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Investigating the role of cholesterol in the formation of non-ionic surfactant based bilayer vesicles: Thermal analysis and molecular dynamics

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ABSTRACT

The aim of this research was to investigate the molecular interactions occurring in the formulation of non-ionic surfactant based vesicles composed monopalmitoyl glycerol (MPG), cholesterol (Chol) and dicetyl phosphate (DCP). In the formulation of these vesicles, the thermodynamic attributes and surfactant interactions based on molecular dynamics, Langmuir monolayer studies, differential scanning calorimetry (DSC), hot stage microscopy and thermogravimetric analysis (TGA) were investigated. Initially the melting points of the components individually, and combined at a 5:4:1 MPG:Chol:DCP weight ratio, were investigated; the results show that lower (90 °C) than previously reported (120–140 °C) temperatures could be adopted to produce molten surfactants for the production of niosomes. This was advantageous for surfactant stability; whilst TGA studies show that the individual components were stable to above 200 °C, the 5:4:1 MPG:Chol:DCP mixture show ~2% surfactant degradation at 140 °C, compared to 0.01% was measured at 90 °C. Niosomes formed at this lower temperature offered comparable characteristics to vesicles prepared using higher temperatures commonly reported in literature. In the formation of niosome vesicles, cholesterol also played a key role. Langmuir monolayer studies demonstrated that intercalation of cholesterol in the monolayer did not occur in the MPG:Chol:DCP (5:4:1 weight ratio) mixture. This suggests cholesterol may support bilayer assembly, with molecular simulation studies also demonstrating that vesicles cannot be built without the addition of cholesterol, with higher concentrations of cholesterol (5:4:1 vs 5:2:1, MPG:Chol:DCP) decreasing the time required for niosome assembly.

1. Introduction

Non-ionic surfactant vesicles (NISVs) or niosomes are generally prepared from non-ionic surfactants such as e.g. monopalmitoyl glycerol, sorbitan esters or ethoxylated sorbitan esters, combined with cholesterol and often charged lipids such as dicetyl phosphate or stearylamine (Uchegbu and Florence, 1995; Brewer and Alexander, 1992; Carafa et al., 1998; Manosroi et al., 2003). Niosomes, like liposomes, have been investigated to enhance the delivery of a range of drugs and vaccines (Uchegbu et al., 1995; Uchegbu and Florence, 1995; Wilkhu et al., 2013b). For example, work from our laboratories has investigated their ability to enhance vaccine efficacy for antigens such as subunit influenza H1N1, H3N2 (Wilkhu et al., 2013a,c) and malarial antigens Merozoite surface protein 1 (MSP1) and glutamate rich protein (GLURP) (Vangala et al., 2006; Wilkhu et al., 2013a,c). We have recently considered the in vivo fate of such antigen-loaded niosomes and our results demonstrate that incorporation of antigen within vesicles enhanced delivery and targeting of the antigen to the Peyer's Patch compared to antigen alone. Delivery to both the Peyer's patches and mesentery lymphatics was shown to be dose dependent at lower concentrations, with saturation kinetics applying at higher concentrations (Wilkhu et al., 2013a).

In the production of niosomes, melting of the surfactants and subsequently homogenising them into an aqueous phase can be used (Uchegbu, 2000); however, quoted melting temperatures used in many of these protocols is high and based on the individual melting points of each of the surfactants used. For example, in the preparation of niosomes composed of monopalmitoyl glycerol (MPG), cholesterol and dicetyl phosphate (DCP) temperatures

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of 120–140 °C have been used (Mann et al., 2004, 2009). Yet, high temperatures may cause surfactant degradation; therefore, consideration of the required temperatures employed in such protocols is a vital component in niosome formulation studies.

To understand further the properties of surfactants used to build niosomes, a range of methods can be adopted. For example, differential scanning calorimetry (DSC) has been widely used in its application in understanding the thermal characteristics of materials (Bouzidi et al., 2005) where an insight into a range of thermal properties including melting temperatures, phase transitions and heat capacity changes can be obtained. Thermogravimetric analysis (TGA) can also be employed to understand thermal degradation and lipid/surfactant decomposition when elevating the temperature of the sample (Skala et al., 1997).

To consider the organisation of surfactants into niosomes, molecular modelling techniques can also be used to mimic the behaviour of molecules on an atomic level (Cai et al., 2011). Currently there are numerous reports in the field of lipid simulations and progress in this area has been summarised in recent reviews (Bennett and Tieleman, 2013; Rabinovich and Lyubartsev, 2013; Schneck and Netz, 2011; Lyubartsev and Rabinovich, 2011; Notman and Anwar, 2013). All-atomic molecular dynamics (MD) simulations are capable of providing the detailed information of each atom in the simulated lipid systems based on an empirical force field (e.g. GROMOS, CHARMM, AMBER), which is hard to obtain otherwise (Cai et al., 2011). Currently, all-atomic MD simulations are able to simulate these systems with hundreds of lipids and water from the order of several nanoseconds to hundreds of nanoseconds (Bennett and Tieleman, 2013, Notman and Anwar, 2013).

