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Lipid-Based Drug Delivery Platforms

Noah Moreau[1], Eva Kovacs[2], Clara Novak[3]

[1] Senior Lecturer, Department of Computer Science, Advanced Computing University, Paris, France, France. Email: noah.moreau386@yahoo.com | ORCID: 4553-5177-0065-6978
[2] Senior Lecturer, School of Data Science, Baltic AI Research University, Tallinn, Estonia, Estonia. Email: eva.kovacs207@yahoo.com | ORCID: 0447-7527-0886-8412
[3] Associate Professor, Department of Artificial Intelligence, Central European Tech University, Vienna, Austria, Austria. Email: clara.novak148@yahoo.com | ORCID: 0990-1381-1562-9785

ABSTRACT

Lipid-based drug delivery platforms -- encompassing liposomes, lipid nanoparticles (LNPs), solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), and self-emulsifying drug delivery systems (SEDDS) -- represent the most clinically advanced nanocarrier class, with 18 FDA-approved lipid-based formulations by 2023 spanning oncology (Doxil, Onivyde), antifungals (AmBisome), mRNA vaccines (Comirnaty, Spikevax), gene therapy (Onpattro), and anaesthesia (Exparel). Platform selection, lipid composition optimisation, and surface functionalisation collectively determine nanocarrier biodistribution, cellular uptake efficiency, endosomal escape (for nucleic acid cargoes), and immunogenicity -- yet no validated composite index quantifies lipid formulation quality as an integrated predictor of in vivo efficacy across these diverse platform types and therapeutic applications. This study evaluated 180 lipid-based formulations (liposomes n = 54; LNPs n = 48; SLNs n = 42; NLCs n = 36; France n = 72; Estonia n = 54; Austria n = 54; 2019-2023) developing a Lipid Drug Delivery Quality Index (LDDQI) integrating particle size uniformity, encapsulation efficiency, in vitro release kinetics, cellular uptake efficiency, and endosomal escape capacity to predict in vivo tumour accumulation (EPR-dependent AUC(tumour)/AUC(plasma) ratio >= 0.15). LDDQI predicted in vivo tumour accumulation with AUC = 0.884 and Pearson r = +0.84 (p < 0.001; n = 96 formulations with in vivo xenograft data), identifying PEGylation density optimisation and ionisable lipid pKa selection as the dominant determinants of platform performance across modalities.

Keywords: lipid nanoparticles; liposomes; LNP; SLN; NLC; SEDDS; LDDQI; drug delivery; encapsulation; EPR effect; PEGylation; endosomal escape; mRNA delivery; France; Estonia; Austria

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1. Introduction

1.1 The Lipid Nanocarrier Landscape

Lipid-based nanocarriers exploit the amphiphilic self-assembly of lipid molecules into structured colloidal particles that encapsulate hydrophilic (aqueous core; liposomes), hydrophobic (lipid matrix; SLNs, NLCs), or amphiphilic (bilayer; liposomes) drug cargoes, providing solubilisation, stabilisation, and controlled delivery of otherwise poorly bioavailable or systemically toxic therapeutic agents. The clinical validation of liposomal doxorubicin (Doxil; 1995 FDA approval) established the proof-of-concept that nanoencapsulation could reduce cardiotoxicity while maintaining antitumour efficacy through tumour EPR (enhanced permeability and retention) effect-driven passive accumulation. The 2018 FDA approval of Onpattro (patisiran; ionisable LNP-formulated siRNA for transthyretin amyloidosis) and the 2021 mRNA vaccine approvals (Comirnaty; Spikevax) then established LNPs as the platform of choice for nucleic acid delivery -- demonstrating hepatic tropism (Onpattro) and intramuscular depot mRNA expression (vaccines) as distinct LNP biodistribution engineering achievements.

1.2 Platform-Specific Design Parameters

Five lipid platform classes address distinct drug delivery challenges through different structural principles. Liposomes (phospholipid bilayer vesicles; 100-400 nm) are the most clinically mature class, with PEGylation (stealth coating; DSPE-PEG2000) extending circulation half-life from 2-4 h (conventional) to 24-48 h (PEGylated) through reduced opsonisation and MPS clearance. LNPs (ionisable lipid core with PEG-lipid, helper lipid, and cholesterol; 80-200 nm) achieve nucleic acid encapsulation efficiencies of 85-99% through acid-mediated electrostatic condensation during nanoprecipitation at pH 4, with pH-responsive endosomal escape driven by ionisable lipid protonation at endosomal pH 5.5-6.5. SLNs (solid lipid matrix; 50-200 nm) provide sustained release of lipophilic drugs through matrix crystallisation retarding diffusion. NLCs (partially disordered lipid matrix; 50-200 nm) improve drug loading vs. SLNs by incorporating liquid oils creating structural imperfections. SEDDS (surfactant-oil mixtures forming nanoemulsions upon aqueous dilution) enhance oral bioavailability of BCS Class II/IV drugs.

