

Semaglutide powder for inhalation manufactured through a novel process and its aerodynamic and *in vitro* performance

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Summary

Semaglutide (SMG) is a glucagon-like peptide-1 (GLP-1) receptor agonist that was modified to increase the half-life from two minutes to approximately one week. It has shown significant results in the treatment of type 2 diabetes and obesity. The opportunity to shift from subcutaneous injection to an inhaled product of SMG for systemic effect would enhance patient adherence and convenience. Nanoform's proprietary Bio platform technology (Bioline) can manufacture formulated biological drug particles of tuneable size and morphology, while retaining biological activity. The technology can be applied across various delivery routes. For inhalation, suitable particle sizes can be easily produced. Formulation development and manufacturing were focused on the production of a physicochemical stable formulation, with improved aerodynamic particle size distribution. No chemical degradation or physical change was observed during the formulation manufacturing process. The powder aerosol showed a low MMAD and high fine particle fraction. A down-selected formulation showed stability at 25°C/60% RH for 3 months. Dissolution testing of the SMG formulation at three dose levels was conducted. The dose was captured on a membrane filter using a modified Andersen Cascade Impactor (mACI) and a pre-separator with a 5 µm cut off. The dissolution testing was performed with USP paddle over disk at pH, 6.0 and 7.5. Dissolution was rapid, 80% is dissolved after 2 minutes and complete dissolution after 30 minutes at both pH's. A stable formulation of semaglutide suitable for inhalation delivery for a systemic effect has been developed and is ready for further preclinical *in vivo* testing.

Key Message

Formulated semaglutide powder suitable for pulmonary delivery by a dry powder inhaler was manufactured using Nanoform's proprietary Bio platform technology. The powder demonstrated an aerodynamic performance advantageous for systemic availability, with at least 3 months 25°C stability. Dissolution was dose linear and rapid at both pH 6 & 7.5.

Introduction

The increased half-life of SMG (a modified GLP-1 agonist), compared to first generation GLP-1 agonists, provides continuous receptor activation, resulting in increased insulin release and slower gastric emptying, that enhances blood glucose level control and promotes greater weight loss [1]. The use of subcutaneously administered modified GLP-1 agonists for treating conditions like type 2 diabetes and obesity has grown substantially [2-3]. Inhaled modified GLP-1 agonists for systemic effect offer advantages over both oral and sub cutaneous delivery routes, by reducing the adverse gastrointestinal effects, and improving patient adherence and convenience.

Nanoform's proprietary Bio platform technology offers among others the opportunity of processing formulations for pulmonary administration by generation of respective feedstock solutions and operating at low temperature, typically <50°C that can maintain activity and stability of heat labile proteins and peptides. It is used for the supply of low bioburden batches for preclinical studies and has been optimised for pilot scale supplies. The implementation of automated GMP manufacturing is ongoing.

To prepare the particles a feedstock solution was modified with respect to various formulation parameters such as pH, API concentration and excipient choice before running on the Bio platform. SMG is a sensitive API; hence development and use of non-heating process was desired. Regular inhalation excipients, including a surface acting agent were used in the screening process. The morphology, impurity levels, aggregate formation, and APSD of SMG-loaded particles were assessed.

A dissolution study was conducted to investigate the rate and extent of dissolution with doses of 2 mg, 8 mg and 14 mg. This was using a mACI with a USP throat, NGI pre-separator with 5 µm cut off. The dissolution experiment will then inform of the dose range for subsequent preclinical *in vivo* testing.

The aim of this project was to develop a physicochemical stable formulation of SMG with suitable APSD and *in vitro* dissolution profiles worthy of further investigation in an *in vivo* preclinical study for systemic exposure assessment.

Experimental methods

A schematic of Nanoform's proprietary Bio platform technology is shown in Figure 1. The formation of droplets is achieved using a liquid nebulizer (vibrating mesh) and a carrier gas. The evaporation of water is slower using drying gas of room temperature. The dried formulation is charged by an in-line ionizer and collected using electrostatic precipitation.

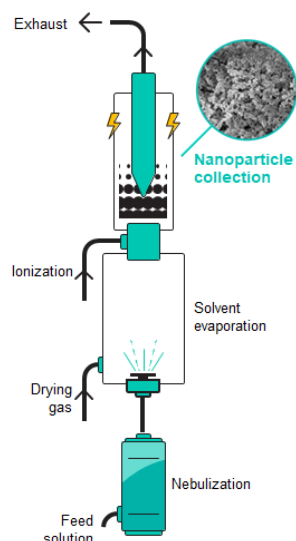


Figure 1. The Bio platform process used during the manufacture of formulated SMG powder

Feedstocks containing formulated API (Aurisco, Yangzhou, China) were prepared and processed using the Bioline. API % w/w investigated ranged from 1-32% w/w, with the 16% w/w API been down selected for the performance measurements and stability study.

