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Optimizing Drug Delivery: Formation and Purification of Custom Liposomes Using a Modular Micro-Continuous Flow Approach

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ABSTRACT:

This study presents a novel modular micro-continuous flow system for the efficient formation and purification of tailored liposomes designed for targeted drug delivery. By optimizing flow conditions and lipid composition, we successfully produced liposomes with controlled size and surface characteristics. The purification process was streamlined using a combination of membrane filtration and chromatography, significantly enhancing the yield and purity of the final product. This approach offers a scalable and reproducible method for liposome production, with potential applications in pharmaceutical delivery systems aimed at improving therapeutic efficacy and patient outcomes.

INTRODUCTION:

Liposomes are a recognised formulation technique used to optimise drug delivery and augment therapeutic efficacy for many substances, including medicines, biopharmaceuticals, and vaccines. Owing to their bilayer vesicular architecture, like that of natural cells, liposomes may encapsulate pharmaceuticals inside their aqueous core and lipid bilayers. Through such methods, the pharmacokinetics of a medicine may be regulated and determined by the liposomal delivery system rather than the properties of the drug itself. This has facilitated the creation of many clinically authorised liposome-based pharmaceuticals, including DOXIL/Caelyx® (doxorubicin), AmBisome® (amphotericin B), and Daunoxome® (daunorubicin), which together generate an annual market value of about $100 million. Nonetheless, despite these benefits, their broader use is constrained by intricate and expensive manufacturing demands. Presently, production techniques include solvent injection, reverse-phase evaporation, and emulsification procedures. These techniques are disadvantageous because to their multi-step processes, significant use of organic solvents, and restriction to batch-release procedures. Moreover, a critical characteristic of an effective liposomal drug system is the vesicle size range, which may be regulated by the manufacturing process, such as sonication (20–40 nm).2, extrusion (70–415 nm)3 or high-pressure homogenisation (20–140 nm)4; and, more recently, microfluidic mixing (20–80 nm)5.6 or for focussing (50–150 nm)7. The pharmacokinetic profile and behaviour of liposomes upon administration are determined by their size; hence, regulating particle size and polydispersity index (PDI) is crucial in their production and a significant parameter in product specifications. To generate liposomes within a certain size range, techniques such as extrusion or homogenisation are often used. This introduces additional stages to the production process and exposes the liposomes and medication components to severe conditions.

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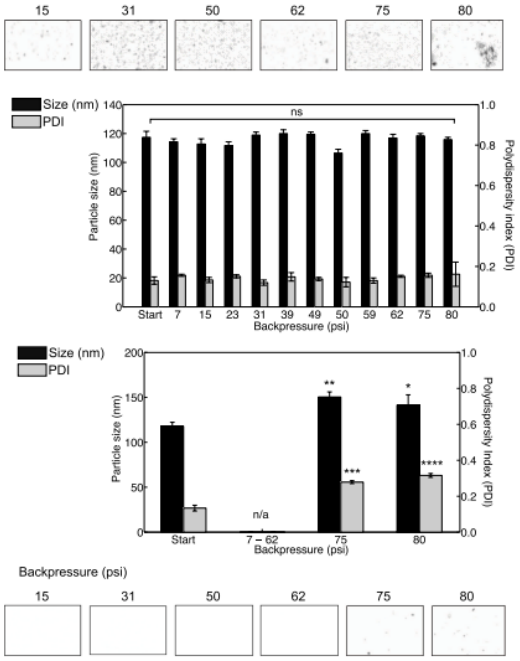

Figure 1. Particle size and polydispersity in relation to rising backpressures in the TFF system, as measured on the retentate side of the membrane. Images from NTA analysis, confirming particles in the permeate (top) and retentate (bottom) streams at escalating backpressures. Particles were detected in the permeate at backpressures over 75 psi. All experimental datasets are provided as mean and standard deviation (mean±s.d.) derived from three separate trials (n=3) under possibly adverse processing circumstances. To resolve these challenges and facilitate the broader use of liposomal systems to enhance healthcare, novel approaches in liposome production are essential.

