Superparamagnetic iron oxide nanoparticles (SPIONs)-loaded Trojan microparticles for targeted aerosol delivery to the lung

Frederic Tewes, Carsten Ehrhardt, Anne Marie Healy

► To cite this version:

Frederic Tewes, Carsten Ehrhardt, Anne Marie Healy. Superparamagnetic iron oxide nanoparticles (SPIONs)-loaded Trojan microparticles for targeted aerosol delivery to the lung. *European Journal of Pharmaceutics and Biopharmaceutics*, 2014, 86, pp.98 - 104. 10.1016/j.ejpb.2013.09.004 . inserm-01102561

HAL Id: inserm-01102561

<https://www.hal.inserm.fr/inserm-01102561>

Submitted on 13 Jan 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1   **Superparamagnetic iron oxide nanoparticles (SPIONs)-loaded**
2   **Trojan microparticles for targeted aerosol delivery to the lung**

3   Frederic Tewes^{1,2}, Carsten Ehrhardt¹ and Anne Marie Healy^{1*}

4   1-   School of Pharmacy and Pharmaceutical Sciences, Trinity College Dublin, Panoz
5   Institute, College Green, Dublin 2, Ireland.

6

7   2-   INSERM U 1070, Pôle Biologie-Santé, Faculté de Médecine & Pharmacie,
8   Université de Poitiers, 40 av. du Recteur Pineau, 86022 Poitiers Cedex, France

9

10   * To whom correspondence should be sent. Ph.: 00 353 1896 1444, e-mail:
11   healyam@tcd.ie

12

Abstract

Targeted aerosol delivery to specific regions of the lung may improve therapeutic efficiency and minimise unwanted side effects. It could potentially be achieved with porous microparticles loaded with superparamagnetic iron oxide nanoparticles (SPIONs) — in combination with a target-directed magnetic gradient field. As a proof of concept of this hypothesis, the aim of this study was to formulate and evaluate the aerodynamic properties of SPIONs-loaded Trojan microparticles after delivery from a dry powder inhaler. Microparticles made of SPIONs, PEG and hydroxypropyl- β -cyclodextrin (HP β CD) were formulated by spray drying and characterised by various physicochemical methods. Aerodynamic properties were evaluated using a next generation cascade impactor (NGI), with or without a magnet positioned at stage 2. Mixing appropriate proportions of SPIONs, PEG and HP β CD allowed Trojan microparticle to be formulated. These particles had a median geometric diameter of $2.8 \pm 0.3 \mu\text{m}$ and were shown to be sensitive to the magnetic field induced by a magnet having a maximum energy product of 413.8 kJ/m^3 . However, these particles, characterised by a mass median aerodynamic diameter (MMAD) of $10.2 \pm 2.0 \mu\text{m}$, were considered to be not inhalable. The poor aerodynamic properties resulted from aggregation of the particles. The addition of $(\text{NH}_4)_2\text{CO}_3$ and magnesium stearate (MgST) to the formulation improved the aerodynamic properties of the Trojan particles, and resulted in a MMAD of $2.2 \pm 0.8 \mu\text{m}$. In the presence of a magnetic field on stage 2 of the NGI, the amount of particles deposited at this stage increased 4-fold from $4.8 \pm 0.7\%$ to $19.5 \pm 3.3\%$. These Trojan particles appeared highly sensitive to the magnetic field and their deposition on most of the stages of the NGI was changed in the presence compared to the absence of the

36 magnet. If loaded with a pharmaceutical active ingredient, these particles may be useful
37 for treating localised lung disease such as cancer nodules or bacterial infectious foci.
38

Introduction

Pharmacotherapy of lung diseases often involves direct delivery of active pharmaceutical ingredients (APIs) by pulmonary inhalation. However, despite the progress in aerosol delivery to the lung, administration systems are still unable to effectively deliver the dose to the optimal areas of deposition within the respiratory tract. Optimising the deposition pattern of API within the most suitable part of the airways should increase the efficacy of the treatment and reduce side effects. This should be beneficial for treating localised lung diseases, such as respiratory infection and lung cancer, i.e. by targeting foci of bacterial infection or tumour nodules.

