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A NEW PREPARATION METHOD FOR PREPARATION OF LIPOSOMES-IN-HYDROGELS PRIMED FOR TREATMENT OF SKIN DISEASES

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/ INTRODUCTION

Liposomes are spherical vesicles (Figure 1) that forms spontaneously when phospholipids are dispersed in an aqueous medium. However, to get liposomes with a more unimodal size distribution, liposomes are usually processed further to reduced size and lammellarity, typically by extrusion, sonication or homogenization.

Liposome formulations for topical application needs a vehicle to assure the desirable retention and adhesion of the drug-loaded liposomes onto the skin, and for this, hydrogels are regarded as promising systems [1]. The hydrogel **"soluble beta-glucan" (SBG)** has caught our attention since it also promotes wound healing on its own [2] (Figure 2).

Dual asymmetric centrifuge (DAC) (Figure 3) utilizes a unique combination of two contra rotating movements of the sample-holder, which results in shear forces that efficiently homogenize and gives a size reduction of liposomes [3]. We have investigated the use of DAC for both use in liposome size reduction and for further mixing of liposomes into hydrogels.

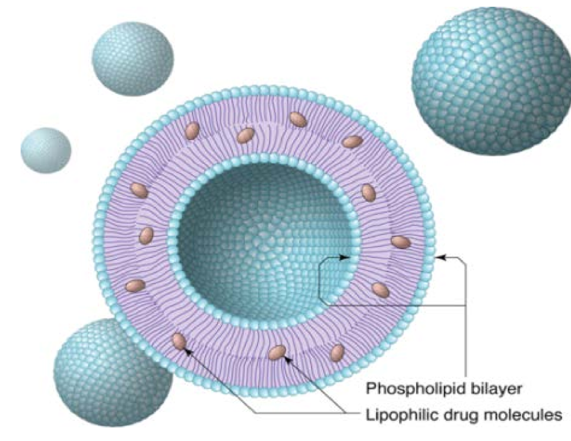

Figure 1: Liposome vesicles

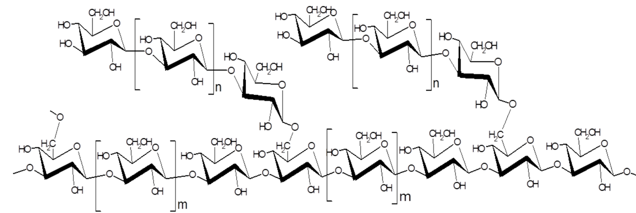

Figure 2: General structural formula for soluble beta 1,3/1,6 glucan (SBG) from bakers yeast (figure provided by Biotec BetaGlucans AS)

Figure 3: The dual asymmetric centrifuge (DAC) (Picture from www.speedmixer.de)

/ EXPERIMENTAL PART

PREPARATION METHODS

Liposome preparation

Liposomes were made from phosphatidylcholine (PC) (Lipoid S-100, Lipoid GmbH, Germany) with and without fluconazole (FLZ) (Sigma-Aldrich GmbH, Germany) as the active compound. PC and FLZ were dissolved in 96% ethanol. After rotary evaporation, the lipid film was hydrated with a mixture of distilled water and propylene glycol (PG) (ratio 1:1:0.75 w/v/v) and transferred to a 100 mL injection vial. The vial was vortexed and thereafter stored at 4 °C over night for hydration of the lipids. Prior to homogenization by DAC (DAC 150 FVZ, Hauschild & Co KG, Germany). 25% (w/w) glass beads was added as mixing aid as described by Massing *et al.* [3]. *Final mixing conditions for the preparation of liposomes were:* 2400 rpm for 2 minutes with the addition of 25% (w/w) glass beads (Ø = 1 mm) as mixing aid. Sample size: 570 mg with FLZ and 550 mg without FLZ.

Mixing liposomes into hydrogel

DAC was also used to mix liposomes and hydrogel (2.5% SBG, Biotec BetaGlucans AS, Tromsø, Norway) and other additives such as glycerol. Mixing conditions used for this operation were: 3540 rpm for 5 minutes. Total sample size: 100 g.

CHARACTERIZATION

Liposome size and polydispersity index (PI) was determined by photon correlation spectroscopy (PCS) using a Submicron Particle Sizer Model 370 with software C-370 v1.51 (NICOMP Particle Sizing Systems, USA). Entrapment efficiency of FLZ was investigated by use of ultracentrifugation (Beckman L8-70M, Beckman USA) at 215578 × g for 1 hour at 10 °C. Drug content in both supernatant and pellet was determined by HPLC (C-18 column) with a Waters 996 PDA detector (λ=260 nm). Mobile phase; Milli-Q water and acetonitrile (ratio 80:20 v/v). Texture properties of the different gel formulations were investigated using a TA.XT Plus texture analyzer (Stabile Micro Systems, UK) with a backward extrusion rig, as described by Hurler et al. [1],but with minor modification. Drug release studies were performed with a Franz-diffusion cells (PermeGear, USA) at 32 °C, applying polyamide membranes as the barrier.