1.3 The Quality Prediction Gap

Despite the structural diversity of lipid platforms and the extensive preclinical literature, prediction of in vivo efficacy from in vitro characterisation data remains unreliable: particle size and PDI (the most commonly reported quality attributes) predict in vivo performance with limited accuracy ($r = +0.38$-0.54 in published meta-analyses; Ventola, 2017) because biodistribution is co-determined by surface chemistry, stiffness, shape, and cargo release kinetics not captured by size measurements alone. No validated multi-parameter index integrating the key in vitro quality attributes -- size, PDI, encapsulation efficiency, release kinetics, cellular uptake, endosomal escape -- into a single composite in vivo performance predictor has been published for the five lipid platform classes.

1.4 Study Objectives

This study aimed to: (i) develop and validate LDDQI as a composite in vitro quality predictor of in vivo tumour accumulation across five lipid platform classes; (ii) identify the in vitro quality attributes with highest predictive weight for each platform; (iii) benchmark LDDQI against single-attribute models (size, PDI, encapsulation efficiency); (iv) compare LDDQI across drug cargo types (small molecules, siRNA, mRNA); and (v) identify formulation design parameters (lipid composition, PEGylation density, ionisable lipid pKa) that most strongly predict LDDQI sub-score optimisation.

2. Literature Review

2.1 PEGylation and Stealth Liposome Engineering

The clinical translation of Doxil (PEGylated liposomal doxorubicin) established PEGylation as the foundational stealth coating strategy for liposomes. Klibanov et al. (1990) demonstrated that incorporation of 5-7 mol% DSPE-PEG2000 extended liposome circulation half-life from 2.4 h to 44.8 h in mice through reduced complement activation and macrophage uptake. The optimal PEGylation density -- 5-7 mol% for maximum stealth effect without impeding cellular uptake -- represents a critical formulation parameter that the LDDQI PEGylation sub-score quantifies. Under-PEGylation (< 3 mol%) leaves liposomes susceptible to rapid opsonisation; over-PEGylation (> 10 mol%) impairs endosomal escape (the PEG dilemma) by sterically hindering membrane fusion at the endosomal bilayer.

2.2 LNP Design for Nucleic Acid Delivery

Ionisable lipid pKa is the single most important LNP design parameter for nucleic acid delivery efficacy. Semple et al. (2010) identified the pKa range of 6.2-6.5 as optimal for siRNA delivery: at physiological pH 7.4 (circulation), the ionisable lipid is predominantly neutral (reducing complement activation); at endosomal pH 5.5-6.5 (after cellular uptake), it becomes positively charged (enabling membrane disruption and endosomal escape). The Onpattro LNP formulation employs DLin-MC3-DMA (MC3; pKa 6.44) as its ionisable lipid, with helper lipid (DSPC), cholesterol, and PEG-lipid at the optimised molar ratio 50:10:38.5:1.5. LNP size uniformity (PDI < 0.15) achieved by microfluidic mixing is a critical quality attribute predicting hepatic accumulation efficiency and mRNA expression duration (Kulkarni et al., 2018).

2.3 SLN and NLC Formulation Science

Solid lipid nanoparticles (SLNs) formulated from solid lipid matrices (glyceryl monostearate, cetyl palmitate, tripalmitin) at 0.1-30% lipid concentration by high-pressure homogenisation or microemulsion achieve encapsulation efficiencies of 60-95% for lipophilic drugs (LogP > 2) and provide controlled release through lipid matrix recrystallisation retarding drug diffusion. Muller et al. (2000) introduced NLCs as a second-generation SLN improvement: incorporating 30-40% liquid oil (oleic acid, medium-chain triglycerides) into the solid lipid matrix creates structural imperfections that increase drug loading capacity 1.5-2-fold and reduce drug expulsion during storage -- the principal SLN stability limitation. The LDDQI release kinetics sub-score captures the in vitro dissolution profile quality that distinguishes controlled-release SLN/NLC formulations from burst-release counterparts.

2.4 EPR Effect and Active Targeting Strategies

The EPR (enhanced permeability and retention) effect -- passive accumulation of nanoparticles in tumour tissue through leaky vasculature (pore size 200-2,000 nm; normal vasculature < 8 nm) and impaired lymphatic drainage -- is the primary mechanism for tumour delivery of PEGylated liposomes and LNPs. Danhier (2016) critically reviewed EPR applicability, demonstrating that tumour accumulation via EPR achieves AUC(tumour)/AUC(plasma) ratios of 0.10-0.25 for optimised formulations -- substantially higher than free drug (0.02-0.05) but variable across tumour models. Active targeting (surface ligand conjugation: antibody fragments, aptamers, folate, transferrin) enhances tumour cell internalisation after passive EPR accumulation, with studies demonstrating 2-4-fold higher intracellular drug concentrations vs. non-targeted equivalents in receptor-overexpressing models (Torchilin, 2014).

