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Encapsulation and Stability of Bioactive Proteins in Gel loaded Liposomes

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The entrapment efficiency and stability of liposomes loaded with alginate and a model bioactive protein alkaline phosphatase (ALP) were compared with that of conventional liposomes loaded with ALP. Multilamellar vesicles (MLV) were prepared by hydration of a thin lipid film of dipalmitoylphosphatidylcholine (DPPC) with a 20 mg/ml solution of ALP with and without 1% alginate. Once prepared the entrapped alginate was cross-linked by addition of CaCl_2 to the vesicle suspension forming liposomes with a gelled core. Entrapment of ALP was shown have 60% greater entrapment efficiency in alginate containing liposomes than liposomes prepared without alginate. Release of ALP from the gel containing liposomes at pH 2 was less than for conventional liposomes however large fluctuations in size was witnessed in the gelled liposomes when stored at pH 3.8 and at pH 7.4, possibly due to shrinking and swelling of the gel respectively. These initial findings have prompted further release and stability studies to determine the activity of the ALP following exposure to simulated gastrointestinal fluids which will reveal the potential of these particles as an oral delivery system for bioactive proteins.

Keywords: liposome, alginate, controlled release, stability, bioactive, encapsulation

1 INTRODUCTION

The incorporation of bioactive proteins in food products and delivery of such proteins in full working order to the physiological target site of the intestine can be problematic. This is due to the fact that often the functionality of bioactive proteins relies on their conformational structure which can easily be denatured by harsh processing conditions [1] and the acidic pH of gastric secretions once ingested [2]. To overcome these difficulties encapsulation strategies can be employed to stabilise and protect functional proteins. Liposomes and microspheres are just two methods used to encapsulate and deliver proteins; however there are limitations to both these delivery systems when used singularly such as stability, scale-up and undesired leaching of the entrapped molecules. The present study aims to develop and characterise a self-assembling delivery system with enteric properties by investigating the potential of loading alginate along with a model enzyme into liposomes, then cross-linking the alginate once encapsulated. Subsequently, immobilising the protein in enteric micro-gel particles, that are coated with phospholipid bilayers.

2 MATERIALS AND METHODS

2.1 Materials

Dipalmitoylphosphatidylcholine (DPPC) was purchased from Avanti polar lipids and the sodium alginate was purchased from BDH and was high G alginate, all other reagents were purchased from Sigma-Aldrich Company (Poole, UK).

2.2 Liposome preparation

DPPC was dissolved in a 9:1 solvent mixture of chloroform and methanol. The solvent was evaporated on a rotary evaporator to obtain a dry film, which was hydrated with a solution of ALP in 100 mM Tris HCl (to give final lipid concentration of 32 $\mu\text{mol/ml}$) in a round bottomed flask at 45°C with intermittent vortexing. Unentrapped ALP was removed from the liposome suspension by repeated centrifugation (13000 rpm for 5 min) decanting the supernatant and washing the pellet with 10 mM tris HCl until no ALP was detected in the supernatant. Liposomes containing alginate were prepared using the same method however the thin lipid film was hydrated with a mixture of 1% Na alginate containing 20 mg/ml ALP. Once unentrapped APL was removed by repeated centrifugation the alginate/ALP loaded liposomes were added to 0.2M CaCl_2 for 15 min to induce gelation of the entrapped alginate. The CaCl_2 was then removed by centrifugation and the liposomes were resuspended in 10 mM Tris HCl.

2.3 Size measurements

The volume mean diameter (VMD) of the liposomes was measured using a Malvern Master Sizer 2000, (Worcestershire, UK) at 25°C, diluting 50 μl of the dispersion to the appropriate volume with double-distilled water.

2.4 Quantification of Entrapment and Release

The concentration of entrapped ALP was determined using the BCA assay. Briefly, test samples of 100 μl vesicle suspension were added

100 ml sodium dodecyl sulphate solution and 100 ml 2% sodium citrate and incubated for 1 h. Samples were then added to BCA reagent incubated at 37°C for 30 min before absorbance measurements were taken at 562 nm. Release of ALP when incubated at pH 2 was also monitored, samples were taken over a 2 h period, centrifuged and the ALP content of the supernatant was quantified using the BCA assay.

2.5 Stability study

Samples of liposomes and alginate containing liposomes loaded with ALP were prepared and stored in Tris HCl maintained at pH 3.8 and 7.4 at 4°C. The liposomes were monitored for changes in size and ALP released over a period of 10 days.

