Optimization of the development of Inhalable EGCG nano-Liposome as a Potential Treatment for Pulmonary Arterial Hypertension by Implementation of Design of Experiments Approach

University of BRADFORD

Fatma Haddad [1], Nura Mohammed [1], R.C. Gopalan [1], Md Talat Nasim [1], K.H . Assi [1]

[1] School of Pharmacy and Medical Sciences, University of Bradford, Bradford.

Background

Epigallocatechin gallate (EGCG), the main ingredient in green tea, holds promise as a potential treatment for pulmonary arterial hypertension (PAH). However, EGCG has many drawbacks including stability issues, low bioavailability, and short half-life [1]. Delivering drugs by inhalable liposomes is considered a smart method since this enhances drug stability and permeability, provides a sustained drug release, prevents local irritation by entrapping the free drug inside the phospholipid's components, targets the drug selectively to the site of action in the lung, in addition, liposomes can encapsulate both lipophilic and hydrophilic drugs [2].

Methods

The design of experiments (DOE) strategy was applied to study the impact of formulation compositions on the liposomal characteristics and to develop the optimum inhalable EGCG nano-liposome formulation. Polynomial models were constructed for the optimization process by employing a 29-run, 4-factor, 3-level Box-Behnken design using design expert software-13. The thin-film rehydration method (**Figure 1**), was used to prepare the inhalable EGCG liposomes. Then it was characterized, and its aerodynamic behavior was identified utilizing the next-generation impactor (NGI) (**Figure 2**). The in vitro effect of the optimum EGCG nano-liposome was determined using the reporter assay.

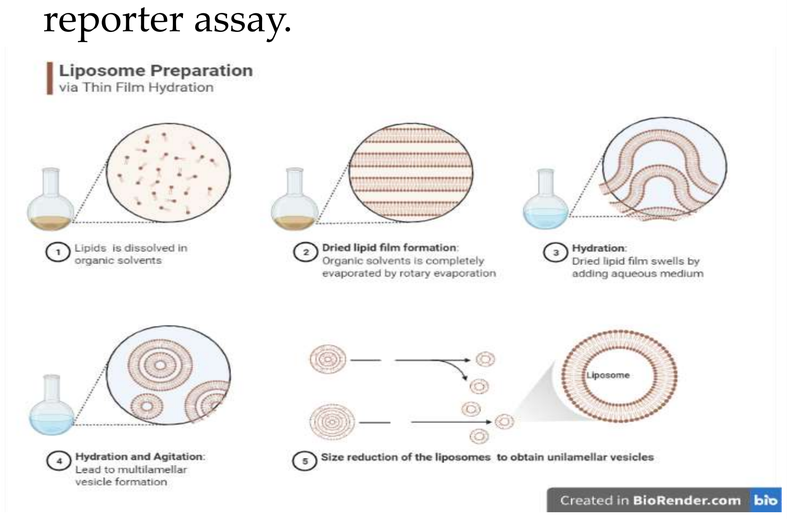

Figure 1. Liposome Preparation via thin film hydration technique

Figure 2. Next Generation Impactor

Results and Discussion

❖ **Optimisation, preparation and characterisation of the inhalable EGCG using DOE:**

The prepared optimum inhalable EGCG liposome has the following experimental characteristics: the average liposome size of 105 nm, polydispersity index (PDI) of 0.18, the zeta potential of -25.5 mV, encapsulation efficiency of 90.5%, and the PDI after three months of 0.19. All the actual results of these responses were in great agreement with the anticipated values by the model.

The result revealed that all the studied formulation factors significantly influenced the characteristics of the prepared EGCG nano-liposome formulations (**Figure 3**).

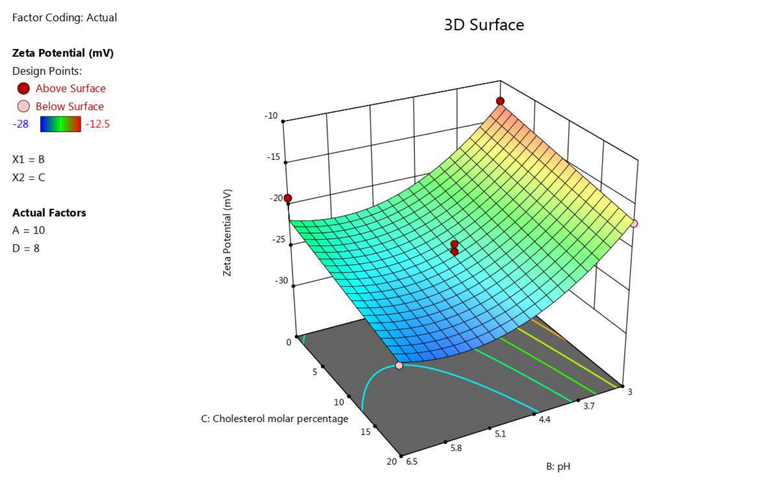

Figure 3. Response surface plot showing the impact of interaction among pH (B) and cholesterol molar percentage (C).

