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# Investigation of nanocarriers and excipients for preparation of nanoembedded microparticles

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ABSTRACT

Colloidal drug delivery systems often face physical and chemical instability as well as challenges with directed delivery. In order to overcome these challenges the colloidal formulations can be processed into microparticulate form (nanoembedded microparticles (NEMs)). In this study, different polymer nanocarriers (poly(lactide-*co*-glycolide), poly(styrene), chitosan and dendrimers) were used for preparing NEMs by spray-drying. Further, distinct matrix excipients were investigated including sugars (i.e., trehalose, sucrose, mannitol) and polymers (poly(vinyl pyrrolidone) and poly(ethylene glycol)), and the characteristics and performance of NEMs were studied in detail. It was found that with increasing hydrophilicity of the polymer nanocarriers, an increasing amount of excipient was necessary to stabilize the nanoparticles. NEMs containing polyplexes and nanogels required a matrix-to-nanoparticle (M:N) ratio of 50:1 and 10:1, respectively, whereas NEMs with poly(styrene) and poly(lactide-*co*-glycolide) only required an M:N ratio of 1:1 and 1:4, respectively. Investigation of different excipients demonstrated that water soluble sugars and polymers can be used to prepare NEMs and that spray-dried amorphous excipients (trehalose, sucrose, poly(vinyl pyrrolidone)) are superior to spray-dried crystalline excipients (mannitol, poly(ethylene glycol)) for stabilizing NEMs. It is therefore important to select an appropriate excipient for stabilization of a given nanoparticle system and identify a suitable level of this excipient to keep the nanoparticles viable.



## 1. Introduction

With the gain in popularity of polymer nanoparticles (NPs) for drug delivery purposes and an increasing number of NP products in development it is important to consider strategies for optimal delivery and stability of NP-based systems for pharmaceutical development (Kumari et al., 2010; Soppimath et al., 2001). There are many challenges associated with polymer NPs intended for drug delivery including physical and chemical stability, premature drug release and a typically tedious multi-step fabrication process including removal of organic solvents (Allen and Cullis, 2004; Sanhai et al., 2008).

Nano-embedded microparticles (NEMs) represent a pharmaceutical dosage form, which is commonly used to stabilize diverse types of NPs (Bohr et al., 2015). NEMs are usually generated from colloidal suspensions by adding stabilizing excipients to form dry microparticles, which demonstrate efficient aerosolization and superior deposition properties in the respiratory tract when delivered via inhalation (Beck-Broichsitter et al., 2012; Tsapis et al., 2002). Additionally, NEMs are used to prevent chemical degradation (Lemoine et al., 1996) and/or physical instability (Abdelwahed et al., 2006b) of NPs, which are otherwise observed when stored as aqueous suspension. Techniques commonly used to form tailored NEM formulations include spray drying and spray-freeze drying, which remove the liquid phase from the colloidal suspensions to form a dry NEMs powder (Ali and Lamprecht, 2014; Bohr et al., 2014; Cheow et al., 2011; Lintingre et al., 2016; Wang et al., 2012). Biocompatible excipients such as sugars and polymers have

*Abbreviations:* APS, aerodynamic particle size; NEMs, nano-embedded microparticles; M:N, matrix to nanoparticle ratio; NPs, nanoparticles; PDI, polydispersity index; PEG, poly(ethylene glycol); PLGA, poly(lactide-*co*-glycolide); PVP, poly(vinyl pyrrolidone); SD, standard deviation; SEM, scanning electron microscopy; TGA, thermogravimetric analysis; TPP, sodium triphosphate penta-basic; XRPD, X-ray powder diffraction.

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previously been spray dried with colloidal suspensions serving as a protecting matrix (Bohr et al., 2015; Freitas and Mullera, 1998). Furthermore, the quality of NEMs is partly influenced by the process of spray drying (Lintingre et al., 2016). Process and formulation parameters have been identified as critical to achieve good-key-quality attributes such as adequate particle size and redispersibility of NPs (Dobry et al., 2009; Vehring, 2008).

To function as NPs at the delivery site, NEMs should be rapidly disintegrated upon contact with physiological fluids and release the payload in unaffected form (Anton et al., 2012; Ungaro et al., 2012). The choice of suitable matrix to stabilize the embedded NPs is depended on the characteristic of NPs and the excipient, as well as the ratio of the two components. As a recent example, Ruge et al. reported the minimal necessary ratio of poly(styrene) NPs to trehalose to achieve complete dissolution/reconstitution in aqueous media (Ruge et al., 2016). Aggregation of NPs takes place when resuspending NEMs in bulk solution to re-obtain discrete NPs due to the breakdown of hydrogen bonds between water molecules and NPs during NEMs preparation by spray drying, and NP–NP interactions then dominate (Abdelwahed et al., 2006b; Ruge et al., 2016). This aggregation resulting from the dehydration of nanosuspensions is not reversible (Jeong et al., 2005; Konan et al., 2002). The addition of an excipient (similar to cryoprotectants used in freeze drying) can substitute water molecules in the nanosuspension (e.g., via hydrogen bonds between the NPs and the matrix) (De Chasteigner et al., 1995, 1996; Jeong et al., 2005; Konan et al., 2002), hence increasing the recovery of NPs from NEMs. The space between NPs in NEMs is separated by the matrix, which results in the reduced interaction between the individual NPs leading to a rapid disintegration (Allison et al., 2000).