HPLC Chromatograms were acquired in a Waters Arc System. The quantification of the API within the powder and during APSD testing was determined using reverse phase HPLC method (limit of quantification 5 µg/ml and linearity 0.99 R² between 5-500 µg/ml). The chemical composition of the API was analysed by reverse phase HPLC method, capable of separating related impurities. The aggregation state of the API was analysed by size exclusion chromatography (SEC).

Scanning emission microscopy was performed to determine morphology of the formulated API. The morphology was evaluated in a ZEISS Sigma 300 SEM with acceleration voltages between 3 – 7 kV. SEM-PSD – a minimum of 200 particles obtained by imaging 3 different points in the stub. The particle size distribution is given by Dn10, Dn50, Dn90, and Dv50.

Water content was determined using Karl Fischer (KF) titration. XRPD was determined using a Malvern PANalytical Empyrean powder X-ray diffractometer equipped with a Cu anode (wavelength of 1.54 Å) in reflection mode.

APSD measurements were determined using a Next Generation Impactor (NGI, Copley Scientific Ltd, UK). Powder, 25mg, was manually loaded with loaded into size 3 HPMC capsules (Capsugel, Belgium). The selected inhaler device was the RS-01 medium resistance inhaler (Berry, Italy), and testing was at 30L/min and 60L/min. The deposited API was dissolved into an aqueous based solvent for quantification. Calculation of APSD parameters was determined using the Inhalytix software (Copley Scientific Ltd, U.K).

Dissolution testing of powder was conducted at a CRO. Testing was carried out in 0.2% Tween80/PBS at pH 7.5 and 6.0 representing *in vivo* relevance of lung pH levels. Testing was carried out using the RS-01 device (as above), medium device resistance to achieve approx. 4 kPa at 65 L/min. Doses tested were 2 mg, 8 mg and 14 mg. The delivered lung dose was achieved by using a mACI, mounted to a USP throat, NGI pre-separator with 5 µm cut off. All particles after the pre-separator with 5 µm cut off

were captured on the Polycarbonate filter membrane. The membrane was transferred into a sandwich, tightened with clips and put into a Erweka DT80 vessel containing pre-heated (37°C) dissolution media. All time point samples were filtered through a syringe filter (0.2 µm) to remove undissolved particles. Total amount of SMG in the vessel was sampled at the last time point, without filtration. Also, the membrane was removed from the dissolution vessel and placed in an extraction solution, not filtered and reported as the total dose.

Results and Discussion

Formulation screening included approved inhalation excipients including a matrix former, phosphate buffer and a surface acting agent. SMG has a low solubility at pH range 2-6 (pI 5.4). The pH was screened and pH 8 was found to be optimal, away from the pI value and lower solubility level. The API % w/w was screened between 1-32% w/w and no effect on stability after manufacture was observed. The water content was < 3% and the powder was amorphous measured by XRPD. The 16% w/w API formulation was down selected based on early predictions of a dose and to minimise the number of capsules per dose. Powder of formulated API was manufactured using the Bio platform process and set down on stability trial.

The sphericity of the particles produced can be adjusted from very spherical to asymmetrical. Here the particles were slightly asymmetrical that can be advantageous for inhalation drug delivery because the shape can influence aerodynamic properties like flowability and deposition patterns within the lungs. The SEM images in Figure 2 show asymmetrical shape with a size distribution of particles. Table 1 gives the precise Dn and Dv50 numbers as determined by an in-house AI particle size software based on SEM images.

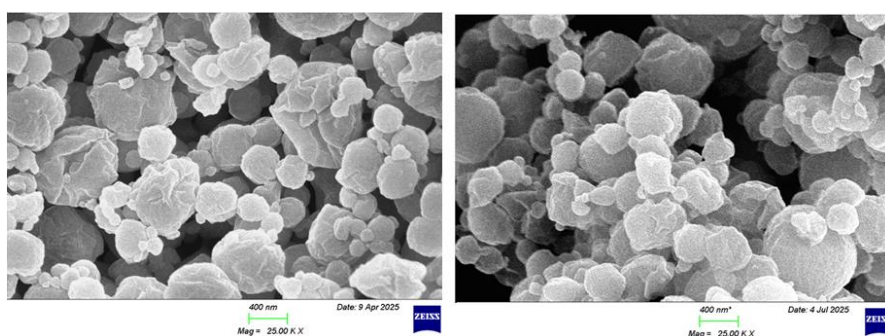


Figure 2. Time zero (left) and 3-month (right) formulated SMG powder by SEM at 25K magnification.

Table 1. SEM-PSD of formulated SMG particles used in the stability trial

	Dn10	Dn50	Dn90	Dv50	Std Dev	Span
Time zero	151	354	640	697	208	1.4
3 Month	167	358	659	676	204	1.4

The related species RP-HPLC is specific for all forms of SMG degradation. The main peak % did not change over the stability trial. Likewise, there was an absence of high molecular weight species.