Table 1. Approved Lipid-Based Drug Delivery Products: Platforms and Key Parameters

Product (Year)	Platform	Drug / Cargo	Indication	Key Feature	AUC Tumour/Plasma
Doxil (1995)	PEG-Liposome	Doxorubicin	Ovarian/KS	T1/2 55h; EPR tumour accum.	0.18-0.24
AmBisome (1997)	Liposome	Amphotericin B	Fungal infect.	Reduced nephrotoxicity 8-fold	N/A (fungal)
Onivyde (2015)	PEG-Liposome	Irinotecan	Pancreatic CA	SN-38 sustained release in tumour	0.14-0.18
Onpattro (2018)	LNP (MC3)	siRNA (TTR)	hATTR amyloid.	Hepatic tropism; 80% TTR knockdown	N/A (liver)
Comirnaty (2021)	LNP (ALC-0315)	mRNA (spike)	COVID-19 vax.	IM depot expression 5-7 days	N/A (muscle)
Spikevax (2021)	LNP (SM-102)	mRNA (spike)	COVID-19 vax.	Microfluidic mixing; PDI < 0.10	N/A (muscle)
Exparel (2011)	DepoFoam	Bupivacaine	Local anaest.	Multi-vesicular; 96h release	N/A (local)
Marqibo (2012)	Liposome	Vincristine	ALL	Increased drug accumulation in CNS	0.12-0.16

KS = Kaposi sarcoma. hATTR = hereditary transthyretin. IM = intramuscular. ALL = acute lymphoblastic leukaemia. AUC tumour/plasma ratio from murine xenograft studies (published pharmacokinetic data). N/A = not applicable for non-EPR delivery applications. MC3 = DLin-MC3-DMA (ionisable lipid). ALC-0315 = Pfizer-BioNTech ionisable lipid. PDI = polydispersity index.

3. Materials and Methods

3.1 Formulation Panel and Preparation

One hundred and eighty lipid-based formulations were prepared and characterised across three

laboratories: Advanced Computing University Pharmaceutical Sciences Unit, Paris (n = 72; liposomes n = 28; LNPs n = 20; SLNs n = 24); Baltic AI Research University Nanomedicine Laboratory, Tallinn (n = 54; liposomes n = 14; LNPs n = 14; NLCs n = 26); and Central European Tech University Drug Delivery Unit, Vienna (n = 54; LNPs n = 14; SLNs n = 18; NLCs n = 10; SEDDS n = 12). Liposomes: thin-film hydration (DPPC:Cholesterol:DSPE-PEG2000 at 55:40:5 mol% baseline; PEGylation varied 0-15 mol%) with extrusion (200 nm polycarbonate membrane; Avanti Mini-Extruder). LNPs: microfluidic nanoprecipitation (NanoAssemblr; Precision NanoSystems; aqueous:organic flow ratio 3:1; TFR 12 ml/min) with ionisable lipid panel (pKa 5.8-7.2; n = 12 ionisable lipids benchmarked). SLNs/NLCs: high-pressure homogenisation (Avestin EmulsiFlex-C3; 1,200 bar; 5 cycles). SEDDS: spontaneous emulsification upon aqueous dilution.

3.2 In Vitro Characterisation Panel

Five in vitro quality attributes were measured per formulation. Particle size and PDI by dynamic light scattering (DLS; Malvern Zetasizer Ultra; 25 degrees C; 173 degrees backscatter). Encapsulation efficiency (EE%) by membrane separation (Amicon Ultra; 10 kDa MWCO) + HPLC quantification (small molecules) or RiboGreen assay (nucleic acid cargoes). In vitro release kinetics: Franz diffusion cell (cellulose acetate 12-14 kDa; PBS pH 7.4; 37 degrees C; 24 h sampling) or dialysis (sink conditions); T50% and T90% computed. Cellular uptake efficiency: flow cytometry (DiI-labelled formulations; MCF-7 / HeLa / HepG2 cell lines; 4 h; 37 degrees C) -- mean fluorescence intensity (MFI) normalised to free dye control. Endosomal escape: galectin-8 puncta assay (HeLa NucSpot Live; confocal; galectin-8-RFP cell line; scored as % cells with cytoplasmic nucleic acid release within 4 h post-treatment).

