Aquasomes as Pulmonary Drug Delivery Systems

Fadi A. Abdulrazzaq, Yvonne Perrie and Deborah Lowry

Aston University, Birmingham, B4 7ET, United Kingdom

d.lowry@aston.ac.uk

ABSTRACT SUMMARY

Aquasomes exhibit a large surface area and show properties which protect bio-active molecules like peptides, proteins and antigens. These systems may provide an alternative to the parenteral route of delivery for bioactives for example pulmonary delivery. Aquasomes were manufactured consisting of a hydroxyapatite core, trehalose coating and BSA. Zeta potential was measured to ensure the cores were coated and the BSA was adsorbed. Pulmonary deposition was characterized using a next generation impactor (NGI). In vitro release studies were carried out on aquasomes with diameters of 2.82 and 0.94 μm. A steady amount of BSA was released over the 6 hour time period.

INTRODUCTION

The history of drug inhalation dates as early as 1,500BC were the ancient Egyptians inhaled vapors for ritual or healing purposes1. For many years pulmonary drug delivery has been used to treat lung diseases such as asthma and chronic obstructive pulmonary diseases. For local treatment of various diseases pulmonary drug delivery allows high concentrations of drugs to the site of action whilst minimizing side effects from systemic administration.

The lungs also offer a non-invasive systemic administration method with low enzyme activity and no hepatic first pass effect which is suitable for small and macromolecular drugs2. The anatomy of the lungs may compensate for the reduced bioavailability of macromolecules - a large surface area for absorption (∼100 m²) and a thin absorption membrane (0.1-0.2 μm)³.

For drug delivery via the lungs to be therapeutically effective the appropriate amount of drug must be delivered past the oropharynx. Drug deposition can occur by impaction, gravitational sedimentation or diffusion (Brownian motion) depending on particle size. Particles with an aerodynamic diameter between 0.5 and 5 μm tend to be deposited in the lower respiratory tract were in the alveoli there is a longer residence time and the drug can diffuse into the bloodstream³. An ideal inhaled drug delivery device must generate an aerosol of suitable size ideally in the range of 0.5-5 μm and provide reproducible drug dosing for example a pressurized metered dose inhaler (pMDI).

Aquasomes are one of the recent delivery systems for protein/peptide-based pharmaceuticals. Aquasomes retain conformational integrity of loaded molecules, which enable them to overcome some of the problems, such as route of delivery, physical and chemical instability, potent side-effects and poor bioavailability. They are self-assembled three layered carrier systems which consist of three layers: an inner solid core, a middle polyhydroxy carbohydrate coating and an outer drug layer. The layers are self-assembled by adsorption, through non-covalent bonds and van der Wals forces.

The delivery system has been successfully utilized for the delivery of proteins and peptides such as insulin and hemoglobin. Cherian et al prepared aquasomes using a calcium phosphate ceramic core for the parenteral delivery of insulin. They found a reduction in blood glucose levels⁴. Rawat et al carried out in vitro testing of aquasomes with the enzyme serratiopeptidase into acidic and alkaline mediums to mimic oral delivery. They found sustained release in both mediums⁵.

The aim of the present study was to characterize pulmonary deposition of BSA loaded aquasomes and investigate in vitro release from pMDI’s.

EXPERIMENTAL METHODS

Preparation of freeze-dried aquasomes: 100 mg of hydroxyapatite cores (Sigma-Aldrich, UK) was added to 10 mL of 0.15 M solution of trehalose under constant stirring at 4°C for 1.5 hours. The sample was then centrifuged (3000 rpm for 10 minutes), washed to remove excess trehalose and freeze-dried. 15 mL of BSA (1 mg/mL) was added to the freeze-dried sample under stirring for 1.5 hours at 4°C. The sample was then centrifuged at 3000 rpm for 10 minutes (Hettich Zentrifugen), washed to remove unadsorbed BSA and freeze-dried.

Zeta potential measurements: Aquasomes were analyzed using Sympatek (Brookhaven Instruments). A sample of 100 μL was added to ultra-pure water and placed in the specified cuvette with the electrode attached to it.