Microfluidic devices function with minimal quantities, provide exceptional control over fluid flow, and use materials, reagents, and energy efficiently. These benefits have been used for the consistent generation of liposomes with a homogeneous size distribution. Liposome production often transpires at the interface between an aqueous phase and a solvent phase containing lipid molecules, and microfluidic devices are adept at creating and precisely regulating these interfaces. In hydrodynamic flow focussing (HFF) devices, the solvent phase is microinjected between two co-flows of aqueous buffer, facilitating the assembly of liposomes with precisely regulated size distributions. Moreover, by altering the ratio of the flow rates of the aqueous buffer and

Lipid	Application	Reference
Dimethyldioctadecyl ammonium bromide(DDA)	Vaccine adjuvant,cationic head group, uptake of vaccine antigens to antigen presenting cells	Smith Korsholm *et al.*[34] Christensen *et al.*[35]
Trehalose 6,6-dibehenate(TDB)	Synthetic immunstimmulator derived from the membrane of mycobacterium	
1,2-dioleoyl-sn-glycero-3-phsphoethanolamine(DOPE)	Fusogenic helper lipid, available in the commercial Lipofectin™ transfection reagent	Henriksen-Lacey *et al.*[36]
1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)	Cationic lipid often used in transfection	
Egg Phosphatidylcholine(PC)	Neutral head group, drug delivery	Senior and Gregoriadis[37] Gregoriadis and Senior[38]
1,2-Dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)(DPPG)	Negative charged head group, drug delivery	Oku *et al.*[39] Kirby *et al.*[40]
1,2- Dipalmitoyl-*sn*-glycero-3-phosphocholine(DPPC)	Neutral head group, drug delivery	
Cholesterol (Chol)	Added for membrane stabilization, known to effect drug encapsulation efficiency in bilayer and aqueous core	Senior and Gregoriadis[37] Kirby *et al.*[40]

Table 1. Lipids investigated in this study.

Backpressure (psi)	7	15	23	31	39	49	50	59	62	75	80
Flow rate (mL min^{-1})	0.01	0.02	0.03	0.1	0.05	1	0.3	2	2.5	0.5	0.1
Capillary I.D. (µm)	50	50	50	63	50	100	63	100	100	63	50
Capillary length (mm)	50	50	50	50	50	50	25	30	25	25	50

Table 2. Backpressures and fow rates through the Tangential Flow Filter (TFF) that were investigated in this study. Liposomes in solution were fed into the TFF device at fow rates ranging between 0.01 and 2.5mLmin−1 . Backpressure was attained by connecting a restrictive capillary with selected (I.D.) and/or length on the retentate side of the TFF outlet.

METHODS

Chemicals. Egg Phosphatidylcholine (PC), CAS: 8002-43-5, 1,2-Dipalmitoyl-sn-glycero-3-phosphorac-(1-glycerol) sodium salt (DPPG), CAS: 67232-81-9, 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), CAS: 63-89-8, and Cholesterol (Chol), CAS: 57-88-5, were gotten from Sigma-Aldrich Organization Ltd. (Poole, UK). 1,2-dioleoyl-sn-glycero-3-phsphoethanolamine (DOPE), CAS: 4004-05-1, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP),

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CAS: 144189-73-1, dimethyldioctadecylammonium bromide (DDA), CAS: 3700-67-2, and trehalose 6,6-dibehenate (TDB), CAS: 66758-35-8 were bought from Avanti Polar Lipids, Inc., (Alabaster, AL), virtue >99% (Table I). Ethanol, CAS: 64-17-5, and methanol, CAS: 67-56-1, were gotten from Fisher Scientifc (Loughborough, UK). TRIS Ultra Unadulterated, CAS: 77-86-1, was gotten from ICN Biomedicals, Inc., (Aurora, Ohio, US). Propofol (2,6-Bis(isopropyl)phenol), CAS: 2078-54-8, and ovalbumin (chicken egg), CAS: 9006-59-1, were gotten from Sigma-Aldrich Organization Ltd., (Poole, UK). Ultrafltration recovered cellulose layers (p\n: U2755-10AE) were gotten from Sigma-Aldrich Organization Ltd., (Poole, UK) (10 kDa, pore size 0.22 μm), and Biomax polyethersulfone ultrafltration film plates with 300 kDa cutof, pore size 0.45 μm (p\n: PBMK06210) from Merck Milipore (Darmstadt, Germany).