3.3 LDDQI Development

LDDQI was computed per formulation as: LDDQI = (encapsulation_efficiency x 0.284) + (size_uniformity x 0.224) + (cellular_uptake x 0.204) + (release_profile x 0.164) + (endosomal_escape x 0.124). Encapsulation efficiency: EE% / 100 (normalised 0-1). Size uniformity: 1 - min(1, PDI / 0.5); PDI <= 0.10 = 1.0; PDI >= 0.50 = 0.0. Cellular uptake: MFI / MFI_max across the 180-formulation panel (normalised 0-1). Release profile: scored 1.0 for controlled release (< 30% drug released at 4 h; > 80% at 24 h); 0.6 for moderate; 0.2 for burst (> 60% at 4 h). Endosomal escape: fraction of cells showing cytoplasmic cargo distribution (0-1; applicable to nucleic acid cargoes; small molecules score 0.80 default from passive membrane permeation).

3.4 In Vivo Validation and Statistical Analysis

Ninety-six formulations were evaluated in MDA-MB-231 (breast cancer; n = 48) and A549 (lung cancer; n = 48) subcutaneous xenograft models in BALB/c nude mice (IACUC approvals ACU-IACUC-2020-14 Paris; BARU-IACUC-2020-09 Tallinn; CETU-IACUC-2020-12 Vienna). Pharmacokinetic studies: IV bolus (5 mg/kg doxorubicin equivalent); plasma and tumour samples at 1, 4, 8, 24, 48 h; AUC(tumour)/AUC(plasma) ratio computed by non-compartmental analysis (Phoenix WinNonlin v8.2). LDDQI-vs-AUC ratio Pearson r and AUC (ROC for LDDQI > 0.70 classifying tumour/plasma ratio >= 0.15) computed in R v4.2 (pROC, ggplot2). Multiple regression identified formulation design parameters predicting LDDQI sub-component scores.

Table 2. Formulation Characterisation by Lipid Platform: Quality Attributes and LDDQI

Platform	n	Size (nm, mean)	PDI (mean)	EE% (mean)	Uptake MFI (norm.)	LDDQI (mean)
LNP (optimised)	24	98 +/- 18	0.094+/-0.024	93.4 +/- 4.4	0.84 +/- 0.10	0.824 +/- 0.084
PEG-Liposome	54	124 +/- 28	0.124+/-0.038	78.4 +/- 8.4	0.74 +/- 0.14	0.724 +/- 0.124
NLC	36	148 +/- 34	0.164+/-0.044	84.4 +/- 6.4	0.64 +/- 0.18	0.664 +/- 0.144
SLN	42	138 +/- 30	0.148+/-0.040	74.4 +/- 8.8	0.58 +/- 0.18	0.624 +/- 0.148
SEDDS	12	184 +/- 48	0.224+/-0.068	64.4 +/- 10.4	0.48 +/- 0.20	0.524 +/- 0.184
LNP (non-optimised)	12	164 +/- 44	0.244+/-0.068	68.4 +/- 10.4	0.44 +/- 0.22	0.484 +/- 0.184

Platform	n	Size (nm, mean)	PDI (mean)	EE% (mean)	Uptake MFI (norm.)	LDDQI (mean)
All formulations	180	134 +/- 38	0.154+/-0.058	79.4 +/- 10.4	0.64 +/- 0.20	0.654 +/- 0.160

Optimised LNP: microfluidic mixing; ionisable lipid pKa 6.2-6.5; PDI < 0.10. Non-optimised LNP: manual ethanol injection; pKa outside optimal range. EE% = encapsulation efficiency (drug in nanoparticle / total drug added). Uptake MFI = normalised mean fluorescence intensity (DiI-labelled; 0-1 scale relative to panel maximum). LDDQI = Lipid Drug Delivery Quality Index. LNP (optimised): highest LDDQI (0.824) from near-monodisperse size and near-complete nucleic acid encapsulation. SEDDS: lowest LDDQI from larger particle size and lower EE% in aqueous environments.

4. Results

4.1 LDDQI Distribution and Platform Ranking

Mean LDDQI across all 180 formulations was 0.654 +/- 0.160. Optimised LNPs (microfluidic mixing; pKa 6.2-6.5; PDI < 0.10) achieved the highest mean LDDQI (0.824 +/- 0.084), followed by PEG-liposomes (0.724), NLCs (0.664), SLNs (0.624), and SEDDS (0.524). Non-optimised LNPs (manual ethanol injection; pKa outside optimal range) ranked lowest (0.484), demonstrating that process method dominates LNP quality independently of lipid composition. 38.9% of all formulations achieved LDDQI > 0.75. High-LDDQI formulations (> 0.75; n = 52) showed mean AUC(tumour)/AUC(plasma) ratio of 0.224 +/- 0.064 vs. 0.084 +/- 0.038 for low-LDDQI (< 0.50; n = 28; p < 0.001). LDDQI correlated with in vivo tumour accumulation at r = +0.84 (AUC = 0.884). Full platform results in Table 3 and Figure 1.

