PTFE) or medical-grade silicone closures under HEPA-filtered cleanroom conditions (Bell et al., 2015a; Klein et al., 2018):

1. **Unsuccussed Vehicle Control:** Pure hydro-alcoholic solvent (e.g., 65% v/v ethanol in ultrapure Milli-Q water) stored in identical vials without mechanical agitation, establishing baseline background particulate counts and dissolved silicate levels.
2. **Succussed Vehicle Control (Potentized Placebo):** Identical hydro-alcoholic solvent subjected to the exact same serial dilution and mechanical succussion regimen as the verum *Carduus marianus* potencies, isolating pure cavitation- and glass-leaching-derived silica nanostructures from source-templated nanoparticles.
3. **Succussed Lactose Trituration Control:** Pure α-lactose monohydrate triturated for 60 minutes per stage without *Carduus marianus* botanical material and subsequently potentized in hydro-alcoholic media, isolating mechanochemically generated lactose nanocrystals from botanical-lactose nanocomposites.
4. **Conventional Extract Reference Control:** Standardized extracted silymarin (characterized by HPLC for silybin A/B, isosilybin A/B, silychristin, silydianin, and taxifolin) dispersed at matched mass concentrations or formulated into benchmark nanocarriers (e.g., phytosomes or NLCs), providing an unequivocal chemical and structural baseline (Kroll et al., 2007; Wianowska et al., 2014).

5.3 Regulatory and Translational Implications

From a regulatory science perspective, the convergence of botanical nanopharmaceutics and homeopathic trituration raises important classification and quality-control questions. Conventional engineered *Silybum marianum* nanomedicines are evaluated under established FDA and EMA nanomaterial guidances requiring rigorous batch-to-batch verification of Critical Quality Attributes (CQAs)—specifically particle size distribution, PDI, zeta potential, encapsulation efficiency, *in vitro* release kinetics, and toxicological clearance (Javed et al., 2011; Gillessen et al., 2020). Conversely, homeopathic pharmacopoeias historically relied on macroscopic organoleptic tests, thin-layer chromatography (TLC), and liquid chromatography assays restricted to mother tinctures (where minimum silymarin content is specified, e.g., $\geq 0.080\%$m/m expressed as silibinin) while lacking physicochemical release specifications for triturated or potentized dosage forms (Klein et al., 2018). Integrating orthogonal nano-characterization metrics (NTA particle concentration, zeta potential, EDS silicon-to-carbon ratios, and Cryo-TEM morphology) into pharmacopoeial monographs would substantially enhance batch reproducibility, prevent manufacturing variability across succussion devices, and bridge the evidentiary gap between traditional botanical preparations and modern nanomedicine (Bell et al., 2015a; Krishnan et al., 2025).

6. Future Perspectives and Integrative Synthesis

The comparative examination of *Silybum marianum* formulations across conventional extraction and homeopathic trituration reveals an intriguing evolutionary arc in pharmaceutical science. Conventional phytochemical extraction initially sought to strip away the complex botanical matrix of milk thistle achenes to isolate pure crystalline silibinin—only to discover that stripping away the natural lipid and polyphenolic milieu created an insoluble, poorly absorbed molecule requiring sophisticated lipid, polymeric, or silica nanocarriers to restore its biological potency (Kidd et al., 2005; Javed et al., 2011; Piazzini et al., 2019a). Conversely, classical homeopathic pharmacy preserved the intact botanical achene matrix and subjected it to high-energy dry mechanochemical grinding in lactose followed by percussive succussion in glass—inadvertently generating self-assembled, silica-coated, and lactose-stabilized botanical nanoparticles capable of triggering low-dose hormetic and allostatic adaptations (Chikramane et al., 2012; Bell et al., 2012; Temgire et al., 2016).

Emerging research at the interface of green nanotechnology and phytomedicine is already synthesizing insights from both traditions. Studies utilizing homeopathic mother tinctures and low-potency botanical dilutions as intrinsic green reducing and capping templates for biogenic nanoparticle synthesis demonstrate that trace phytochemical

nanostructures retain sufficient surface reactivity to direct crystal nucleation and enhance biological efficacy (Bhattacharyya et al., 2012; Das et al., 2013; Goyal et al., 2025). Simultaneously, solvent-free mechanochemical co-grinding (ball milling) of silymarin with hydrophilic carbohydrates, mesoporous silica, or phospholipids—a direct industrial scale-up of classical dry trituration—is gaining prominence as an environmentally sustainable, green pharmaceutical manufacturing route that achieves nanoscale amorphization without toxic organic solvents (Chikramane et al., 2012; Ibrahim et al., 2020; Seo et al., 2024).

Future experimental investigations should prioritize head-to-head, blinded, multi-parametric characterization studies comparing standardized *Silybum marianum* extracts, engineered lipid/silica nanocarriers, mechanochemically co-milled silymarin-lactose nanocomposites, and serially triturated/potentized *Carduus marianus* preparations (spanning 1X, 3X, 6X, 6C, 30C, and 200C) under identical analytical conditions. Deploying Cryo-TEM, single-particle ICP-MS (spICP-MS), surface-enhanced Raman spectroscopy (SERS), and transcriptomic profiling in hepatocyte and hepatic stellate cell models will definitively map the structure–activity continuum linking particle size, silicate surface chemistry, dose-response kinetics, and therapeutic hepatoprotection.

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