Liposome bunch definitions for characterisation of the Distracting Stream Filtration (TFF) gadget. Multilamellar vesicles (MLV) were arranged utilizing the lipid flm hydration method33. Lipids were gauged and disintegrated in a chloroform/methanol (9:1 v/v) combination. Cationic liposomes involved DDA:TDB (8:1 molar proportion) and anionic liposomes included DPPG, DPPC, Chol (1:1:1.3 molar proportion). Te natural dissolvable was accordingly taken out by turning dissipation under vacuum (100 RPM, 180 mBar, Rotavapor R-100, BÜCHI Labortechnik AG, Switzerland), trailed by fushing with nitrogen for evacuation of dissolvable deposits (5minutes). Te meager lipid flm on the lower part of a round base fask was hydrated with 10mMpH 7.2 TRIS bufer. Little liposomes were shaped by means of test sonication (Soniprep150plus, MSE, UK; 5 min at adequacy of 5). Ethanol was physically added to the liposome detailing to a fnal grouping of 20% (v/v) to recreate dissolvable items normally coming about because of the microfuidics creation technique. Ovalbumin (100 μg mL−1) was utilized as a model protein, and propofol (1 mg mL−1) as a model medication. Tese were added to the liposome definition after creation to impersonate the circumstances post liposome producing by microfuidics.

Gadget creation. As recently detailed, the fltration framework was intended to seal layers set up through mechanical clamping24. Two poly(methylmethacrylate) (PMMA) plates, with a straight channel (1 mm width, 1 mm profundity, 45 mm length) and a 1 mm opening processed at each end were cinched together utilizing M3 screws along the edges (Force 10 Ncm). A 1 mm wide and 0.75 mm profound slicing was utilized to hold the PDMS gasket set up, which was utilized to get the layer set up (Strengthening Figure S4). Diferent industrially accessible film sheets were sliced to the necessary size utilizing a CO2 laser stamping head (Synrad Inc., Mukilteo, WA, USA). Te layers utilized here of investigations had a cut of 10 kDa or 300 kDa, for medication or protein fltration, separately. Te films were cleaned afer each analysis by back-fushing with water and put away inside the TFF framework in 0.8M saline arrangement, prepared for the following trial.

Furthermore, a clasping framework was produced using PMMA (two plates kept intact by screws [M3]) for the stunned herringbone micromixer (SHM) chip utilizing a micromilling machine (M3400E, Folken IND, Glendale, USA). Te gasket for the fltration unit was

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fabricated from poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning, Midland, USA), as per the producer's directions and cast in PMMA molds, made as portrayed previously. Interconnect ports (processed from 5 mm PMMA), with two openings tapped with a M3 string were utilized for association with the fltration unit; a M6 strung opening was utilized for standard association fttings (P-221, Upchurch Scientifc, Oak Harbor, WA, USA).

Backpressure guideline. Backpressures were managed through vessels, which were appended to the retentate outlet of the fltration gadget (see Valuable Fig. S3). Tese vessels limited the fow as they were chosen with inner breadths less than the polytetrafuoroethylene (PTFE) tubing (1/16 in.×0.031 in., p\n: 58700-U, Sigma-Aldrich Int.) which associated the TFF gadget with helper siphons and assortment vials. Backpressure was determined utilizing Hagen-Poiseuille's Regulation