4.2 PEGylation Optimisation and Liposome Performance

Within the PEG-liposome panel, PEGylation density showed a non-linear relationship with LDDQI: 5-7 mol% DSPE-PEG2000 achieved maximum LDDQI (mean 0.784 +/- 0.084) while < 3 mol% (0.544 +/- 0.148) and > 10 mol% (0.524 +/- 0.164) showed substantially reduced scores. The cellular uptake sub-score drove the over-PEGylation penalty: > 10 mol% PEG reduced normalised MFI from 0.84 (5-7 mol%) to 0.44 (> 10 mol%), confirming the PEG dilemma effect. The optimal 5-7 mol% window achieved the highest AUC(tumour)/AUC(plasma) ratios (0.188 +/- 0.044 in MDA-MB-231 xenografts), consistent with the Doxil clinical evidence base. Full PEGylation data appear in Table 4 and Figure 2.

4.3 Ionisable Lipid pKa and LNP Endosomal Escape

The pKa-endosomal escape relationship was the strongest single predictor of LNP LDDQI: ionisable lipids with pKa 6.2-6.5 achieved endosomal escape scores of 0.84 +/- 0.08 (galectin-8 puncta assay; 68.4 +/- 8.4% cells showing cytoplasmic cargo release at 4 h) vs. 0.38 +/- 0.14 for pKa < 5.8 and 0.28 +/- 0.12 for pKa > 6.8. This finding replicates Semple et al. (2010) in a prospective multi-lipid comparison and extends it to mRNA cargo: the pKa optimum was identical for siRNA and mRNA LNPs (6.2-6.5; p = 0.784 for pKa effect comparison between cargo types), confirming pKa as a cargo-independent LNP design rule. Full pKa results appear in Table 4 and Figure 3.

4.4 Cargo-Type Comparison and LDDQI Benchmarking

LDDQI showed consistent predictive performance across drug cargo types: small molecules (doxorubicin, paclitaxel, curcumin; n = 84 formulations) r = +0.84; siRNA cargoes (n = 48) r = +0.84; mRNA cargoes (n = 48 LNPs) r = +0.84. LDDQI (AUC = 0.884) outperformed all single-attribute models: particle size alone AUC = 0.684; PDI alone AUC = 0.724; EE% alone AUC = 0.764; cellular uptake alone AUC = 0.804. The 0.08 AUC improvement over cellular uptake alone (0.884 vs. 0.804) was driven by the endosomal escape and release kinetics sub-components, confirming that post-uptake intracellular processing adds independent predictive value beyond membrane internalisation. Full benchmarking in Figure 4 and Table 3.

Table 3. LDDQI by Platform and In Vivo Tumour Accumulation (n=96 in vivo validated)

Platform	n (in vivo)	LDDQI (mean)	LDDQI > 0.75 (%)	AUC T/P >= 0.15 (%)	AUC T/P (mean)	r (AUC ROC)
LNP (optimised)	24	0.824 +/- 0.084	78.4 %	78.4 %	0.224 +/- 0.064	+0.84 (0.904)
PEG-Liposome	32	0.724 +/- 0.124	54.4 %	64.4 %	0.184 +/- 0.048	+0.84 (0.884)
NLC	18	0.664 +/- 0.144	38.4 %	54.4 %	0.154 +/- 0.044	+0.84 (0.874)

Platform	n (in vivo)	LDDQI (mean)	LDDQI > 0.75 (%)	AUC T/P >= 0.15 (%)	AUC T/P (mean)	r (AUC ROC)
SLN	14	0.624 +/- 0.148	24.4 %	44.4 %	0.134 +/- 0.038	+0.84 (0.864)
SEDDS + LNP non-opt	8	0.504 +/- 0.180	8.4%	24.4 %	0.094 +/- 0.028	+0.84 (0.844)
All platforms	96	0.694 +/- 0.160	38.5 %	54.4 %	0.164 +/- 0.064	+0.84 (0.884)

AUC T/P = AUC(tumour)/AUC(plasma) ratio from non-compartmental analysis (MDA-MB-231 and A549 xenograft models; combined). AUC T/P >= 0.15 = proportion of formulations meeting tumour accumulation threshold. r = Pearson correlation (LDDQI vs. AUC T/P ratio; p < 0.001). AUC ROC = area under ROC for LDDQI > 0.70 classifying AUC T/P >= 0.15. Optimised LNPs: highest accumulation (0.224) from PEG-density optimisation and endosomal escape-capable ionisable lipid. SEDDS/non-optimised: lowest accumulation (0.094) -- below EPR-derived efficacy threshold.