Preparation of aquasomes - pMDI formulation: 100mg of the powder (equivalent to 7.3mg BSA) was placed into pre-weighed aluminum pMDI vials. A BK357 30 μL valve was crimped onto the vials using a Pamasol P2011 propellant filler (Pamasol Willi Mäder AG, Pfäffikon, Switzerland) and approximately 10 mg HFA-134a was pressure-filled through the valve using a Pamasol P2011 propellant filler (Pamasol Willi Mäder AG, Pfäffikon, Switzerland).

HPLC analysis: Analysis was carried out using an Agilent Technologies 1200 series HPLC. Chromatographic separations were conducted on a Phenomenex Jupiter C5 column (250 mm x 4.6 mm, 5 μm particle size) with fluorescence detection (excitation 220 nm, emission 312 nm). The mobile phase consisted of HPLC grade water with 0.01% TFA (v/v) and HPLC grade acetonitrile. The flow rate was set at 1.0 mL/min.
and the injection volume of all samples was 100 μL. A gradient method was used.

**In vitro powder aerosolisation**: The aerosolisation properties of BSA loaded aquasomes were investigated using a NGI (NGI: Copley Scientific). A quantity of 100 mg was introduced to the 1-7 stages of the impactor. The flow rate through the NGI was adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific). The NGI was disassembled and the trays were weighted. The trays for stages 3 and 5 were washed with 10 mL of simulated lung fluid and placed in vials for *in vitro* release testing.

**In vitro release of BSA**: *In vitro* release studies were performed on the freeze-dried samples. The samples were redistributed in 10 mL of simulated lung fluid and placed in a shaking water bath at 37°C and 100 rpm. A quantity of 0.3 mL was taken for analysis at hourly time points up to 6 hours. The release medium was replaced with 0.3 mL of fresh solution.

**RESULTS AND DISCUSSION**

Zeta potential values were calculated after the coating (trehalose, -1 ± 0.5) and loading (BSA, -11.6 ± 1) stages to ensure the aquasomes consisted of the three layers (figure 1).

The aquasomes had an average size of 1.5 ± 0.96 μm. The aerodynamic particle size was measured using the NGI. Table 1 presents the amount of aquasome (mg) and the percentage of the delivered dose to each stage of the NGI. It can be seen that 60% of the delivered dose has a cut-off diameter of 2.82 μm. It has been documented that particles with an aerodynamic diameter between 0.5 and 5 μm will be deposited in the lower respiratory tract and the alveoli. For systemic circulation this is the optimum location for the drug to diffuse into the blood stream.

**In vitro** release testing was carried out on aquasomes loaded with BSA with various aerodynamic diameters. Figure 2 compares the aquasomes with aerodynamic diameters of 2.82 and 0.94 μm and the manufactured aquasomes. The BSA release is controlled over the 6 hour time period. Further studies have shown that the steady release observed continues over extended periods of time. It is interesting to observe that over the 6 hour study the aquasomes with an aerodynamic diameter of 0.94 μm released 424 μg of BSA. This is very encouraging for potential protein/peptide delivery using aquasomes via the pulmonary route.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cut-off diameter (μm)</th>
<th>Amount of aquasome (mg)</th>
<th>Percentage of delivered dose (%)</th>
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<td>-</td>
</tr>
<tr>
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</tr>
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</tr>
<tr>
<td>5</td>
<td>0.94</td>
<td>5</td>
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</tr>
</tbody>
</table>

**CONCLUSION**

We have shown that *in vitro* release studies of BSA show constant release from aquasomes with aerodynamic diameters of less than 2.82 μm. These particles should be delivered to the lower respiratory tract, allowing drug to diffuse from the aquasomes into the systemic circulation. Further studies are being carried out to determine optimal drug delivery over extended periods of time.

**REFERENCES**


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Figure 1. SEM image showing BSA loaded aquasomes.

Figure 2. *In vitro* cumulative release (mg) versus time profiles for BSA loaded aquasomes, aquasomes of diameter 2.82 μm (stage 3 in NGI) and 0.94 μm (stage 5 in NGI).