where μ, L, d and Q are the powerful thickness of the medium at 25 °C, the length and interior breadth of the confining slim, and the volumetric fow rate, separately. We utilized Hagen-Poiseuille's condition (1) to choose the slender sizes and the fow rates to accomplish the backpressure range from 5 to 80 psi. For each backpressure examination, a narrow was associated with the TFF retentate outlet utilizing PTFE tubing, ferrules (p\n: P-200, IDEX Europe GmbH, Germany) connectors (Flangeless Nuts, p\n: P-247, Look, M6 Level Base, for 1/16 in. OD, IDEX Europe GmbH, Germany) and metric associations (Metric Association, M6 Port, p\n: P-602, IDEX Europe GmbH, Germany). Te gulf of the TFF was associated through a Luer-lock ftting and polytetrafuoroethylene PTFE tubing to a solitary utilize plastic needle. Water was taken care of in the TFF gadget at discrete fow rates going from 0.01 to 2.5 mL min−1 achieved by a needle siphon (Nemesys, Cetoni GmbH, Germany). Backpressures were estimated tentatively with a tension sensor (40PC100, Honeywell, NJ, USA) associated on the retentate side; the information was logged with a LabVIEW virtual instrument (Public Instruments, TX, USA). We thought about the hypothetical backpressures from condition (1) to the deliberate backpressures, and the deliberate qualities surpassed their determined partners from 20% to 6.25% while expanding the applied backpressure from 5 to 80 psi, separately (Beneficial Figure S5, and Strengthening Table S6). One of the TFF power source was deliberately fixed with a fat base fitting (p\n: P-314, M6, IDEX Europe GmbH, Germany) while a solitary outlet associated through a ferrule (p\n: P-200), nut (p\n: P-247) and tubing into an assortment vial for fluid going through the film.

Filtration. Filtration was acted in diafltration mode to examine the liposome conduct in the laid out pressure and fow rate range (Table 2). For this trial, seat top arranged liposomes in watery arrangement were spiked with medication, protein or dissolvable, and were brought into the TFF through needle siphons (Nemesys, Cetoni GmbH, Germany), connectors and vessels as depicted before. A hairlike was associated with the TFF, in cis-confguration (on a similar side of the film), and shut the circle of the retentate fuidic line (see Strengthening Fig. S3). Retentate from the TFF was gathered in a halfway assortment vial and could be infused in the gadget thus considering different passes,

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alluded in this article as diafltration cycles. Transmembrane pressure was achieved by controlling the fow rates in the siphon; likewise by adding a tightening fine of known calculation, for example inside breadth and length. Retentate and saturate divisions were gathered in Eppendorf tubes, surveyed by weight, and utilized for additional examination, for example zeta potential, size, polydispersity, quantifcation by means of HPLC. A volume of TRIS bufer, 10mMpH 7.2, was added afer every diafltration cycle to make up for how much fluid going through the film (in penetrate) and to support consistent fixation levels (in retentate) during the nonstop purifcation process.

Consistent cycle fow confguration. To test the consistent handling of liposome development followed by liposome purifcation, a SHM and a TFF gadget were associated in grouping. Te SHM (Accuracy Nanosystems Inc., Vancouver, Canada) comprised of two gulfs, a bifurcated channel with herringbone designs, and single outlet formed in PDMS. Te directs were 200 μm in width and 79 μm in level with herringbone elements of 50 μm in width, 31 μm in level, 45° point, deviation file 2:1 (as per Accuracy Nanosystems, Inc.). Luer-lock ftting and polytetrafuoroethylene (PTFE) tubing (1/16 in.×0.031 in., Sigma-Aldrich Int.) were utilized to connect dispensable 1mL needles with the two bay ports of the chip; fow rates and fow rate proportions were constrained by needle siphons (Nemesys, Cetoni GmbH, Germany) and the entire framework was prepared with Tris bufer (10mM, pH 7.2) before activity. Natural stage, a gauged measure of lipids in ethanol, was infused into the frst channel of the SHM gadget, while in the second delta watery stage (TRIS bufer, 10mM, pH 7.2) was infused. Te micromixer was held set up utilizing a clasping gadget made from PMMA. Te micromixer was associated with the extraneous fow fltration unit by means of a middle of the road assortment vial (2.0 mL Eppendorf) for extra usefulness. Tis permits the expansion of different parts, for example, of microfuidics-fabricated liposomes preceding the fltration framework for purifcation. A bi-directional milliGAT siphon (VICI Valco, Valco Instruments Co.) was associated in-accordance with the retentate circle of the TFF through a hairlike at the lower part of that transitional assortment vial. Transmembrane pressures was shifted by confining the fow of the retentate utilizing diferent little measurement vessels associated in-accordance with the TFF outlet. Te retentate fowed through the narrow and was gathered in the middle of the road vial, while saturate went through the layer and was accumulated in a different cylinder. The two portions were broke down for content of liposomes, propofol, protein, lipid and ethanol.

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