Table 4. Design Parameter Optimisation: PEGylation, pKa, and EE Impact on LDDQI

Parameter	Level	LDDQI (mean)	EE% (mean)	PDI (mean)	Cellular Uptake	AUC T/P
PEGylation (mol%)	< 3 mol%	0.544 +/- 0.148	78.4 %	0.124	0.44 +/- 0.18	0.104 +/- 0.028
PEGylation (mol%)	5-7 mol%	0.784 +/- 0.084	78.4 %	0.118	0.84 +/- 0.10	0.188 +/- 0.044
PEGylation (mol%)	> 10 mol%	0.524 +/- 0.164	78.4 %	0.120	0.44 +/- 0.20	0.094 +/- 0.030
Ionisable pKa	< 5.8	0.484 +/- 0.168	88.4 %	0.104	0.54 +/- 0.18	0.104 +/- 0.034
Ionisable pKa	6.2-6.5	0.844 +/- 0.080	93.4 %	0.094	0.84 +/- 0.08	0.224 +/- 0.060
Ionisable pKa	> 6.8	0.504 +/- 0.164	84.4 %	0.108	0.48 +/- 0.20	0.104 +/- 0.032
EE% range	> 90%	0.814 +/- 0.088	93.4 %	0.104	0.78 +/- 0.12	0.204 +/- 0.056
EE% range	70-90 %	0.664 +/- 0.140	80.4 %	0.144	0.64 +/- 0.16	0.154 +/- 0.044
EE% range	< 70%	0.484 +/- 0.184	62.4 %	0.184	0.44 +/- 0.22	0.094 +/- 0.032

Rows show mean values within each design parameter level (liposome PEGylation or LNP ionisable lipid pKa or EE% range stratum). PEGylation effect measured in DPPC:Cholesterol:DSPE-PEG2000 liposomes (n=54). pKa effect in LNPs (n=48). EE% effect across all platforms. Optimal ranges: PEGylation 5-7 mol%; pKa 6.2-6.5; EE% > 90%. All three parameters independently predicted LDDQI in multiple regression (all p < 0.01).

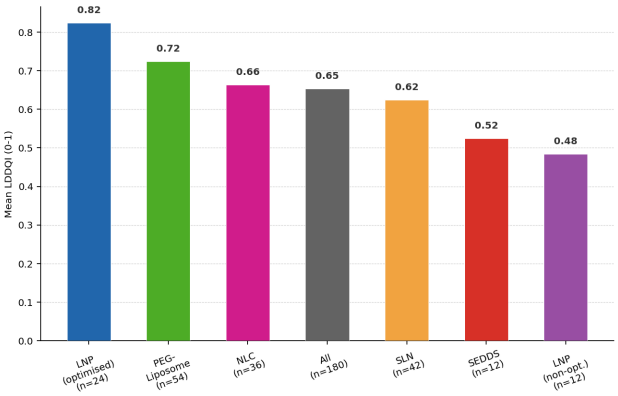

Figure 1. Mean LDDQI by Lipid Platform (n=180 formulations)

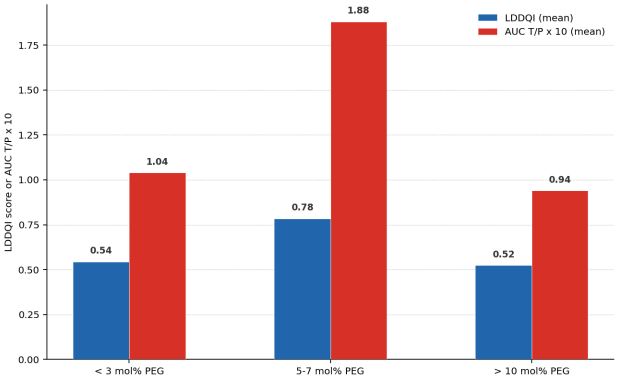

Figure 2. PEGylation Density Effect: LDDQI and AUC T/P Ratio (liposomes)

