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Point of use production of liposomal solubilised products

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ABSTRACT

With the progression towards personalised and age-appropriate medicines, the production of drug loaded liposomes at the point of care would be highly desirable. In particular, liposomal solubilisation agents that can be produced rapidly and easily would provide a new option in personalised medicines. Such a process could also be used as a rapid tool for the formulation and pre-clinical screening of low soluble drugs. Within this paper, we outline a novel easy-to-use production method for point of use production of liposome solubilised drugs. Our results demonstrate that pre-formed multilamellar liposomes, stored in a fresh or frozen format, can be bilayer loaded with low solubility drugs using a simple bath sonication process. Sonication is undertaken in a sealed vial allowing the contents to remain sterile. Liposomes around 100 nm were prepared and these liposomes were able to increase the amount of drug dissolved by up to 10 fold. These liposomal solubilisation agents were stable in terms of size and drug solubilisation for up to 8 days when stored in the fridge making them an easy to use and robust small-scale tool for drug solubilisation.

1. Introduction

Both the pre-clinical development and clinical use of many drugs remains hindered by their low solubility. Indeed, the ability to produce medicines in a liquid format remains a major consideration in pediatric and children's medicines. Liquid dosage forms can also offer advantages as age-appropriate formulations, they offer flexibility in dosing and provide wider options for those who suffer from dysphagia. Tablets in particular can cause issues for pediatric dosing; for example, the World Health Organization noted that 4 children under 36 months in age died due to choking in a deworming campaign in Ethiopia during 2007 (WHO, 2007). They also noted that medical personnel are having to either break up tablets, dissolve them in solvents, or administer the powder contained in a capsule to young children as a relevant liquid drug delivery system isn't available for that drug. However, there are a number of risks associated with these methods including difficulties in splitting and dividing of tablet doses and ensuring the drug can be reconstituted in water in a homogeneous system. Therefore, new solutions for such medicines are needed to overcome these issues.

Similar issues are faced with low solubility drugs in early pre-clinical development. Therefore a standard solubilizing agent that can be adopted for poorly soluble active pharmaceutical ingredients at a range of concentrations, that avoids the use of solvents, and that is non-toxic and easy to use would accelerate preclinical formulation time-lines. There are a number of different techniques used for formatting low solubility drugs, and suspension formulations are commonly used in the early discovery phase, owing to their ease of preparation. However, disadvantages associated with these systems can include batch-to-batch variability (including particle size) and stability issues.

Liposomes have been extensively investigated for the delivery of both hydrophobic and hydrophilic drugs due to their hydrophilic core and hydrophobic bilayer structure (Gregoriadis and Perrie, 2010). However, despite these advantages, their wide-scale use as clinically approved products remains limited to a small number of high-cost products: a key issue that has hindered their application is their cost-effective manufacture. As a result, the application of liposomes as solubilisation agents is generally cost-prohibitive. Yet liposomes offer the potential to act as solubilisation agents in a range of applications, including point-of-care medicine manipulation and point-of-use pre-clinical studies. For example, in early work from our group (Mohammed et al., 2004), we were able to load ibuprofen into the liposome bilayer and use liposomes as a lipophilic drug carrier. These studies identified key factors to consider in the formulation of liposomal solubilising agents. This included cholesterol bilayer content, lipid alkyl length and the presence of charged lipid head-groups (Mohammed et al., 2004). Following on from this, we considered a range of drugs

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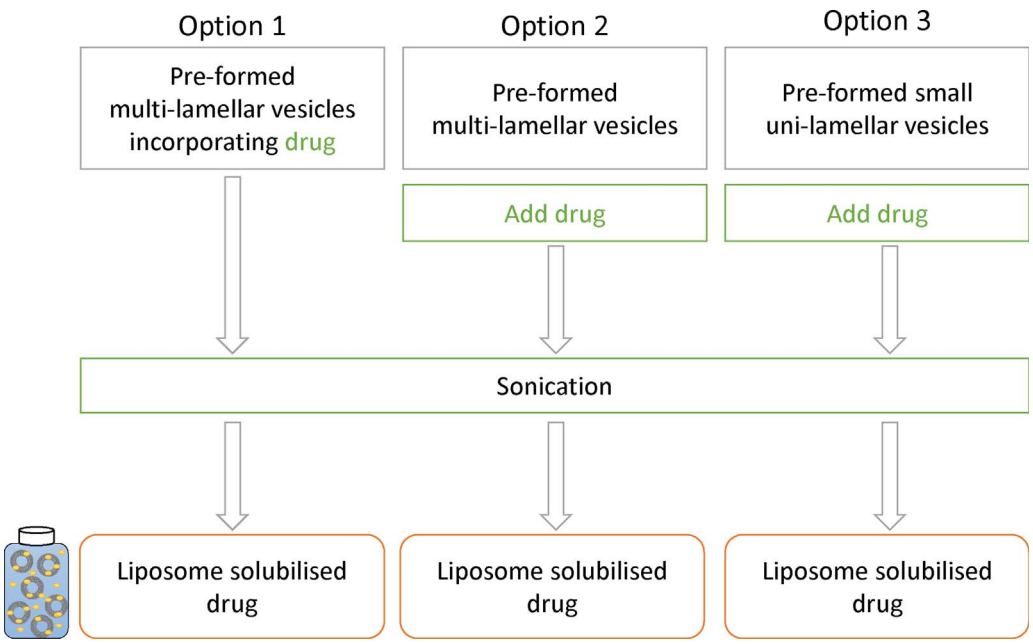