Acknowledgement

We would like to thank Lipoid GmbH (Ludwigshafen, Germany) for generously supplying the phospholipids and Biotec BetaGlucans AS (Tromsø, Norway) for providing sample batches of soluble beta-glucans (SBG).

AIM

To evaluate the suitability of dual asymmetric centrifugation (or speed mixing) as a novel preparation method for the making "liposomes-in- hydrogel" drug formulations intended for topical application.

/ RESULTS AND DISCUSSION

Liposome size

Optimal liposome size, when intended for topical application, is claimed to be in size-range around 300 nm [4] . We thus aimed for liposomes to be between 200-300 nm in diameter. This aim was met with a DAC mixing time of 2 minutes and a rotation speed 2400 rpm (Table 1). Liposomes containing FLZ were slightly smaller than empty liposomes (Table 2). When sampled from the SBG-hydrogel, both empty liposome and FLZ-loaded liposomes had a larger particle size, according to the PCS measurements. This might be due to a change in surface properties, with SBG or glycerol molecules forming a layer on the surface of the liposomes (Table 2). However, all liposomes showed an acceptable size distribution.

Table 1. Effect of centrifugation time on liposome size (measured by PCS, volume-weighted, Gaussian distribution) (n= 3)

Centrifugation time (min)	Average particle size ± S.D. (nm)	Polydispersity index ± S.D.
2	231.9 ± 3.9	0.226 ± 0.015
5	184.5 ± 2.1	0.173 ± 0.011
15	135.1 ± 0.7	0.163 ± 0.013
20	129.4 ± 3.6	0.187 ± 0.031

Table 2. Effect of drug and hydrogel on liposome size (measured by PCS, volume-weighted, Gaussian distribution) (n= 3)

Formulation	Average particle size ± S.D. (nm)		Polydispersity index S.D.	
	from dH_2O	from hydrogel (SBG)	from dH_2O	from hydrogel (SBG)
Empty liposomes	269.0 ± 8.9	327.8 ± 52.5	0.270 ± 0.028	0.394 ± 0.094
Drug loaded liposomes	220.0 ± 10.9	251.0 ± 16.7	0.246 ± 0.036	0.270 ± 0.028

Texture properties

Texture analysis of the different formulations showed no significant difference between gels with and without liposomes. However, a significant change in the gels texture properties was observed when 20% (w/w) glycerol was added as compared to 0, 5, and 10% (Figure 4). Based on these results, and visual observation of the gels, 10% glycerol was selected as optimal for this formulation and used in further studies.

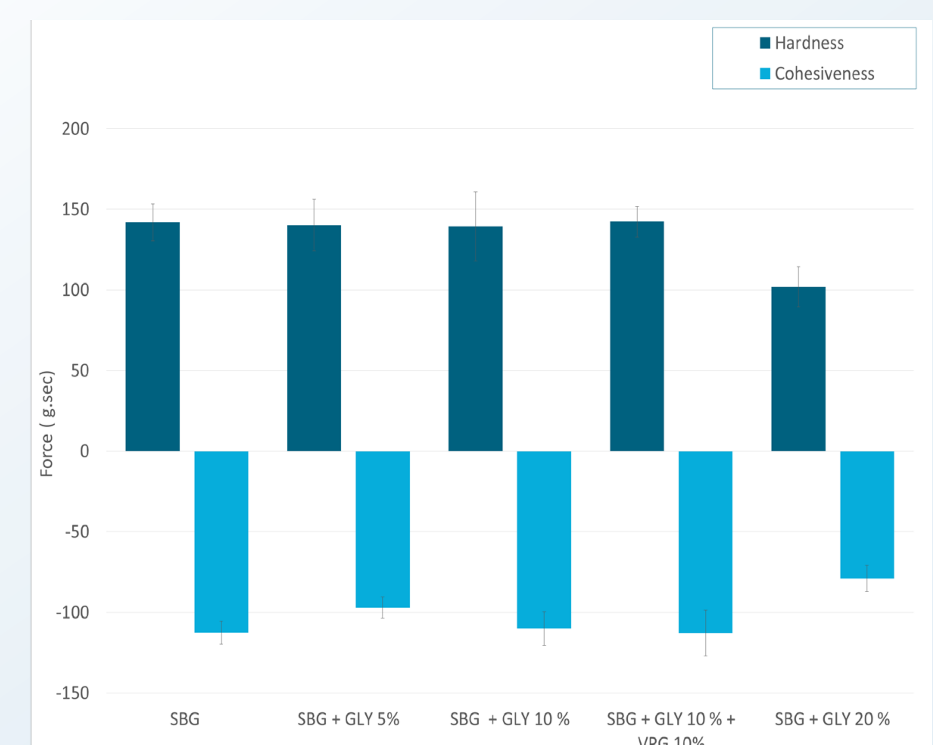

Figure 4: Texture results; hardness and cohesiveness from different liposome formulations (n=3)