Superparamagnetic iron oxide nanoparticles (SPIONs), such as nanoparticles of maghemite ($\gamma\text{-Fe}_2\text{O}_3$) or magnetite (Fe_3O_4) offer attractive magnetic properties. However, SPIONs dispersions are unstable at physiological pH and surface modifications are required to increase their stability in aqueous media. SPION coated dispersions are one of the few FDA approved nanoparticles for use as MRI contrast agents [1]. Lately, SPIONs were also envisaged as sensitive devices for magnetic drug targeting after intravenous nanoparticles administration, in combination with a target-directed magnetic gradient field [2-6].

This concept has also been applied in an attempt to target dry nanoparticle aerosols within the lung using magnetically driven deposition [7, 8]. However, it has been demonstrated that, even with optimised magnet design, the resulting magnetic forces would not be sufficient to efficiently guide individual SPIONs in a dry-powder aerosol because of their small magnetic moment [9]. In contrast, when a multitude of SPIONs are assembled in an liquid aerosol droplet as a nanomagnetosol, the magnetic moment of the

assembly increased, which resulted in aerosols which were guidable by medically compatible magnetic fields [9]. Also, recently, inert SPIONs added to the nebuliser solution were used to guide the aerosol to the affected region of the lung by means of a strong external magnetic field. Various therapeutic agents have been administrated by this technique [10].

However, the small size of nanoparticles can lead to particle–particle aggregation, making their physical handling difficult in liquid and dry powder forms [11]. The delivery of API to the lung may be achieved by using dry powder inhalers (DPIs), metered dose inhalers or nebulisers. In the solid form, as in a DPI, pharmaceutical ingredients are usual more stable than in liquid form. Therefore, with the same concept of increasing the magnetic moment, targeted aerosol delivery to a specific area of the lung could also be achieved with Trojan microparticles loaded with SPIONs. Trojan particles are microparticles composed of nanoparticles and additional components used to maintain the nanoparticles together. Once in the body, the microparticles disaggregate and release the nanoparticles. Trojan particles were previously formulated and reported as being an efficient means of administering nanoparticles to the lung by inhalation [11]. These nano-in-microparticle systems should allow for higher aerosolisation and delivery efficiency than nanoparticles and permit the focalization of the drug reservoir in the targeted area.

To reach the deep lung alveolar region, particles require a 1-5 μm aerodynamic diameter range. This aerodynamic diameter range corresponds to spherical particles of unit density having a 1 - 5 μm geometric diameter range. Iron oxide density is 4.9 g/cm^3 and 5.2 g/cm^3 for maghemite and magnetite, respectively. Therefore, the best way to nebulise

85 microparticles composed of iron oxide is to develop porous hybrid particles with a
86 reduced density. In fact, to optimise the efficacy of dry powder inhalation, porous
87 particles with low apparent density were developed [12]. For instance, tobramycin
88 powder was produced using the emulsion-based PulmoSphere technology, producing
89 highly dispersible porous particles [13]. Excipient-free nanoporous microparticles
90 (NPMPs) [14-17] prepared by a spray drying process had improved *in vitro* deposition
91 properties compare to non-porous microparticles. Trojan large porous particle composed
92 of polymeric nanoparticles were also formulated by spray drying and exhibited much
93 better flow and aerosolisation properties than the nanoparticles from which they were
94 prepared [11]. Therefore, the aim of this study was to formulate SPIONs-loaded Trojan
95 porous microparticles for aerosol lung magnetic targeting after administration using a dry
96 powder inhaler.

97

1- Materials and methods

2.1 Materials

Hydroxypropyl- β -cyclodextrin (HP β CD) with an average degree of substitution of 0.65 (Encapsin™ HPB) was purchased from Janssen Biotech, Olen, Belgium. Linear PEG 10kDa (PEG), alkanes with 99.8% purity (hexane, heptane, octane, nonane, decane and undecane), maghemite (Fe₂O₃) nanoparticles (SPIONs) with a mean diameter of 50 nm, magnesium stearate (MgST) and ammonium carbonate ((NH₄)₂CO₃) were all purchased from Sigma-Aldrich, (Dublin, Ireland).

