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Investigating the effects of size, charge, viscosity and bilayer flexibility on liposomal delivery under convective flow

M. Narenji[a], M.R. Talaee[b], H.R. Moghimi[a,*]

[a] Department of Pharmaceutics and Nanotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Railway Engineering Rolling Stock, Iran University of Science and Technology, Tehran, Iran

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ABSTRACT

Convective flow is one of the main mechanisms of mass transfer employed in drug delivery (e.g. osmotic pumps) and working in material transport in the body (e.g. blood circulation). Although convective flow has been investigated extensively, not much data is available on convective behavior of nanoparticles, the subject of the present investigation.

Here, liposomes with different sizes, charges, bilayer flexibilities and medium viscosities were encountered convective flow and changes in their properties were monitored over 48 h. For large particles (>350 nm), neutral liposomes (NL) showed significant phase separation and decreased lipid content over time, while positively or negatively charged liposomes remained homogenous. Reduction of size in NLs to about 100 nm resolved phase separation problem, but their lipid content still showed reduction; no such a problem was observed in charged small liposomes. When bilayer flexibility of large NLs was increased, neither phase separation nor decreased lipid content was observed. Increasing medium viscosity for large NLs from 3.4 cP to 45.2 cP again solved the problem and a uniform liposomal delivery was observed. These results indicate that size, charge, bilayer flexibility and viscosity affect convective liposomal delivery and that uniform delivery is possible even in large liposomes by optimizing such factors.

1. Introduction

Convection is one the main mechanisms of mass transport, which is conducted by spatial gradient in pressure (Topp, 2000). In addition to different pharmaceutical processes such as drying, dissolution, filtration and mixing, convection is one of the most important mechanisms in intracellular and extracellular transport and has a great role in the in-vivo fate of drug (Siepmann et al., 2009). Convection is also used as a mechanism in development of controlled release drug delivery systems. Controlled drug delivery systems has been investigated and used for more than 3 decades, using different mechanisms to control the concentration and time-course of their cargo in biophase. Convective delivery (e.g. osmotically driven systems) is one of these mechanisms which provide many advantages over diffusion including, but not limited to, drug release profile independent of molecular properties (weight, charge, ...), zero-ordered release and possibility of delivery of macro molecules and nanoparticles (Thummar et al., 2013; Patel et al., 2012)

Nanotechnology has a developing trend in drug delivery due to the numerous benefits that nanoparticulate systems offer as therapeutic approaches. Such as passive targeting, selective and also intracellular drug delivery and enhancing the drug permeation

* Corresponding author at: Department of Pharmaceutics and Nanotechnology, School of Pharmacy, Shahid Beheshti University of Medical Sciences, P.O. Box: 14155-6153, Tehran, Iran.
E-mail address: hrmoghimi@sbmu.ac.ir (H.R. Moghimi).

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through (at cell or tissue level) biological barriers. Also due to their large variety in forms, materials and mechanisms of action, the nanotechnological approaches make a very promising platform for a great improvement in delivery of all drugs (Lamprecht, 2009). Like any other drug delivery system, there are some obstacles that limit applications of these systems. Such as rapid clearance of the plain nanoparticles form the circulation. These particles usually represent foreign particles and will be opsonized rapidly before completing their action. Thus the longevity and the long circulating time is a still growing area in biomedical research (Torchilin, 2006).

Among the several drug delivery systems, liposomes have drawn a lot of interest as advanced and versatile carriers for pharmaceuticals. At the present, liposomal formulations span multiple areas, from clinical application of the liposomal drugs to the development of various multifunctional liposomal systems for therapy and diagnostics (ElBayoumi and Torchilin, 2010). The special properties of liposomes have generated numerous applications of liposomes as drug delivery systems (Madelmont et al., 2003) which are feasible with various drug molecules and routes of administration and as models for biological membranes (Tamaddon et al., 2007). The application of liposomes are growing rapidly as complex and sophisticated liposomal formulations such as dendrosomes (Movassaghian et al., 2011, 2013), magnetoliposomes (Azizian et al., 2012) and prolonged-release gel-based liposomal systems (Alinaghi et al., 2014, 2013). Liposomes can face convective flow in different conditions. Blood circulation and convection-mediated drug delivery systems (such as osmotic pumps), are examples of these conditions. Loading liposomes into the convection-based drug delivery systems could fix their rapid clearance from blood flow and also optimize their therapeutic properties as drug delivery systems. For small molecules, it is postulated that drug molecules follow the flow of fluid, but it is obvious that such a behavior is not expected from large particle; e.g. aerosol in airways during breathing. Interestingly, although nanoparticles are faced to convective flows, there is not much data available on behavior of these particles in convective flow. Nanoparticles behave differently from both molecules and microparticles and it is not possible to extrapolate behavior of molecules or large particles to nanoparticles, including liposomes. Size and surface properties of nanoparticles have been shown to be highly effective on bio-distribution and fate of nanoparticles (Kim and Khang, 2015). Charge of nanoparticles can affect the pharmacokinetic properties of nano systems. RES clearance, cellular and tumor uptake, targeted drug delivery to brain or topical drug delivery can be influenced by the charge of nanoparticles. The effect of some of these variables (size, charge and bilayer flexibility) on the in-vivo fate of liposomes has been investigated (Honary and Zahir, 2013), but there is no data available on the effects of such parameters on the behavior of liposomes under convective flow. The main idea of this study is to investigate the behavior of nanoparticles in convective flow and the effects of liposomes' charge, size, viscosity and bilayer flexibility on their behavior in convective flow.

