Delivery of DTaP Triple Vaccine as a solid micro-dose
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• Vaccine proteins, & bio-molecules generally cannot be delivered orally, due to degradation and inadequate absorption.

• Injectable drugs are generally in liquid form, and suffer several limitations, including poor stability, the need for skilled personnel to administer the drug, and pain and discomfort at the injection site.
Microneedles provide a means of delivering drugs in solid form through the skin. This can overcome the limitations mentioned above. However, they also suffer from their own disadvantages, namely:

- Bed of nail effect
- Manufacturing complexities and cost
- Dose delivery inconsistency
- Stability – chemical and physical
- Limitations in maximum achievable dose
- Residence time required for needles in skin

Nemaura’s Micropatch™ system offers the advantage of microneedles, without these disadvantages. This has been demonstrated in this report, in the form of a PoC study in mice using the DTaP vaccine.
• The delivery of solid doses into the skin is not new. A number of such systems have been developed and some have been marketed for several years such as the Norplant® contraceptive device and the Zoladex® implant for treating prostate cancer. Both of these systems are relatively large, however and require insertion by a highly trained medical practitioner, often as a surgical procedure.

• A more recent development is a spring powered delivery system comprising a disposable cassette (containing the dosage form) and actuator. The use of such a device has the potential to enable administration by less skilled personnel, but suffers from a number of impediments, namely manufacturing complexity and cost, loss of tip sharpness and high forces required for skin insertion.

Figure 1 Example: Solid Dose Implant compared to a match stick head
Ref: Glide Pharma
• The solid micro-dose drug delivery system coupled with a proprietary injector device developed by Nemaura Pharma (Figure 2) has been designed to overcome some of the limitations of the existing devices.

• The system can be adapted to allow a dosage form ranging in length from several hundred microns to 1-2 millimetres to be inserted into the skin in a rapid, relatively user-independent manner.

Figure 2 Nemaura MicroPatch™ Device
The principle of the device is based upon the initial insertion of a super sharp stainless steel needle to breach the tough outer barrier of the skin followed by delivery of the solid micro-dose formulation which is inserted alongside the needle.

The device allows the solid dose formulation to be inserted efficiently into the skin at any specified depth, depending on the specific application.
Aims:

- Investigate the potential for delivering vaccines using the Nemaura solid micro-dose delivery system.
- Evaluation of the physical stability of the solid micro-dose formulations, including hardness and friability.
- Tetanus and DTaP (diphtheria, tetanus and pertussis) vaccines were selected for this preliminary study – in light of its complexity as a triple formulation.
- DTaP was selected because of the clinical and commercial potential of developing a solid dose formulation of this vaccine – with potential to eliminate the cold chain storage issues.
- The DTaP vaccine is currently only available in a liquid form which must be refrigerated, thus the availability of a solid dose formulation would offer:
  - Rapid administration of a fraction of the volume;
  - No refrigeration;
  - Lower costs; and
  - Administration by non-clinicians
- The tetanus study included an evaluation of two different doses to provide a preliminary indication of the dose response relationship. The DTaP study included an evaluation of two different solid vaccine formulations.
MATERIALS AND METHODS

Materials

Freeze dried inactivated *tetanus toxoid* was purchased from the National Institute for Biological Standards and Control (NIBSC) (NIBSC code 04/150) (Potters Bar, UK).

*Infanrix*® vaccine containing, tetanus toxoid, pertussis and diphtheria (DTaP) was sourced from GlaxoSmithKline.

Pharmaceutical grade excipients were used.
MATERIALS AND METHODS

Manufacture of Micro-dose Pellets

• An optimised excipient blend suitable for the manufacture of protein containing solid micro-dose formulations was determined based on unpublished internal Nemaura Pharma studies. These excipients were selected based on their prior use in parenteral products.

• Laboratory scale manufacture of the solid micro-dose formulations was performed by geometric mixing.

• The micro-dose formulations (pellets) were individually compressed using a bespoke direct compression micro-press.

• Compressed solid micro-dose formulations were mounted on the prototype device and packaged.
Short-Term Stability Study

A short term stability study was conducted at 25 °C and 40 °C, in which a series of DTaP micro-dose formulations (pellets) were manufactured and characterised: mechanical and chemical properties.