2.2 Methods

2.2.1 Spray drying

Various suspensions containing SPIONs were spray dried using a B-290 Mini spray dryer (Büchi, Flawil, Switzerland) set in the closed cycle mode with a 2-fluid nozzle. The liquid phase of the suspensions was composed of butyl acetate/methanol/water mixture with a volume ratio 5:5:1., as used previously [18]. The composition of the suspensions is described in Table 1. The spray dryer was operated as follows: Inlet temperature was 65°C; feeding pump was set at 30%; spraying N₂ nozzle flow rate was 15 L/min; N₂ flowing at 670 NL/h was used as the drying gas. These conditions resulted in an outlet temperature ranging from 36 to 39°C.

Table 1: Concentration (g/L) of materials in the spray dried solutions and formulation code.

	25P- 75H- 5F	25P- 75H- 30F	50P- 50H- 50F	50P- 50H- 50F- CO ₃	100P- 50F	100P- 50F- CO ₃	100H- 50F	50P- 50H- 50F-ST	50P- 50H- 50F- CO ₃ -ST
Fe ₂ O ₃	5	30	50	50	50	50	50	50	50
PEG	25	25	50	50	100	100	0	50	50
HPβCD	75	75	50	50	0	0	100	50	50
(NH ₄) ₂ CO ₃	0	0	0	25	0	25	0	0	25
MgST	0	0	0	0	0	0	0	6	6

2.2.2 Scanning electron microscopy (SEM)

SEM micrographs of samples were taken using a Tescan Mira XMU (Tescan s.r.o., Czech Republic) electron microscope. The samples were fixed on aluminium stubs and coated with a 10 nm-thick gold film. Primary electrons were accelerated under a voltage of 5 kV. Images were formed from the collection of secondary electrons.

2.2.3 Powder X-ray diffraction (XRD)

XRD measurements were conducted on samples placed in a low background silicon holder, using a Rigaku Miniflex II desktop X-ray diffractometer (Rigaku, Tokyo, Japan). The samples were scanned over a range of 5 – 40° 2θ at a step size of 0.05°/s as previously described [19].

2.2.4 Particle size distribution analysis

The geometric particle size distributions (PSD) were determined by laser diffraction using a Mastersizer 2000 (Malvern Instruments, Worcestershire, UK) with the Scirocco 2000 dry powder feeder to disperse the particles as described previously [19]. The dispersive air pressure used was 3 bar and vibration feed rate was set to 50%. Data were analysed based on the equivalent volume median diameter, D_{50} , and the span of the PSD. Calculation was performed using Mie theory and refractive index part of 2 and absorption

part of 1 as optical particle properties ($n=2$).

2.2.5 Particle true density

The true density of the materials was measured using an Accupyc 1330 Pycnometer (Micromeritics®) with helium (99.995% purity) to determine the volume of accurately weighed samples. Samples were dried prior to measurement for 24 h in a Gallenkamp vacuum oven operating at 600 mbar and 25°C ($n=2$).

2.2.6 Surface free energy measurement

Measurements were performed at 0% RH or 40% RH and 30°C, ($n = 3$) using an inverse gas chromatography (iGC) instrument (SMS Ltd., London, UK). Powders were packed into a silanized glass column (300mm x 3mm), and then pre-treated for 1 h at 30°C and 0% RH. Then, 250 μ L of the probe vapour-helium mixture was injected into the helium flow. All injections of probe vapours were performed at 0.03% v/v of the saturated probe vapour. A flame ionization detector was used to monitor the probes' elution. In acid-base theory, the total surface free energy of a solid (γ_s^T) has 2 main components: a dispersive contribution (γ_s^d) and specific or acid-base contribution (γ_s^{AB}) which are independent and additive. In order to calculate γ_s^d of the particles, alkane probes with a known dispersive contribution (γ_p^d) and a nil specific contribution (γ_p^{AB}) were used. Methane was used as inert reference. At this low % of saturation (0.03% v/v), iGC was used in infinite dilution conditions and γ_s^d was calculated using the method developed by Schultz *et al.* [20].