Fig. 1. Method overview. Schematic representation of the three processes tested for promoting liposome-bilayer drug loading in a rapid small-scale format.

(propofol, ibuprofen, phenytoin, diazepam and midazolam and rifampicin) and demonstrated that fatty alcohols could be used as bilayer stabilisers as an alternative to cholesterol (Ali et al., 2013). This work also showed that drug molecular weight is a key factor influencing drug loading within liposomes bilayers, with the larger molecules such as rifampicin showing low drug bilayer loading compared to smaller molecules such as propofol and ibuprofen (Ali et al., 2010; 2013). Kaess and Fahr (2014) has also shown that by taking advantage of the increased lipophilic relative area afforded to small liposomes compared to larger ones, they were able to load temoporphin into a number of different liposomal formulations.

Thus, whilst liposomes offer the potential to act as solubilisation agents, there remains a lack of appropriate, rapid and cost-effective production methods to allow the use of liposomes as solubilisation agents. Formation of large vesicles followed by size-reduction via sonication is a well-established method for the production of small-unilamellar liposomes in the laboratory setting. Yet this method is generally not suitable for the production of liposomes beyond the laboratory due to its multi-step process and lack of scalability. Sonication is a commonly used small-scale tool for size reduction and can be split into two options; bath and probe sonication. Probe sonication is the usual method employed due to its ease of use. It is also a well characterized and rapid method (e.g. Lapinski et al., 2007; Paini et al., 2015; Mendez and Banerjee, 2017). However, it can be limited by a lack of temperature control and the need to remove contamination post sonication (e.g. titanium particles that have sheared off the probe (Philippot et al., 1994)). Furthermore, this method cannot be conducted under sterile conditions due to the contact required between the sample and the probe. In contrast, bath sonication, can be conducted under sterile conditions (Lasic, 1998) and offers the opportunity to produce liposomes solubilizing drug at the individualized patient scale. It also offers the ability to work with low levels of active pharmaceutical ingredient, as is often the case in early formulation studies. Therefore, given that there is a need for the rapid and simple formulation of low solubility drugs, the aim of this current study was to develop a simple and rapid method for producing liposome solubilized drug formulations in a point of use setting. Our objectives were to investigate if drugs could be solubilized into pre-formed liposomes via sonication and the impact of drug and lipid selection had on this process.

2. Materials and methods

2.1. Materials

Ibuprofen, midazolam, propofol and cholesterol were purchased from Sigma-Aldrich, Dorset, UK. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-distearoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (DSPG), were obtained from Avanti Polar Lipids, Alabama. All chemicals used were of analytical grade and were used without further modification.

2.2. Methods

2.2.1. Preparation of multilamellar vesicles

MLV were generated using a technique based on the established film method and modified for low solubility drugs. Briefly the lipid entities were dissolved in a chloroform:methanol (9:1) at appropriate ratios and the solvent evaporated on a rotary evaporator to yield a dry film as per the standard lipid film hydration method. To entrap drugs within the bilayer, the required amount of drug was added to the solvent mixture and subsequently hydrated. Liposomes were formed from DSPC:Chol; 4:1 M ratio, or DSPC:Chol:DSPG; 6:4:2.5 M ratio. In all cases, the film was hydrated with 2 mL of phosphate buffer saline (PBS) to give final lipid concentration of 2 mg/mL unless otherwise stated.

2.2.2. Preparation of small unilamellar vesicles

To prepare small unilamellar vesicles (SUV), 1 mL of MLV were subjected to sonication for 15 sonication cycles (90 s/cycles) with 30 s stop time between each sonication cycle using a Bioruptor® Plus sonicator at 40 °C. Using this system we are able to uniformly sonicates multiple samples (3–12 samples) of volume from 100 uL to 20 mL in sealed tubes. This system uses ultrasounds derived from magnets placed below the water tank and indirectly transfers ultrasonic energy to samples. The control of the temperature and the distribution of the energy inside the water bath and the continuous rotation of tubes promoted even sonication of samples. Each of the 3 different drugs were added independently to liposomes using 3 different options; option 1: drug was added during MLV preparation followed by sonication; option 2: drug was added post MLV formation but prior sonication; and option 3: drug was added to pre-made SUV and subjected to sonication (Fig. 1).