Given that previous work from our laboratories has investigated using niosomes for the delivery of vaccines using a surfactant combination of MPG:Chol:DCP at a 5:4:1 wt ratio (Wilkhu et al., 2013c,a), the aim of this current work was to investigate the thermal conditions required for the production of non-ionic based vesicles prepared via hot-melt homogenisation and to consider the molecular interactions in the system, via MD simulations, with particular consideration of the role of cholesterol within the system.

2. Materials and methods

2.1. Surfactants

The surfactants used in the study were monopalmitoyl glycerol (MPG) (Larodan Labs, Sweden), cholesterol (Synthecol) (Sigma Aldrich, UK) and dicetyl phosphate (DCP) (Sigma Aldrich, UK).

2.2. Differential scanning calorimetry investigations of surfactants and surfactant blends

The surfactants were analysed in the solid state using a TA Instruments Q200 Thermal Analysis DSC. The DSC was calibrated using sapphire and indium for a cell constant and temperature calibration based on the heat flow and type of cooler in place on the system. The individual surfactants were weighed into T-Zero aluminium pans and then hermetically sealed. All experimental runs started at an initial temperature of 20 °C, purged under nitrogen gas, with a scan rate of 10 °C/min to 120 °C.

After the individual surfactants were analysed for melting, different blends of the surfactant mixtures were further analysed based on a design of experiments template created where the ratio of surfactants was changed. The surfactants were mixed at the appropriate ratios and a sample of this blend was placed into the pan and investigated.

2.3. Thermogravimetric analysis of surfactant blends

The three surfactants (MPG, Chol and DCP), either individually or at a 5:4:1 ratio, were placed onto the Perkin Elmer TGA and analysed for their stability across a temperature range of 20–250 °C. Samples were also heated to 90 °C, 120 °C or 140 °C and held isothermally for 10 min at each of the three temperatures respectively to consider the stability at melting commonly reported (Mann et al., 2004, 2009). All samples were run in triplicate to determine degradation and reproducibility, and all formulations were carried out using nitrogen gas and air.

2.4. Design of experiments

To afford better understanding of the temperatures required to melt the surfactants employed in the preparation of MPG:Chol:DCP niosomes, further studies adopting Design of Experiments using a D-optimal design were employed. The MPG ratio was fixed at a wt ratio of 5 whilst cholesterol varied from a wt ratio of 2–4 and the DCP wt ratio from 0 to 3, hence effects of the individual components could be analysed for melting onset temperature, melting enthalpy, crystallisation enthalpy and reheat enthalpy.

2.5. Investigation of surfactant packing in monolayers

Langmuir monolayer studies have been used widely to understand the packaging of surfactants when mixed together by spreading insoluble amphiphilic molecules in chloroform onto an aqueous water subphase (Gopal and Lee, 2006). Monolayer studies of the individual surfactants (MPG, Chol and DCP), and a mixture of surfactants in the ratio 5:4:1 of MPG:Chol:DCP respectively, were carried out using a KSV mini trough Langmuir system (KSV Instruments Ltd., Helsinki, Finland) equipped with a platinum Wilhelmy plate in an isolated area. Ultrapure water 18 Ω (Milipore, UK) formed the subphase within these studies and the temperature of the trough was 20 ± 1 °C. Stock solutions of the individual surfactants were prepared at a 0.5 mg/mL in chloroform and a mixture was also prepared in chloroform at the set ratio. The method used was adapted from Ali et al. (2010) where 20 μL of the surfactant stock solutions was spread onto the air/water interface using a glass Hamilton syringe precise to ±0.2 μL (Ali et al., 2010). Upon spreading of the samples onto the interface, the chloroform was left to evaporate and the hydrophilic barriers were set to close at a speed of 10 mm/min to form monolayer isotherms. Each sample ran until it reached its collapse pressure and triplicate samples were tested.

2.6. Simulation of molecular dynamics

The molecular dynamics (MD) simulations utilised the AMBER11 software package (Case et al., 2005) with the general AMBER force field (gaff) (Wang et al., 2005) for all molecules. All molecules and models were built by Discovery Studio Visualizer 3.1. For simulation of the melt process, the simulated annealing method was used to mimic the hot melt preparation method of solid dispersion in the experiments (Ouyang, 2012). In the minimisation procedure, the structures were subjected to 1000 steps of steepest descent minimisation followed by 1000 steps of conjugate gradient minimisation. After minimisation, 1 ns simulated annealing simulation was performed. Langevin dynamics was used with a time step of 2 fs and a cut off of 12 Å for non-bonded interactions. Firstly, the system was gradually heated from 0 to 400 K in 200 ps, and then kept at a temperature of 400 K for 300 ps to equilibrate the systems. Next, the system was cooled from 400 to 300 K in 100 ps and finally the systems were kept at a temperature of 300 K