2.2.7 Aerodynamic particle diameter analysis

The aerodynamic diameter (AD) distribution of the particles was measured using a Next Generation Impactor (NGI) as previously described [19]. The flow rate was adjusted to get a pressure drop of 4 kPa in the powder inhaler (Handihaler®, Boeringher Ingelheim, Ingelheim, Germany) and the time of aspiration was adjusted to obtain 4 L. The inhaler

was filled with gelatin n°3 capsule loaded with 20 ± 2 mg of powder ($n = 3$). After inhaler actuation, particle deposition on the NGI was determined by the SPIONs assay as described below. The amount of particles recovered on each stage expressed as a percentage of the emitted recovered dose was considered as the fine particle fraction (FPF). The mass median aerodynamic diameter (MMAD) and FPF were calculated as previously described [19]. Additional experiments were performed in the presence of a neodymium iron boron magnet (e-Magnets UK, Hertfordshire UK) of 20 mm of diameter and 20 mm of length having a maximum energy product ($BH_{\max}$) of 413.8 kJ/m^3 placed on the bottom of the stage 2 of the NGI.

2.2.8 SPIONs concentration assay

SPIONs assay was performed by turbidity measurements at 510 nm. SPIONs were dispersed in 2% w/w poly (vinyl alcohol) (10kDa) solution containing 0.1M of NaOH using a sonicator bath. In the case of formulations containing MgST, particles were dispersed in a 1/1 (v/v) mixture of ethanol and an aqueous solution composed of 2% w/w PVA (10kDa) and 0.1M of NaOH. In these conditions, stable suspensions were obtained. Calibration curves were constructed with standard suspensions composed of SPIONs dispersed in the same media as the formulations and having concentrations ranging from 0.001 to 0.05 mg/mL.

2.2.9 Statistical data analysis

Data were statistically evaluated by a two-way ANOVA using Excel® software (Microsoft). Significance level was $\alpha < 0.05$.

2- Results and discussion

Powder XRD patterns recorded for the powders made of PEG, HP β CD and SPIONs (Fig. 1) had curved baselines and diffraction peaks at 30.3 and 35.6 2θ degrees corresponding to maghemite iron oxide [21]. The intensity of the diffraction peaks increased with an increasing amount of SPIONs incorporated in the formulation. PEG residual crystallinity was also observed in formulations containing more than 33 wt% of PEG from the presence of the two major diffraction peaks at 19.2 and 23.3 2θ degrees [18, 19]. The absence of diffraction peaks corresponding to the HP β CD, suggested that this excipient was in the XRD amorphous state.

For spray dried systems comprising PEG, HP β CD and SPIONs, SEM pictures showed individual spherical microparticles surrounded by SPIONs nanoparticles (Figure 2A-B-D). This morphology was obtained due to the high Peclet number of the SPIONs relative to the other excipients, resulting in accumulation on the surface of the microparticles [11, 22]. Without PEG in the formulation, heterogeneous blends of free SPIONs and HP β CD microparticles were obtained (data not shown). This failure to form the Trojan microparticles may be as a result of the van der Waals forces between the SPIONs accumulated on the microparticles being too low to enable them be retained on the surface [11] and could also be due to the weak adhesion between HP β CD and SPIONs. Volume weighted particle size distributions of these particles (100H-50F) showed a large proportion of nanoparticles (Fig. 3F). In the absence of HP β CD, large particle aggregates were produced and the presence of SPIONs was not visible (Figure 2C). These aggregates were thought to result from the low and broad melting temperature of the PEG, leading to the formation in the spray dryer of partly solidified sticky particles [18,

19]. The absence of visible SPIONs suggests that these soft particles embed SPIONs in their bulk, before complete solidification. These particles (100P-50F) had the highest geometric median diameter, as measured by laser diffraction, compared to the other formulations containing PEG and HP β CD (Fig. 3 - curve A), resulting also in the lowest specific surface area (Table 2). Due to their large size, these particles were considered to be not suitable for pulmonary administration. These particles presented the lowest dispersive surface free energy (γ_s^d) values (36 ± 1 mJ/m²), similar to values found previously for PEG alone (37.7 ± 4 mJ/m²) [18], confirming that their surfaces were mainly composed of PEG molecules.

Table 2: Physicochemical properties of the Trojan particles. * Data from supplier.