2. Material and methods

2.1. Materials

Egg phosphatidylcholine (EPC, >80%), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC, >99%), 1,2-distearoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DSPG, >99%) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP, >99%) were purchased from Lipoid GmbH (USA), cholesterol (>99%) and propylene glycol (PG) were purchased from Sigma (USA). methanol (99%), chloroform (99–99.4%), ammonium thiocyanate (98.5%) and ferric chloride hexahydrate (>99%) were purchased from Merck (Germany). All other analyticals were of pharmaceutical grade.

2.2. Preparation of liposomes

This liposomal formulations were prepared by lipid film hydration method. The lipids were dissolved in chloroform: methanol mixture (2:1) and the organic solvent was evaporated at 60 °C under vacuum to form a lipid film using a rotary evaporator (Heidolph, Germany). The film was then hydrated by deionized water at the same temperature (Lasch et al., 2003). Total lipid content of liposomes was 20 mM.

To investigate the effects of charge, viscosity and bilayer flexibility, different liposomal formulations were prepared here. Neutral liposomes were formulated using DSPC: cholesterol (70:30 molar ratios). The lipid phase of negatively charged liposomes was consisted of DSPC: DSPG: cholesterol (65:10:25 molar ratios) and the positively charged liposomes were prepared using DSPC: DOTAP: cholesterol (65:10:25 molar ratios).

Fluidity of liposomes depends on their lipid composition and phase transition temperature (Tm) of phospholipids. To prepare liposomes with different fluidity, two lipids with different transition temperatures were chosen here: EPC with Tm of about 0 °C and DSPC with Tm of about 55 °C (Avanti Polar Lipids Inc., 2016). EPC: cholesterol (50:50) was used to obtain more flexible liposomes.

To prepare high viscosity liposomes, water in neutral liposomes consisted of DSPC: cholesterol (70:30), has been replaced with propylene glycol.

Extrusion (Northern Lipids, Canada) of liposomes was conducted to adjust their size. To prepare small particles with a particles size of around 100 nm, liposomes were extruded through polycarbonate filters (Sigma, USA) with pore size of 1 μm for 10 times, followed by 5 times extrusion through membrane with pore size of 200 nm and 5 times through 100 nm polycarbonate filters. To obtain liposomes with larger particle size, liposomes were extruded 10 times through polycarbonate filters with pores size of 1 μm.

2.3. Convective flow model

This study has employed pressure as the driving force for the convective flow using a syringe pump (FNM, Iran). This pump injects the liposomes at a flow rate of 0.01 ml/h, from a cylindrical polymeric syringe, 0.4 cm diameter, 5 cm height and total volume of about 0.5 ml (21G" needle), similar to properties of commercial osmotic pumps in which the size of reservoir is in range of 1.5–5.1 cm in length and 0.6–1.4 cm in diameter, and the orifice is identical to a 21G" needle. Samples were collected in defined periods of time over 48 h and their liposomal size distribution, zeta potential and phospholipid concentration were measured.

2.4. Characterization of liposomes

2.4.1. Size distribution and zeta potential

Size distribution of the particles and their zeta potential were measured using Nanozetasizer (Malvern, UK). Zavg (mean diameter), changes in nanoparticles' size populations and zeta potential were chosen as factors to study the behavior of formulations in convective flow.

2.4.2. Phospholipid content

Phospholipids are the main ingredient of the prepared liposomes in this investigation. It is also usual in liposomal studies to assume that all lipids follow the same fate as long as liposomes are intact. Therefore, as an indicator of liposomes concentration, the amount of phospholipids was measured here using the Stewart method (Stewart, 1980) and total lipids (liposome content) was calculated using phospholipid data. This method uses the reaction