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Effect of Process Parameters on the critical attributes of the Liposomal formulations

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SUMMARY

This study investigated the effect of process parameters on the size, distribution, zeta potential and loading efficiency for the co-delivery of liposomal formulation of Ran-RCC1 inhibitory peptide (RANIP) and doxorubicin (DOX) to breast cancer. The results show that drug concentration, pH and ultrasound intensity are all critical factors to control the attributes of the liposomes.

INTRODUCTION

Liposomes are widely used for enhancing the delivery of biopharmaceuticals and chemotherapy. They help reducing the metabolism of APIs and/or their detection by the immune system. Furthermore, liposomes can be functionalised for targeted delivery to specific sites/cells (Eloy et al., 2017). Liposomes can be produced by various methods including thin film rehydration method. This study focuses on investigating the effect of process parameters such as drug concentration, pH of the rehydration media and the intensity of the ultra sound energy used to break the lipid phase on the size, size distribution, zeta potential and loading efficiency of the liposomes. The resultant liposomes are to be used for the co-delivery of RANIP and doxorubicin for the treatment of chemo resistant breast cancer (Haggag et al., 2020).

MATERIALS AND METHODS

Liposomes formulation

Phospholipids and cholesterol (DPPG: DOPE:cholesterol) (Sigma, UK) of (4:4:2 molar ratio) were dissolved in chloroform and methanol (3:1 v/v) in round bottom glassware. The organic solvent was evaporated at 40°C using rotary evaporation. The resultant thin film was maintained under vacuum for 6 h to ensure removal of residual solvents. The thin film was hydrated in a solution of RANIP in PBS for 1 h at 60°C using a bath sonication (150 W). The sample was further sonicated using a probe sonication for 90sec 40% amplitude until completely clear. The sample was centrifuged at 22,000 g for 30 minutes at 4°C for purification and removal of the non-encapsulated drug.

Characterization of drug-loaded liposomes

Average size and population spread (polydispersity index) of liposome preparations were determined using dynamic light scattering (Nanosizer ZS, Malvern Instruments, UK). Encapsulation effecinecy was measured using LCMS.

RESULTS AND DISCUSSION

The results shows that the increase in the pH value of the media from 6 to 7.4 resulted in the reduction of the average particle size of the liposomes regardless

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of the drug loading level. Surprisingly the zeta potential showed no significant change with changing the pH values within the investigated range. The Encapsulation efficiency, however, increased with increasing the pH from 6 to 7.4 (Table1). The drug loading level was also critical with the increase in loading level causing a reduction in the overall encapsulation efficiency. This is critical as the naked peptide was shown to be metabolised quickly by the enzymes in the cell media.

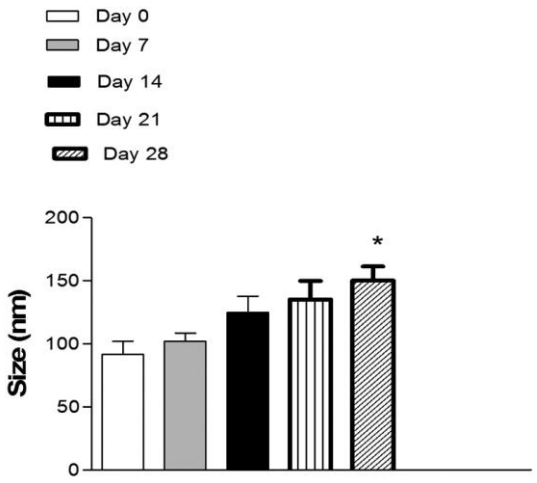

Fig. 1. *Particle size measurements of the F11 liposome formulation over 28 days of storage at 4oC. Values are mean ± SD for (n = 3). *p < 0.05 compared to day 0.*

Table 1. *Process variables & physicochemical characterizations of RANIP-loaded liposomes.*

Formulation	PH	Drug Loading	Zeta P.	Size (nm)	E.E (%)
F1	6	3%	−27.55 ± 3.44	138.47 ± 21.11	52.13 ± 7.96
F2	6.8	3%	−28.11 ± 5.69	119.50 ± 10.69	54.17 ± 5.89
F3	7.4	3%	−26.21 ± 2.58	80.55 ± 11.23 *	93.15 ± 6.10**
F4	6	5%	−29.11 ± 4.23	185.56 ± 16.33	41.25 ± 4.58
F5	6.8	5%	−26.39 ± 3.12	177.22 ± 13.44	43.47 ± 6.11
F6	7.4	5%	−25.22 ± 3.69	116.75 ± 11.99	72.36 ± 7.89 Δ
F7	6	7%	−27.16 ± 4.55	244.88 ± 9.78	32.15 ± 4.19
F8	6.8	7%	−28.14 ± 5.37	226.75 ± 13.74	30.36 ± 9.74
F9	7.4	7%	−28.21 ± 1.36	127.51 ± 14.35	64.78 ± 2.14

To ensure the stability of this formulation, a sample was stored in a fridge at 4°C for 4 weeks and its particle size was measured periodically. The results show that the particle size showed a non-significant increase after 3 weeks compared to freshly prepared liposomes ($p > 0.05$). The short-term stability was enough to run the in vitro tests on the cell lines. However long-term stability must be improved if this drug was to be commercialised. This could be achieved by freeze drying of the liposomal preparations. Finally, the effect of ultrasound exposure on the size of the liposomes was investigated using freeze thawing /prob sonication (figure 2). The figure shows that prob sonication was more effective at achieving smaller particle size with fewer steps compared to the traditional freeze thawing method using water bath. This can be attributed to the direct application of the ultrasound leading to higher intensity and more efficient energy transfer to the liposomes.

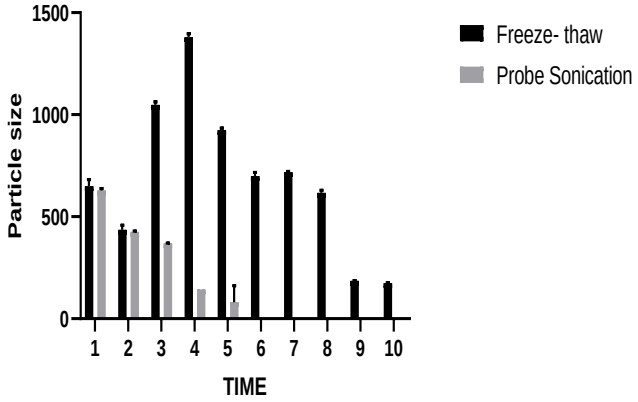

Fig. 2. *liposomal size reduction approaches freeze-thaw approach vs. probe sonication*

CONCLUSIONS

This study has successfully produced liposomal formulation for the co-delivery of RANIP and doxorubicin for the treatment of resistant breast cancer cell lines. pH value, drug loading level and ultrasound intensity were critical factors in determining the size, zetapotential and encapsulation efficiency of the liposomes.

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