	25P-75H-5F	25P-75H-30F	50P-50H-50F	100P-50F	50P-50H-50F-CO3	50P-50H-50F-ST	50P-50H-50F-CO3-ST	SPION
True density (g/cm ³)	1.39 $\pm$ 0.01	1.64 $\pm$ 0.01	1.68 $\pm$ 0.01	1.69 $\pm$ 0.01	1.76 $\pm$ 0.01	1.66 $\pm$ 0.01	1.71 $\pm$ 0.01	2.70 $\pm$ 0.01
Specific surface area (m ² /g)	4.5 $\pm$ 0.2	4.8 $\pm$ 0.2	3.9 $\pm$ 0.1	1.4 $\pm$ 0.2	3.2 $\pm$ 0.1	6.8 $\pm$ 0.4	4.6 $\pm$ 0.3	46.6*
geometric median diameter (μ m)	1.9 $\pm$ 0.2	2.0 $\pm$ 0.4	2.2 $\pm$ 0.4	7.2 $\pm$ 0.7	2.8 $\pm$ 0.3	1.54 $\pm$ 0.2	2.15 $\pm$ 0.3	0.05*
γ_s^d (mJ/m ²)	119 $\pm$ 2	149 $\pm$ 27	43 $\pm$ 5	36 $\pm$ 1	39 $\pm$ 2			
MMAD (μ m)					10.2 $\pm$ 2.0	3.4 $\pm$ 1.1	2.2 $\pm$ 0.8	9.3 $\pm$ 3.1
GSD					2.4 $\pm$ 1.1	2.4 $\pm$ 0.5	2.1 $\pm$ 0.4	4.3 $\pm$ 1.8

The use of PEG allowed SPIONs to stick together to form the Trojan microparticles, but produced large aggregates in the absence of HP β CD. Also, an appropriate ratio between PEG and HP β CD was necessary to obtain isolated and spherical Trojan microparticles.

Particle deposition on the NGI impactor stages was dependent on the particle type. For SPIONs alone, only 30% of the nanoparticles emitted out of the gelatin capsule were deposited beyond the first impactor stage, which has a cut-off aerodynamic diameter of 8.9 μ m (Fig. 5A). According to equation 1, these nanoparticles with a geometric diameter of 50 nm and having a true density of 4.9 g/cm³ should have an aerodynamic diameter of

110 nm and deposit mainly on the filter stage of the impactor. The large deposition on stage 1 is presumably due to the agglomeration of the nanoparticles in non-inhalable large clusters. In fact, SPIONs had a high γ_s^d (147.5 ± 42 mJ/m², Table 1), favouring their aggregation. The increase in PEG concentration in the formulation reduced γ_s^d (Table 2), decreasing the ability of the particles to aggregate. Besides preventing the particles from aggregating, the PEG should also play a role in preventing nonspecific and irreversible adsorption of foreign protein onto the particles, reducing opsonisation and particle phagocytosis.

In order to reach the pulmonary alveoli, particles must have an aerodynamic equivalent diameter (d_a) in the 1-5 μ m range [12]. The aerodynamic diameter is the diameter of a sphere of unit density, which reaches the same velocity in the air stream as the particle analysed, which can be nonspherical and have a different density [23, 24]. It is linked to the volume-equivalent geometric diameter (d_g) by the particle shape and density, as described by the following equation [23, 24]:

$$d_a = d_g \sqrt{\left(\frac{\rho_p}{\rho_0 \cdot \chi} \right)} \quad \text{Eq. 1}$$

Where ρ_0 is the standard particle density (1g/cc), χ is the particle shape factor that is 1 for a sphere and ρ_p is the apparent particle density. ρ_p is equal to the mass of a particle divided by its apparent volume, i.e. the total volume of the particle, excluding open pores, but including closed pores [24], which can be less than the material density (true density) if the particle is porous. Aerodynamic diameter decreases with increasing χ . For an irregular particle χ is always greater than 1 [24]. Also, in order to decrease d_a of large or

dense particles below 5 μ m, several studies focused on enhancing the particle's porosity to increase χ and decrease ρ_p [11, 15-17].