Overall, the influence of distinct excipients on different NP systems forming NEMs has not been reported in detail so far and thus, remains a question for the strategy of finding an appropriate excipient to embed NPs with different physico-chemical properties into microparticulate form. Accordingly, the current study aimed at investigating four NP-based formulations for preparing NEMs with special emphasis on the influence of their properties, including their degree of hydrophilicity, on the ability to form NEMs. Furthermore, focus was placed on the performance of different excipients (i.e., water-soluble sugars and polymers) used as excipient in the preparation of NEMs. The feasibility of three commonly used water-soluble sugars (trehalose (Tomoda et al., 2009, 2008), mannitol (Boukari et al., 2015; Grenha et al., 2007) and sucrose (Yin et al., 2014)) as well as two water-soluble polymers (poly(vinyl pyrrolidone) (PVP) (Thirumala et al., 2009) and poly(ethylene glycol) (PEG) (Lee et al., 2009)) previously studied as cryoprotectants was investigated as excipients during NEM fabrication. The redispersibility of different NP systems from NEMs prepared using different excipients was studied and compared. Moreover, the NEMs were characterized with regards to their physical structure, moisture content and solid-state form to assess the impact of their characteristics on the performance of NEMs.

## 2. Materials and methods

### 2.1. Materials

Poly(lactide-*co*-glycolide) (PLGA) Resomer® RG502H was acquired from Evonik (Darmstadt, Germany). Poly(styrene) NPs were obtained from Polysciences (Polybead®, Warrington, USA). Trehalose dihydrate and mannitol were purchased from VWR (Leuven, Belgium). Sucrose, PVP (MW 10,000 Da), PEG (MW 20,000 Da), PAMAM dendrimer (generation 3), low molecular weight chitosan, sodium triphosphate pentabasic (TPP) and sodium alginate (MW 10,000–600,000 Da) were purchased from Sigma-Aldrich (Missouri, USA). Scrambled siRNA was acquired from Eurogentec (Liege, Belgium). Ultrapure water used in this study was generated by a Barnstead® Nanopure® instrument (Thermo Fisher, Rockford, USA). All other chemicals and solvents were of analytical grade and used as received.

### 2.2. Preparation of PLGA nanoparticles by anti-solvent precipitation

PLGA NPs were prepared by a modified method from Govender et al. (Govender et al., 1999). Briefly, 5 ml acetonitrile solution containing PLGA at a concentration of 10 mg/mL was added dropwise into water under stirring resulting in an acetonitrile: water ratio of 1:4 (v/v). After an overnight evaporation, water was added to a final volume of 25 ml.

### 2.3. Preparation of chitosan-alginate nanogels

Low molecular weight chitosan was dissolved in a 1% acetic acid solution at a chitosan concentration of 0.05% (w/v). Nanogels were then formed via bulk mixing of the chitosan solution with a solution of TPP (0.06% w/v) and sodium alginate (0.015% w/v). The two solutions were mixed at a ratio of 9:2 (v/v) and vortexed resulting in a ratio of 3:1 (w/w) between chitosan and alginate + TPP. The nanogels were left to form for 20 min at room temperature (Islam et al., 2016). The final pH of all nanogel suspensions was pH 3.2.

### 2.4. Preparation of siRNA-dendrimer polyplexes

Polyplexes of siRNA and PAMAM dendrimer were prepared by mixing aqueous solutions of siRNA and dendrimer via bulk mixing using RNAse free water (Jensen et al., 2010). The dendrimer solution (200 μl) was pipetted into the siRNA solution (800 μl), vortexed and then left at room temperature for 20 min for polyplexes formation. The final siRNA and dendrimer concentrations were 1 μM (17.9 μg/mL) and 16 μM (227.4 μg/mL), respectively, resulting in an amine to phosphate (N/P) ratio of 20.

### 2.5. Size, polydispersity index and zeta-potential measurements

Dynamic light scattering and laser Doppler velocimetry (NanoZS/ZEN3600, Malvern Instruments, Worcestershire, UK) were used to measure the hydrodynamic diameter (size), polydispersity index (PDI) and zeta-potential of the NP systems. All measurements were performed at 25 °C.

### 2.6. Preparation NEMs and blank free matrix microparticles

The four NP systems were entrapped into excipient matrices to form microparticles by spray drying. Matrix solutions were prepared in 0.5 ml water and mixed with the NP suspension (25 ml) via mild shaking before spray drying. The spraying feed concentrations ranged between 3 and 6 mg/mL. The NPs were mixed with the excipient solutions in order to achieve matrix:NP (M/N) ratio of 1–100 depending on the utilized NP system. Spray drying was performed using a Büchi B-290 (Büchi, Switzerland) with a Büchi B-296 dehumidifier (Büchi, Switzerland). All samples were prepared using an inlet temperature of 80–110 °C (outlet temperature was controlled in the range of 39–42 °C) with a feed rate of 3–4 ml/min and atomized at a gas flow rate of 450 l/h using compressed air and a drying air flow rate of 22.5 $m^3$/h. The free excipients used in this study were spray dried using the same condition except the concentration of spray drying solution was set to 2% (w/v).