3 RESULTS & DISCUSSION

The results given in Table 1 show a large difference in size between the standard MLV and MLV containing alginate. This increase in size is likely to be due to aggregation where residual alginate is present. Entrapment was also seen to increase in the vesicles containing alginate. This is thought to be a consequence of immobilisation of the ALP within the alginate gel network ultimately reducing leakage through the liposome bilayer. Multilamellar vesicles are expected to be more stable than other liposome types due to the many bilayers surrounding the encapsulated material [2]. However the addition of alginate appears to increase the stability releasing 7% less ALP when incubated for 2 hours at 37°C at pH 2. This stability was highlighted further by evaluating release over 48 h at pH 2 which revealed the alginate containing liposomes retaining 70% ALP compared with 32% retained by the standard liposomes (results not shown).

Sample	Mean Diameter (µm)	% Entrapment of ALP	% Released in 2h at pH 2
Liposomes	30.8 ± 3.1	0.9 ± 0.3	19.6 ± 1.4
Gelled liposomes	13.4 ± 0.1	1.6 ± 0.3	13.8 ± 1.3

Table 1: Liposomes and liposomes containing alginate gel. Comparison of particle size, % entrapment of ALP and release following 2 h at pH 2. All measurements were obtained for at least three independently synthesized batches and Mean ± S.D. is reported for each sample.

The effects of pH on the stability of both vesicle types was additionally analysed by monitoring size over a period of 10 days. Fig.2 shows samples stored at pH 3.8. The alginate containing vesicles reduced in size, possibly a result of the alginate gel contracting. The standard MLV revealed a slight increase in size when stored at pH 3.8 which is a possible indication instability. When this is compared with MLV stored at pH 7.4 (Fig.3) the

particle size remains constant for 10 days. Interestingly, the alginate containing liposomes increased from 30 µm to 45 µm after 10 days at pH 7.4. This is may be due to the alginate gel gradually swelling. Further studies are in progress monitoring release of ALP from the particles at pH 7.4 and the activity of the encapsulated ALP following exposure to simulated gastric fluid.

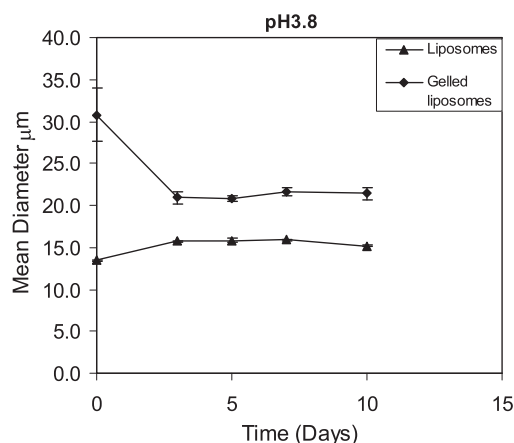


Figure 2: Mean diameter of samples stored at pH 3.8 for a period of 10 days for standard liposomes (filled triangles) alginate containing liposomes (filled diamonds).

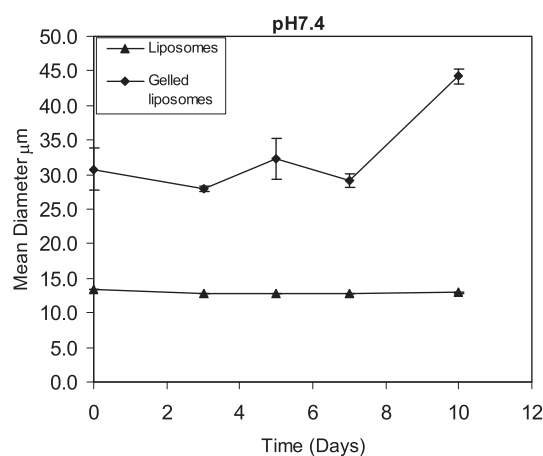


Figure 3: Mean diameter of samples stored at pH 7.4 for a period of 10 days for standard liposomes (filled triangles) alginate containing liposomes (filled diamonds).

4 CONCLUSION

Liposomes loaded with alginate that is then gelled by addition of CaCl₂ may be a potential approach for the encapsulation of bioactive proteins, to protect them from harsh processing environments and acidic pH of the stomach once ingested.

References

- [1] D.A. Parkins and U.T. Lashmar: Pharm. Sci. Technol. Today 3 (4) (2000) 129-137.
- [2] Yu et al: Colloid Surface B. 68 (2) (2009) 245-249.
- [3] Kokkona et al: Eur J Pharm Sci. 9(3) (2000) 245-252.