The aerodynamic properties for the prepared optimum liposome were as follows: the mass median aerodynamic diameter (MMAD) was 4.41 μm, and the fine particle fraction (FPF) was 53.46%, which demonstrates that the prepared optimum EGCG liposome has all the properties required to be inhalable, and it is expected to be deposited in the narrower airways (**Figure 3**). The optimum EGCG nano-liposome inhibits TGFβ signaling in cell-based studies and thus holds promise as a potential treatment for PAH (**Figure 4**).

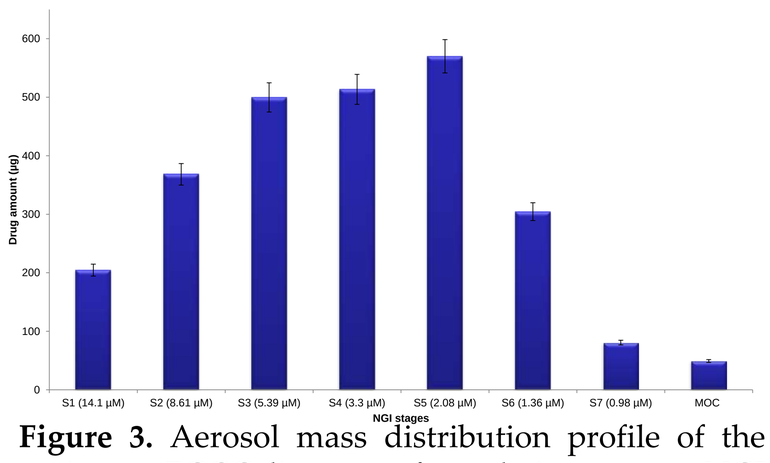

Figure 3. Aerosol mass distribution profile of the optimum EGCG liposome formulation among NGI stages from Aeroneb® GO nebulizer at flow rate of 15 L/min.

❖ ***In vitro* test of the effectiveness of optimum EGCG nano-liposome formulation**

Whilst both the free EGCG and optimum EGCG nano-liposome inhibited the reporter activity at 10 μM, the EGCG nano-liposome formulation elicited a higher efficacy in inhibiting the TGFβ signaling at 1 μM (*p-value* <0.05).

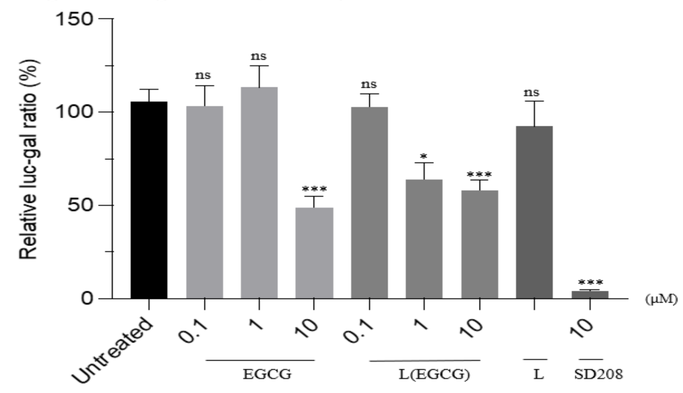

Figure 4. The effects of EGCG nano-liposome formulation on TGF-β signalling After 24 hours of seeding the HEK293T cells in a 96 half-area plate, cells were then transfected with TGFBRII, SBE-Luc and βgal. Following 24 hours these were treated with compounds, EGCG: free EGCG, L(EGCG): EGCG nano-liposome and Liposome at various concentrations.

Conclusion

❖ An inhalable nano-liposome has been optimised and formulated using high-phase transition phospholipids to maintain its stability during nebulization.

❖ The result revealed that all the studied formulation factors significantly influenced the characteristics of the prepared nano-liposome formulations.

❖ Whilst both the free EGCG and optimum EGCG nano-liposome inhibited the reporter activity at 10 μM, the EGCG nano-liposome formulation elicited a higher efficacy in inhibiting the TGFβ signaling at 1 μM.

References

1 Granja A, Pinheiro M, Reis S. *Epigallocatechin gallate nanodelivery systems for cancer therapy.* Nutrients 2016;8(5):307.

2 Mehta PP, Ghoshal D, Pawar AP, Kadam SS, Dhapte-Pawar VS. *Recent advances in inhalable liposomes for treatment of pulmonary diseases: concept to clinical stance.* J Drug Deliv Sci Technol 2020;56:101509.

printed by MegaPrint Inc. www.postersession.com