Entrapment and drug release

A drug entrapment of 20.2 ± 1.2% was obtained into the liposomes after optimizing the DAC-preparation method. This encapsulation was of the same magnitude as obtained with extrusion and sonication (results not shown), however the DAC-method was less time-consuming and work-intensive.

A sustained liposomal drug release was observed from the Franz diffusions cells when comparing the optimized liposome-in-hydrogel formulation SBG/glycerol/liposomes (ratio 80:10:10 w/w), to the release of free drug in hydrogel (SBG), containing 10% glycerol (Figure 5).

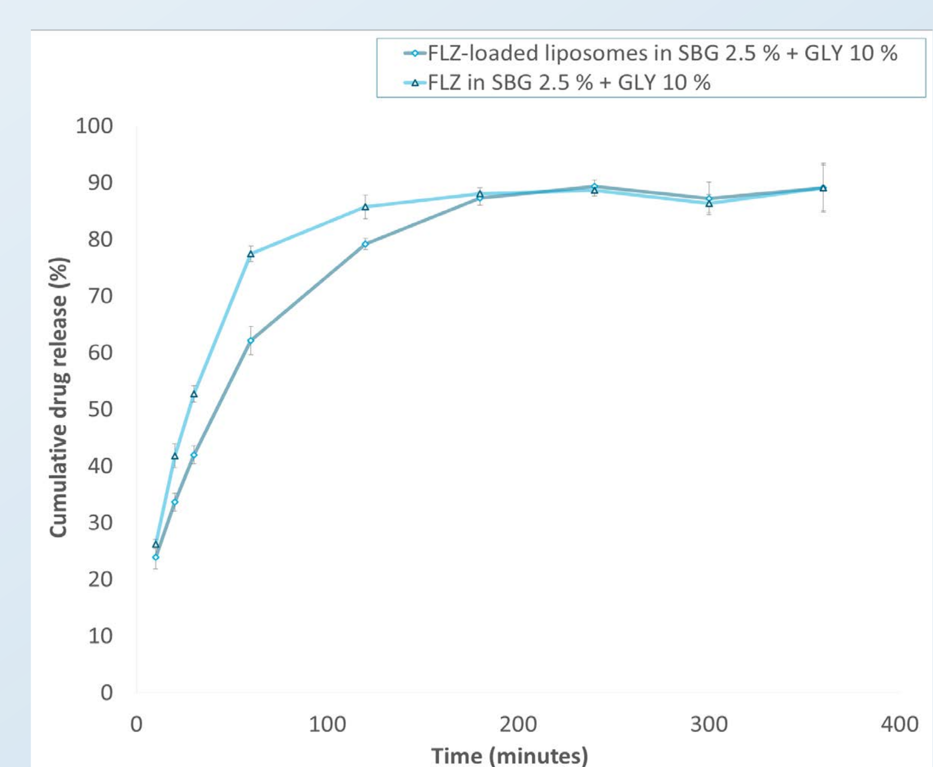

Figure 5: Drug release from hydrogels (SBG with 10% glycerol) when added as free drug or as a liposome dispersion (n=3)

CONCLUSION

DAC has been proven to be a promising tool for preparing novel liposomes-in-hydrogel formulations for topical administration to the skin, assuring a desired liposome size distribution and a sustained drug release.

REFERENCES

1.Hurler, J., et al., Improved burns therapy: Liposomes-in-hydrogel delivery system for mupirocin. J. Pharm. Sci., 2012. 101(10): p. 3906-3915.
2.Zykova, S.N., et al., Macrophage stimulating agent soluble yeast β-1,3/1,6-glucan as a topical treatment of diabetic foot and leg ulcers: A randomized, double blind, placebo-controlled phase II study. J. Diabetes Investigation, 2013. DOI: 10.1111/jdi.12165 (online version).
3.Massing, U., S. Cicko, and V. Ziroli, Dual asymmetric centrifugation (DAC)—A new technique for liposome preparation. J. Control. Release, 2008. 125(1): p. 16-24.
4.du Plessis, J., et al., *The influence of particle size of liposomes on the deposition of drug into skin.* International journal of pharmaceutics, 1994. **103**(3): p. 277-282.

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