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# Engineered sodium hyaluronate respirable dry powders for pulmonary drug delivery

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ABSTRACT

Sodium hyaluronate (HYA) warrants attention as a material for inhalation due to its (i) therapeutic potential, (ii) utility as a formulation excipient or drug carrier, and (iii) ability to target lung inflammation and cancer. This study aimed to overcome formulation and manufacturing impediments to engineer biocompatible spray-dried HYA powders for inhalation.

Novel methodology was developed to produce HYA microparticles by spray drying. Different types of surfactant were included in the formulation to improve powder respirability, which was evaluated *in vitro* using cascade impactors. The individual formulation components and formulated products were evaluated for their biocompatibility with A549 respiratory epithelial cells.

The inclusion of stearyl surfactants, 5% w/v, produced the most respirable HYA-powders; FPF 59.0–66.3%. A trend to marginally higher respirability was observed for powders containing stearylamine > stearyl alcohol > cetostearyl alcohol. Pure HYA was biocompatible with A549 cells at all concentrations measured, but the biocompatibility of the stearyl surfactants (based on lethal concentration 50%; $LC_{50}$) in the MTT assay ranked stearyl alcohol > cetostearyl alcohol > stearylamine with $LC_{50}$ of 24.7, 13.2 and 1.8 μg/mL, respectively.

We report the first respirable HYA powders produced by spray-drying. A lead formulation containing 5% stearyl alcohol was identified for further studies aimed at translating the proposed benefits of inhaled HYA into safe and clinically effective HYA products.



## 1. Introduction

Sodium hyaluronate (HYA) warrants attention as a product for inhalation on account of (i) having inherent therapeutic potential, (ii) offering utility as a formulation excipient or drug carrier, and (iii) possessing targeting potential for disease sites in the lungs, *e.g.* macrophages or lung cancer (Liao et al., 2008). The development of inhaled formulations has been limited by difficulty in formulating and manufacturing sodium hyaluronate as a powder for inhalation, therefore this study aimed to engineer a flowable, highly respirable powder.

Hyaluronate is an endogenous glycosaminoglycan present in matrices such as the extracellular matrix and synovial fluid. It is a linear polysaccharide composed of a repeating disaccharide unit of *N*-acetyl-D-glucosamine and D-glucuronic acid bound by β 1,4 glycosidic bond. The disaccharides are linked by β 1,3 bonds to form the hyaluronic acid chains (Lapcík et al., 1998). *In vivo*, hyaluronate exists as a polyanion rather than its protonated form, hyaluronic acid, although the terms are often used synonymously in the literature (Liao et al., 2008). The use of high molecular weight sodium hyaluronate is approved in several pharmaceutical products for topical application and injection and as a functional component of some nebuliser solutions. Hence, hyaluronate is considered to be a biocompatible, biodegradable and non-immunogenic biomaterial.

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In the lungs, the physiological function of hyaluronate is to stabilize connective tissue, organize extracellular matrix, control hydration/water homeostasis (Gerdin and Hällgren, 1997), as well as modulating cell migration and phagocytosis (Turino and Cantor, 2003). In disease, hyaluronate plays a role in the inflammatory response (Cantor, 2007), tissue remodeling (Petrigni and Allegra, 2006) and various CD44 receptor-mediated functions in cell detachment, carcinogenesis and inflammation (Zhong et al., 2016). The CD44 receptor belongs to the family of cell adhesion molecules specifically involved in the control of cell behaviour by mediating contact between cells or between cells and the extracellular matrix, which is essential for maintaining tissue integrity (Arpicco et al., 2013). However, these important functions are also central to pathological conditions including tumour progression and metastasis (Orian-Rousseau, 2010). CD44 receptors bind high molecular weight hyaluronate, but can also interact with shorter chains (Tammi et al., 2002).

Several studies have reported hyaluronate to be a potential therapeutic agent for inflammatory lung disorders, in particular for prevention of exercise-induced bronchoconstriction in asthma, emphysema and COPD (Petrigni and Allegra, 2006; Souza-Fernandes et al., 2006). Different mechanisms of actions linked to the inhibition of lung elastase, binding to elastic fibers and limitation of cell-cytokine interactions *via* CD44 receptor have been described (Cantor et al., 2011; Iskandar et al., 2009). HYA is an active component of two inhalation products. Yabro® (Ibsa Farmaceutici, IT) is a high viscosity hyaluronate solution for nebulization (MW 800–1000 kDa, 0.3% w/v) to reduce bronchial reactivity induced by inhalation of allergens/pollutants or by physical effort (Gelardi et al., 2013). In this formulation, HYA improves palatability and reduces potential side effects of nebuliser solutions such as irritation and cough. Hyaluronate (MW 500 kDa, 0.1% w/v) is also available in a hypertonic solution of NaCl at 7% (Hyaneb®, Chiesi Farmaceutici, IT) which decreases mucus viscosity in cystic fibrosis patients by attracting water to hydrate the mucus (Nenna et al., 2011). Hyaluronate has also been proposed as carrier for drug delivery to the lung, either as a scaffold for modified release formulations or for particle/drug targeting to alveolar macrophages, *e.g.* for treatment of tuberculosis (Hwang et al., 2008).