To increase the porosity of the particles, ammonium carbonate (NH₄)₂CO₃ was added to the formulation. In fact, (NH₄)₂CO₃ is commonly used as a blowing agent, [12, 25] pore-forming agent [12] or process enhancer [14, 15]. This compound decomposes at 60°C and produces gases during spray drying, thus it is able to create porous or hollow particles. The addition of (NH₄)₂CO₃ to the PEG-SPIONs mixture did not change the morphology of the particle aggregates (Figure 4A). It would appear that the partially solidified PEG did not allow the formation of void cavities in the particles, which are usually made by material solidification around the gas bubbles produced by the (NH₄)₂CO₃ decomposition on spray drying. However, the addition of ammonium carbonate to the 50P-50H-50F formulation produced individual spherical and hollow microparticles (Figure 4B). These microparticles had a median geometric diameter of 3 μ m (Fig. 3) and SPIONs were observable on their surface, but appeared more entrapped than when processing was undertaken in the absence of ammonium carbonate (Figure 2D). The deeper penetration of the SPIONs within the microparticles may allow for the avoidance of SPIONs desorption from the surface of the microparticles due to the mechanical stress produced during the dry powder inhalation. The large pores in these microparticles should enhance their aerodynamic properties, and be favourable for alveolar particle deposition.

The formulation of SPIONs-loaded microparticles made of PEG and HP β CD with (NH₄)₂CO₃ (50P-50H-50F-CO₃) decreased the amount of particles collected on stage 1 of the impactor from 49.8 $\pm$ 9.0% (for SPIONs alone) to 35.1 $\pm$ 6.4%, and increased the

amount collected on the other stages, with a gradual decrease in the amount deposited with the decrease in the stage cut-off diameter (Fig. 5B). The application of a magnetic field on stage 2 of the impactor changed the particle deposition profile. The percentage of particles on stage 1, 3 and 4 decreased and the percentage on stage 2 significantly increased, 2.5-fold compared to the percentage obtained without magnetic field. Thus, this type of particles was sensitive to the magnetic field; however, their aerodynamic properties were not appropriate for targeting the deep lung area. The aerodynamic properties of the 50P-50H-50F-CO₃ microparticles showed a large measured MMAD value ($10.2 \pm 2.0 \mu\text{m}$) compared to the d_a ($3.66 \mu\text{m}$) calculated with equation 1 using the median volume-weighted geometric diameter D_{50} , considering the particles to be spherical and using the particles' true density (Table 2). This difference can be attributed to the aggregation of the microparticles. Therefore, in order to reduce particle aggregation, magnesium stearate (MgST) was added to the formulation.

MgST is used in marketed DPI products (Seebri® Breezhaler®, Novartis; Foradil® Certihaler®, Novartis), and is commonly used to reduce the surface free energy [26] and agglomeration of particles [27]. The carboxylate group of MgST may be coordinated to the iron atom on the SPIONs surface via four different structures, as was previously observed with oleic acid [28]. This would make the SPIONs surface less polar and less adhesive and would change the SPIONs behaviour. However, it was found that the dispersive part of the surface free energy (γ_s^d) of the microparticles was not changed by the addition of MgST. This component of the surface free energy of the Trojan microparticle was already low due to the presence of PEG at the surface. The addition of MgST to the formulations changed the particle morphology. SEM micrographs (Fig. 4C-

D) showed individual microparticles with surfaces that appeared more porous compared to particles formulated without MgST (Fig. 4B). This increase in porosity induced an increase in the specific surface area, as measured by nitrogen adsorption (Table 2). Also, rough or irregular particles may have very low effective van der Waals adhesion forces [29], facilitating particle aerosolisation.

The addition of MgST into the formulation significantly decreased particle deposition on stage 1 of the NGI, from $49.8 \pm 9.0\%$ for nanoparticles alone to $1.8 \pm 0.3\%$ of the recovered dose for 50P-50H-50F-ST and increased the amount of particles recovered on the other stages (Figure 5C). For the 50P-50H-50F-ST particles the main deposition occurred on stages 2 and 3 (22.9 ± 3.4 and $20.9 \pm 3.4\%$, respectively), having a cut-off of 4.46 and 2.82 μm , respectively, with a gradual decrease in the amount of powder recovered on the other lower cut-off stages. These particles have a MMAD of $3.4 \pm 1 \mu\text{m}$ which was higher than the aerodynamic diameter (2.0 μm) calculated using equation 1. In the presence of magnets on stage 2, the amount of particles recovered on this stage increased from 22.9 ± 3.4 to $32.6 \pm 4.9\%$ and decreased on the following stages.