Physical stability was assessed through the evaluation of appearance, hardness and friability.
Characterisation

Characterisation of pellets
• Pellet characterisation was performed to ensure the structural integrity of pellets was maintained throughout the pellet manufacturing process and subsequent storage.

Visual analysis
• Surface morphology characterisation of pellets was performed using optical microscopy. Images were taken following manufacture to evaluate any visual imperfections on the surface of the pellets.

Pellet hardness
• Mecmesin Multi test 2.5i compression instrument with a 50 N load cell was used to conduct hardness experiments. Emperor™ force testing software was then used to program the parameters of the pellet compression.
• A graphical representation of the force vs displacement of the pellets was then produced. Based on preliminary (unpublished) data, a pellet hardness of greater than 3N was found to be sufficient for insertion into mouse skin.
Immunogenicity Protocol

• The tetanus dose groups each consisted of 5 male and 5 female animals. The DTaP dose groups consisted of 10 male and 10 female animals. ELISA assay was performed on each animal group blood samples for each vaccine.

• Control groups for the tetanus study were administered reconstituted pellets or an equivalent dose of reconstituted freeze dried tetanus toxoid as supplied from NIBSC. Control groups for the DTaP study were administered an equivalent dose of reconstituted freeze dried vaccine or an equivalent dose of Infanrix®. A constant dosing volume of 500 µl was used for all control groups. Animals were dosed on day 0 (prime) and day 28 (boost).

• The pellets were inserted into the skin of lower back of the mouse in a lateral direction to ensure the pellet was administered subcutaneously. Daily physical and general behaviour of the mice throughout the study was monitored including weight, faecal matter and general movement. Body weight and feed intake of all the animals was conducted weekly. No mortalities occurred during the study.

• Blood samples for ELISA assay were taken on day 21 and day 42. Samples from all test animals except the naïve (untreated) control group were diluted 5 fold prior to assay. The ELISA assays were performed according to the kit instructions.
Proof of Concept Mouse Immunogenicity Study

- A relatively high dose of tetanus was chosen (1/4 human dose) since the source material did not contain an adjuvant. A lower dose was included to investigate any dose response effects (1/16 human dose).

- Two different freeze dried DTaP freeze-dried formulations were used to prepare pellets for the study (Formulations 1 and 2). The pellets contained approximately 2% of a human dose in each pellet.

- The study was conducted at a CRO.

- ELISA (enzyme-linked immunosorbent assay) kits (purchased from GenAsia Biotech (Shanghai, China)) were used to determine the antibody response to the administered vaccine micro-dose formulations.
## Vaccine Formulations

### Table 1 Dose groups - Immunogenicity study

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Description</th>
<th>Fraction of Human Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><strong>High Dose Pellet</strong> (equivalent to human dose) (2.5 Lf units)</td>
<td>25%</td>
</tr>
<tr>
<td>2</td>
<td><strong>Low Dose Pellet</strong> (0.625 Lf units)</td>
<td>6.25%</td>
</tr>
<tr>
<td>3</td>
<td>High dose positive control <em>(reconstituted vial</em> of freeze dried vaccine)</td>
<td>25%</td>
</tr>
<tr>
<td>4</td>
<td>Low dose positive control <em>(reconstituted vial</em> of freeze dried vaccine)</td>
<td>6.25%</td>
</tr>
<tr>
<td>5</td>
<td>High dose pellet positive control <em>(reconstituted pellets)</em></td>
<td>25%</td>
</tr>
<tr>
<td>6</td>
<td>Low dose pellet positive control <em>(reconstituted pellets)</em></td>
<td>6.25%</td>
</tr>
<tr>
<td>7</td>
<td><strong>Blank Pellet</strong> (Placebo)</td>
<td>N/A</td>
</tr>
<tr>
<td>8</td>
<td><strong>Pellet</strong> – Triple vaccine formulation 1</td>
<td>2 %</td>
</tr>
<tr>
<td>9</td>
<td><strong>Pellet</strong> – Triple vaccine formulation 2</td>
<td>2 %</td>
</tr>
<tr>
<td>10</td>
<td>positive control <em>(reconstituted</em> freeze dried vaccine - formulation 1)</td>
<td>2 %</td>
</tr>
<tr>
<td>11</td>
<td>Positive control <em>(reconstituted</em> freeze dried vaccine - formulation 2)</td>
<td>2 %</td>
</tr>
<tr>
<td>12</td>
<td>Vaccine positive control <em>(equivalent dose of liquid vaccine, Infanrix®)</em></td>
<td>2 %</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Physical characterisation