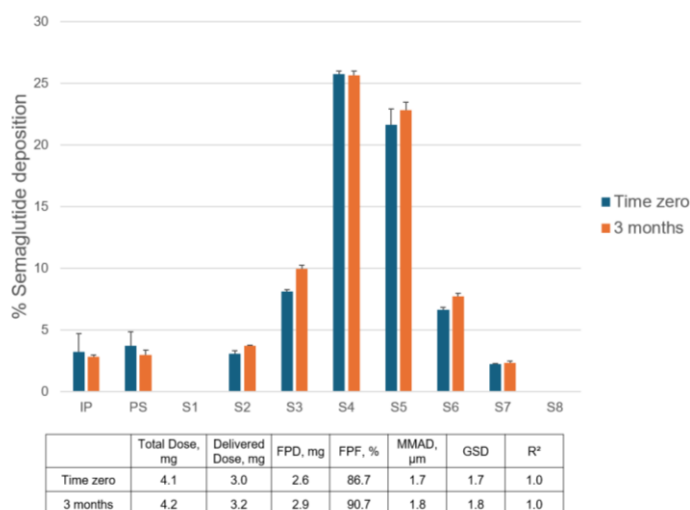


Figure 3. Aerodynamic parameters at 60 L/min, medium resistance RS-01 device, aerosolising 25 mg of powder, approx. 4 mg API into an NGI (n=3).

The APSD assessment of formulated SMG powder on stability trial showed very consistent results. There is low deposition in the induction port and pre separator. For the 60 L/min flow rate the MMAD was 1.8 μm . The main deposition in the NGI is between stages 4 and 5. The FPF was high >85%. The low MMAD can lead to better lung deposition, particularly in the lower airways and alveoli, thereby increasing the potential of systemic uptake.

At 30 L/min the MMAD was 2.6 μm , the FPF approx. 74% and FPD 2 mg. Again, the stability trial data at time zero and 3 months was very consistent. The profile is very similar to the 60 L/min result, but the overall deposition is lower. There is a flow rate dependency using the specific device and capsule selected and improvements can be investigated.

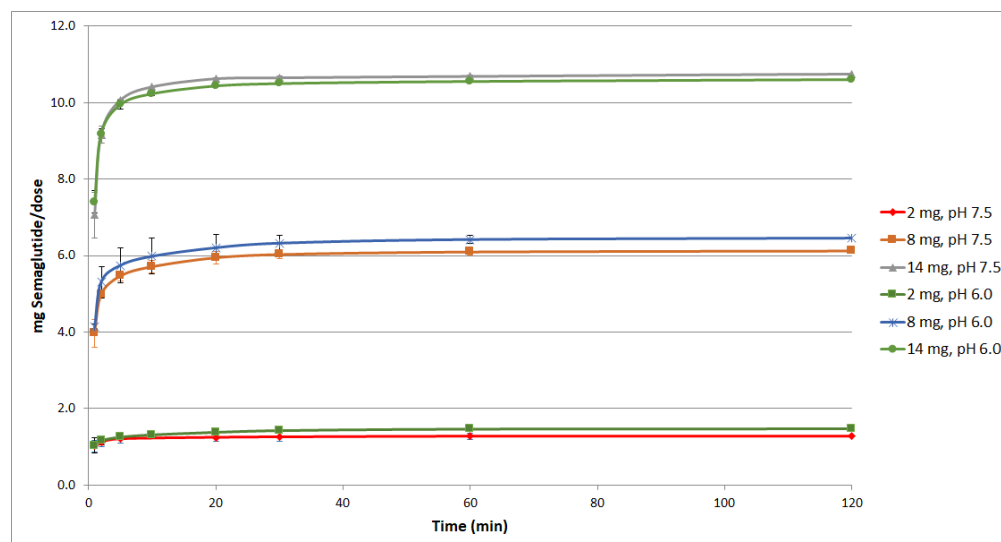


Figure 4. Dissolution profile of SMG at pH 6.0 and 7.5. Mean $\pm$ SD dissolved amounts (mg SMG/dose). n=4.

The aim of the dissolution study was to characterise the dissolution performance of the SMG formulation at the absorption site in the lung. The range of SMG dose were 2 mg, 8 mg and 14 mg. The upper dose range was explored to cover any unexpected lower uptake via the lung versus subcutaneous delivery. The two pH levels are of *in vivo* relevance of normal and diseased states. The dissolution was very fast at both pH 7.5 and pH 6.0. Approx. 80% dissolved was achieved after 2 minutes and complete dissolution after 30 minutes at both pH 7.5 and pH 6.0. No significant difference in dissolution rate and extent of dissolution was observed when comparing between the two pH values. The dissolution rate was dose linear.

Conclusions

Nanoform's proprietary Bio platform technology was used to manufacture dry powder particles of formulated SMG with excellent physicochemical stability data and consistent APSD data. The lower MMAD and high FPF indicates deep lung alveolar deposition, a prerequisite for achieving systemic availability *in vivo*. The dissolution experiment highlighted that absorption is rapid and dose linear.

References

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