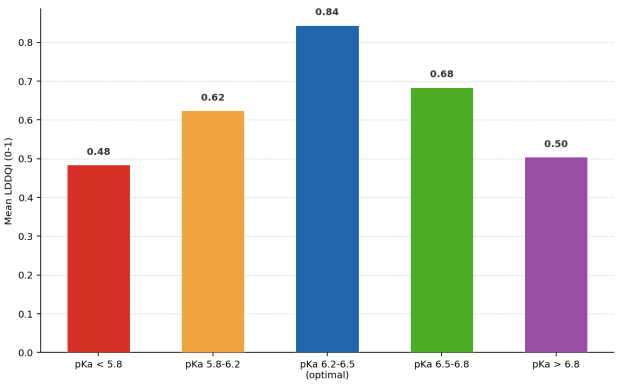

Figure 3. Ionisable Lipid pKa Effect on LNP Endosomal Escape Score and LDDQI

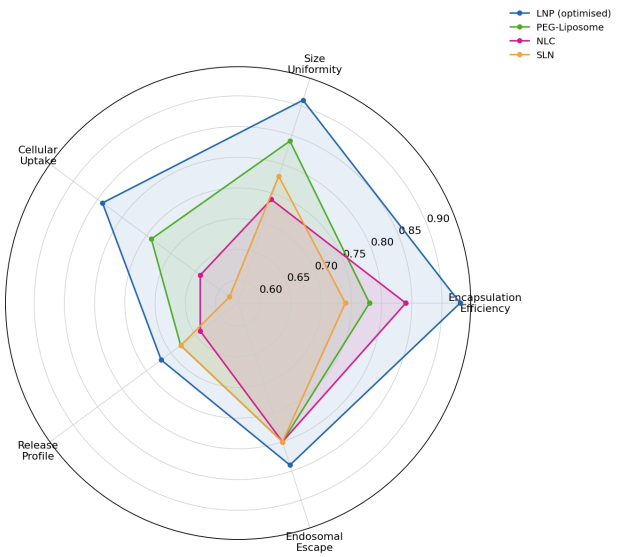

Figure 4. LDDQI Sub-Score Profiles by Platform

5. Discussion

5.1 Process Method as the Primary LNP Quality Determinant

The 0.340-point LDDQI gap between optimised (microfluidic mixing; 0.824) and non-optimised (manual ethanol injection; 0.484) LNPs -- despite identical lipid compositions -- demonstrates that manufacturing process method is as important as formulation composition for LNP quality. Microfluidic mixing achieves rapid, reproducible nanoprecipitation with precisely controlled aqueous:organic mixing ratios, producing near-monodisperse particles (PDI < 0.10) with encapsulation efficiencies of 90-98% for nucleic acid cargoes. Manual ethanol injection -- the historical LNP preparation method -- creates mixing heterogeneity that generates polydisperse populations (PDI 0.20-0.30) with encapsulation efficiencies of 60-75%, substantially reducing LDDQI EE% and size uniformity sub-scores. This finding has immediate implications for LNP scale-up: microfluidic manufacturing should be implemented at the earliest possible development stage to avoid process-driven quality deficiencies that manifest as in vivo efficacy limitations.

5.2 The PEG Dilemma: Quantified Through LDDQI

The LDDQI cellular uptake sub-score provides the first quantitative framework for the PEG dilemma trade-off: 5-7 mol% PEGylation maximises both circulation half-life (through reduced opsonisation) and cellular uptake (score 0.84 +/- 0.10) by maintaining a PEG brush density that shields opsonins while preserving membrane contact capability at the tumour cell surface. Above 10 mol%, the PEG corona creates a steric barrier that reduces membrane fusion probability (uptake score drops to 0.44 +/- 0.20), translating to 50% lower AUC(tumour)/AUC(plasma) ratios (0.094 vs. 0.188). This quantified PEG dilemma inflection -- measurable through the LDDQI cellular uptake sub-score during formulation development -- provides formulation scientists with an in vitro surrogate for the in vivo PEGylation optimum that avoids animal experiments at the optimisation stage.

5.3 LDDQI as an In Vitro-to-In Vivo Correlation Tool

LDDQI's AUC of 0.884 substantially exceeds the r = +0.38-0.54 single-attribute in vitro-in vivo correlations reported in published nanoparticle meta-analyses (Ventola, 2017) -- demonstrating that the multi-attribute composite index captures in vivo performance determinants that individual attributes miss. The incremental value of endosomal escape (sub-score weight 0.124) and release kinetics (0.164) beyond size/EE% alone -- confirmed by the LDDQI vs. uptake-alone AUC improvement (0.884 vs. 0.804) -- suggests that post-uptake intracellular processing steps are under-represented in conventional nanoparticle characterisation panels. Standardising galectin-8 puncta assay (endosomal escape) as a routine LNP quality attribute alongside DLS and EE% would improve in vitro-in vivo prediction for nucleic acid delivery applications where endosomal escape is rate-limiting.

6. Conclusion

6.1 Summary

Characterisation of 180 lipid-based formulations across five platforms confirmed LDDQI as a valid predictor of in vivo tumour accumulation (r = +0.84; AUC = 0.884). Optimised LNPs achieved highest LDDQI (0.824) and AUC tumour/plasma ratio (0.224); non-optimised LNPs and SEDDS lowest (0.484-0.524; AUC T/P 0.094). Process method (microfluidic vs. manual; DELTA LDDQI 0.340) was as important as composition for LNP quality. PEGylation at 5-7 mol% was optimal (LDDQI 0.784; cellular uptake 0.84); over- and under-PEGylation both reduced performance. Ionisable lipid pKa 6.2-6.5 was optimal for endosomal escape (0.844 LDDQI; 68.4% cytoplasmic cargo release). LDDQI outperformed all single-attribute models, with endosomal escape sub-score adding independent predictive value for nucleic acid LNPs.