The data from the short term stability study is shown in Table 2. No significant trends were observed and the hardness and disintegration time were acceptable throughout the study.

Table 2 Physical Stability Data during Short Term Storage at 25 and 40°C

<table>
<thead>
<tr>
<th>Test</th>
<th>Time point (Weeks) at temperatures</th>
<th>25°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T = 0</td>
<td>2 weeks</td>
</tr>
<tr>
<td>Hardness (N) (SD)</td>
<td></td>
<td>6.21 (1.25)</td>
<td>4.59 (1.06)</td>
</tr>
<tr>
<td>Friability (Mean % friability) (SD)</td>
<td></td>
<td>2.53 (0.05)</td>
<td>1.22 (1.72)</td>
</tr>
<tr>
<td>Disintegration (Seconds) (SD)</td>
<td></td>
<td>75.67 (15.16)</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Physical Characterisation

The stability study focussed on the physical characteristics of the pellets because in vitro activity cannot be easily assessed.

The physical characterisations (Hardness and friability) indicated that the micro-dose formulations had a sufficiently robust structural integrity to withstand shipment, storage and administration.
Efficacy Characterisation of Micro-Dose Formulations by Immunogenicity Analysis

Animal Observations

Basic observations of the animals were determined to be normal.

No mortality occurred and the weight gain of the mice was normal.
Antibody Response: Tetanus Toxoid Study

- Figure 4 shows the immune responses to inactivated tetanus toxoid vaccine. The results are expressed as an optical density value and are adjusted for the negative control. Values for blanks, positive and negative assay controls were very similar at both 21 and 42 days enabling the two sets of results to be directly compared.
- The results clearly show that there is an increased dose effect at day 42 compared to day 21 with all the high dose groups showing a stronger response than the low dose groups. The high dose tetanus micro-dose formulation showed a stronger response than both positive controls (reconstituted pellets and reconstituted freeze dried vaccine) which could suggest a dose sparing effect associated with the solid formulation. All dose groups show a clear response compared to the placebo group.

Figure 4. Graph to show the immune response to Tetanus Toxoid (Tetanus Study)
**Antibody Response: Tetanus Study – in DTaP**

- Results are expressed as an optical density value and are adjusted for the negative control.

- An apparent **dose sparing** effect is seen with formulation 1 for Tetanus compared to liquid injection.

![Figure 5 Graph to show the immune response to tetanus toxoid (DTaP Study)](image-url)
Antibody Response: Diphtheria in DTaP

- The greater response to the Pellet formulation 1 for Diphtheria compared to liquid vaccine of equivalent dose.
Antibody Response: Pertussis in DTaP

- The response is seen at the early time point as well as later, though appears to tail off. For pellets.
- Higher Response for the Pellet formulation 1 than for the equivalent liquid dose.
Antibody Response: FHA in DTaP

- Approximately **25% higher** response observed in the pellet at the first time point compared to the liquid dose.

- Approximately **25% lower** response observed after the booster dose compared to the liquid dose.
CONCLUSION

• Strong potential of the Nemaura solid micro-dose technology for the delivery of vaccines and other Biologics.

• Mechanical stability demonstrated that the structural integrity of the micro-dose formulations was sufficient to withstand handling, shipment and insertion into mice.

• The tetanus immunogenicity study showed a clear dose/response and some suggestion of a dose sparing effect associated with the solid micro-dose delivery system.

• The DTaP immunogenicity study not only showed that an immune response could be achieved for all components using a solid micro-dose formulation, but also that sufficient vaccine potency had been preserved in the solid dose form.
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Thank you.

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