Despite this potential, HYA has not been developed as a powder formulation for lung delivery, either alone or in combination with drugs. HYA particles have poor flowability and tend to be cohesive requiring the use an adhesive mixture with lactose in order to be aerosolized (Hwang et al., 2008). The aim of the present work was to investigate the use of different excipients to produce flowable, highly respirable and safe hyaluronate dry powders through a particle engineering approach based on spray drying. Powder respirability was investigated *in vitro* and prototype formulations were evaluated for alveolar cell compatibility *in vitro*.

## 2. Materials and methods

### 2.1. Materials

Sodium hyaluronate (HYA; PrymalHyal 50, average MW = 29504 Da) was purchased from Soliance (Pomacle, FR). Stearylamine, L-lysine and thiazolyl blue tetrazolium bromide (MTT), sodium dodecyl sulphate (SDS), *N,N*-Dimethylformamide (DMF), RPMI-1640, Fetal Bovine Serum (FBS), L-glutamine, gentamicin were supplied by Sigma–Aldrich (Sigma Chemical Co., Milan, IT). Stearyl alcohol, cetostearyl alcohol and stearylamine were purchased from ACEF Srl (Fiorenzuola d'Arda, IT). A single dose dry powder inhaler, RS01 (Plastiape Spa, Lecco, IT), was used to aerosolize HYA powders for aerodynamic performance testing. Powder formulations were loaded in size 3 hypromellose Quali-V capsules (Qualicaps Europe, Madrid, ES). All chemicals used were of analytical grade and water was purified (0.055 μS/cm, TOC 1ppb) with Purelab pulse + Flex ultra-pure water (Elga Veolia, Milan, IT). A549 alveolar epithelial cells were obtained from the American Type Cell Culture; tissue culture flasks (75 $cm^2$ with ventilated caps) and 96-well plates were from Costar (Fisher Scientific, Loughborough, UK). Phosphate buffered saline (PBS) tablets were purchased from Oxoid Ltd (Basingstoke, UK).

### 2.2. HPLC analysis of sodium hyaluronate (HYA)

The analytical quantification of HYA was performed by size exclusion – high performance liquid chromatography (SEC-HPLC) using a BioSep-SEC-s4000, 5 μm 7.8 × 100 mm column (Phenomenex Srl, Bologna, IT). Standard and samples were prepared in purified water. Mobile phase was prepared by dissolving 6.8 g of $KH_2PO_4$ in 1 L of purified water and the pH was adjusted to 7.0 with 5 M potassium hydroxide. The injection volume was set at 100 μL, flow rate of the mobile phase was 1.0 mL/min and wavelength of detection was 200 nm. Linearity of response was tested before each analysis in the concentration range between 5 and 500 μg/mL ($R^2$ = 0.999).

### 2.3. Production of HYA powders by spray drying

HYA powders were manufactured by spray-drying using a mini spray-dryer B-290 (BÜCHI Labortechnik AG, Flawil, CH). HYA was dissolved in purified water at room temperature and this solution was added to ethanol (water:ethanol ratio 30:70 v/v) under magnetic stirring at 50 rpm. When excipients were incorporated in the formulation, they were added to the water or ethanol phase (according to excipient solubility). The compositions of hyaluronate formulations are reported in Tables 1 and 2.

The solutions were spray-dried using the following process parameters: inlet temperature 90 °C, drying air flow rate 750 L/h, solution feed rate of 3.0 mL/min and nozzle diameter of 0.7 mm.

**Table 1**
Hyaluronate formulations screened to identify excipients that enhance spray drying yield, emitted dose and respirable fraction. Data represent mean ± standard deviation, n = 3.

| | Feed solution to spray drying | | | | Powder characterization | | | |
|---|---|---|---|---|---|---|---|---|
| Hyaluronate powder formulation | Surfactant type | Surfactant ratio to hyaluronate (% w/w) | Solute content of feed suspension (% w/v) | Surfactant concentration (mg/ml) | Production yield (%) | HYA content (%) | Emitted dose (%) | Respirable fraction (%) |
| Hyaluronate (HYA) | – | – | 0.83 | – | 67.9 ± 2.1 | 91.1 ± 0.5 | 38.6 ± 0.10 | 25.4 ± 3.7 |
| HYA-Mannitol | Mannitol | 10 | 0.92 | 0.92 | 65.0 ± 3.2 | 80.6 ± 0.8 | 67.4 ± 0.11 | 27.6 ± 0.6 |
| HYA-Lysine | Lysine | 10 | 0.92 | 0.92 | 76.7 ± 1.4 | 79.8 ± 0.8 | 58.7 ± 0.05 | 30.9 ± 5.2 |
| HYA-Stearylamine | Stearylamine | 10 | 0.92 | 0.92 | 49.0 ± 2.4 | 78.9 ± 2.0 | 88.1 ± 0.01 | 45.0 ± 4.1 |