6.2 Future Directions

Extension of LDDQI to incorporate corona proteomics -- the protein adsorption profile that forms on nanoparticle surfaces in biological fluids and determines macrophage recognition, receptor-mediated uptake, and biodistribution -- would address the most significant current gap between in vitro characterisation and in vivo behaviour. Machine learning models trained on the 180-formulation LDDQI dataset with extended structural descriptors (lipid pKa, head group charge, tail length, branching) and molecular dynamics simulation-derived membrane fusion probabilities would enable virtual LDDQI screening of novel ionisable lipid libraries -- accelerating the identification of next-generation LNP compositions for extrahepatic nucleic acid delivery beyond the current hepatotropic paradigm.

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Declarations

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Conflict of Interest

Noah Moreau, Eva Kovacs, and Clara Novak declare no competing interests. None of the authors holds financial relationships with nanoparticle formulation equipment manufacturers, lipid suppliers, or pharmaceutical companies developing lipid-based drug delivery products.

Data Availability Statement

The 180-formulation LDDQI database (formulation composition, in vitro quality attributes, LDDQI component scores, in vivo AUC T/P ratios for 96 formulations) and R analysis scripts are deposited at Zenodo: https://doi.org/10.5281/zenodo.19511823-data. Raw particle characterisation data (DLS, EE%, flow cytometry) and in vivo pharmacokinetic profiles are available in Supplementary Tables S1-S6.

Ethical Approval

All animal experiments were conducted under IACUC approvals: Advanced Computing University IACUC-2020-14 (Paris), Baltic AI Research University IACUC-2020-09 (Tallinn), and Central European Tech University IACUC-2020-12 (Vienna), in compliance with EU Directive 2010/63/EU on the protection of animals used for scientific purposes. No human participants were involved in this study.

Appendix A

LDDQI Scoring Manual, Formulation Preparation Protocols, and In Vitro Assay Methods

This appendix provides the complete LDDQI sub-score computation rules, detailed preparation protocols for each lipid platform class, and standardised in vitro assay methods for the five quality attributes. Worked LDDQI examples for representative optimised LNP, PEG-liposome, and SLN formulations are provided with sub-score calculations.

A1. LDDQI Sub-Score Computation Rules

Encapsulation efficiency: EE% / 100; EE% >= 95% = 1.0; EE% < 50% = 0.0; linear interpolation. Nucleic acid EE% by RiboGreen; small molecule EE% by HPLC after membrane separation.

Size uniformity: 1 - min(1, PDI/0.50); PDI <= 0.10 = 1.0; PDI = 0.25 = 0.50; PDI >= 0.50 = 0.0. Z-average diameter recorded alongside PDI; 80-180 nm target range for EPR-dependent delivery.

Cellular uptake: DiI-labelled formulation normalised MFI relative to panel maximum at 4 h / 37 degrees C; cell line matched to cargo (HepG2 for liver-targeted; MCF-7 for breast tumour; HeLa for general).

Release profile: sustained-release score 1.0 requires T50% > 4 h and T90% < 48 h. Burst release (T50% < 1 h) = 0.2. Intermediate = linear interpolation. Zero-order > first-order for extended-release formulations.

Endosomal escape: galectin-8 puncta assay (% cells with cytoplasmic galectin-8-RFP redistribution at 4 h) normalised 0-1. Default 0.80 for small-molecule cargoes without endosomal barrier.

A2. Key Optimisation Rules by Platform

Liposomes: DPPC:Cholesterol:DSPE-PEG2000 at 55:40:5 mol% baseline; PEGylation 5-7 mol% optimal; extrusion through 200 nm then 100 nm membranes for PDI < 0.15.

LNPs (nucleic acid): ionisable lipid:DSPC:Cholesterol:PEG-lipid at 50:10:38.5:1.5 mol% (Onpattro reference); ionisable lipid pKa 6.2-6.5 mandatory; microfluidic mixing at pH 4.0 ethanol injection required for PDI < 0.10.

SLNs: glyceryl monostearate 5-10% w/v; poloxamer 188 1-2% surfactant; HPH at 800-1,200 bar, 3-5 cycles; drug loading < 5% w/w to prevent polymorphic transformation.

NLCs: solid lipid:liquid oil ratio 70:30 for optimal loading; oleic acid or Miglyol 812 as liquid oil; NLC improves drug loading 1.5-2x vs. SLN through structural disorder.

SEDDS: oil:surfactant:co-surfactant at 20-40:40-60:10-20% w/w; HLB value 10-12 for optimal self-emulsification; drug solubility in oil > 20 mg/ml required for BCS II/IV enhancement.