The addition of $(\text{NH}_4)_2\text{CO}_3$ and MgST to the formulation improved further the aerodynamic properties of the Trojan particles, leading to the main deposition being centred on stage 4 ($23.4 \pm 3.5\%$) which has a cut-off of 1.6 μm (Figure 5D). In the *in vivo* situation, it would be expected that these particles would be spread out within the whole lung. In the presence of a magnetic field on stage 2, the amount of particles deposited at this stage significantly increased 4-fold from $4.8 \pm 0.7\%$ to $19.5 \pm 3.3\%$. These Trojan particles appeared more sensitive to the magnetic field than particles formulated without

(NH₄)₂CO₃ and MgST and the deposition on the NGI stage was significantly altered in the presence of the magnet (Figure 5D).

These particles may be useful for treating localised lung disease, by targeting foci of bacterial infection or tumour nodules. SPIONs released from the Trojan microparticles should be eliminated from the lungs via mechanisms such as mucociliary clearance and macrophage phagocytosis [30]. Even though it has already been used in inhalation in humans, the inhalation of SPIONs may raise some toxicological concerns [31]. A previous toxicological study [32] showed that intratracheally instilled Fe₂O₃ nanoparticles of 22 nm diameter could pass through the alveolar-capillary barrier into the systemic circulation at a clearance rate of 3.06 mg/day. The authors of this study suggested that this absorption was probably due to macrophages clearance function overloading, potentially resulting in lung cumulative toxicity of the nanoparticles.

PEG used in the formulations discussed here could be useful to prevent early stage particle phagocytosis by alveolar macrophage, by forming a swelling/viscous crown around the drug loaded microparticles to temporarily repulse macrophages [33, 34]. Thus, the use of PEG in the current formulations could decrease the macrophage overloading by slowing down the rate at which the particles are phagocytosed. After complete solubilisation, PEG of 10 kDa should diffuse from the lung into the blood circulation and be eliminated by renal excretion, as its molecular weight is lower than 30kDa [35, 36].

Other study performed on rats exposed to magnetite particles with a MMAD of 1.3 µm for 13-week of inhalation showed no mortality, consistent changes in body weights, or systemic toxicity. Elevations of neutrophils in bronchoalveolar lavage appeared to be the most sensitive endpoint of the study [37]. Particle size appears to be determinant in

SPIONs toxicity. For example, ultra small superparamagnetic particle of iron oxide having a diameter of 5 nm were not toxic to human monocyte-macrophages *in vitro* and did not activate them to produce pro-inflammatory cytokines or superoxide anions [38]. Nevertheless, we suggest that this approach of lung drug targeting by an external magnetic field would be acceptable only in the case of a clear benefit such as in the case of anticancer drug delivery [39] and if used at a low frequency.

3- Conclusion

This study demonstrates the feasibility of formulating SPIONs-loaded microparticles which may be useful in the treatment of localised lung disease, such as foci of bacterial infection or tumour nodules. The Trojan microparticles formulated were aerosolised using a dry powder inhaler to produce inhalable particles. These particles were sensitive enough to the magnetic field produced by a commercial magnet to induce a significant change of their distribution on a cascade impactor.

352   **4- Acknowledgement**

353   The authors acknowledge funding by a Strategic Research Cluster grant (07/SRC/B1154)
354   under the National Development Plan co-funded by EU Structural Funds and Science
355   Foundation Ireland.
356

357   **5- References**

- 358   [1] L. Yildirimer, N.T.K. Thanh, M. Loizidou, A.M. Seifalian, Toxicological
359   considerations of clinically applicable nanoparticles, *Nano Today*, 6 (2011) 585-607.
- 360   [2] I. Chourpa, L. Douziech-Eyrolles, L. Ngaboni-Okassa, J.F. Fouquenot, S. Cohen-
361   Jonathan, M. Soucé, H. Marchais, P. Dubois, Molecular composition of iron oxide
362   nanoparticles, precursors for magnetic drug targeting, as characterized by confocal
363   Raman microspectroscopy, *Analyst*, 130 (2005) 1395-1403.
- 364   [3] E. Munnier, F. Tewes, S. Cohen-Jonathan, C. Linassier, L. Douziech-Eyrolles, H.
365   Marchais, M. Soucé, K. Hervé, P. Dubois, I. Chourpa, On the interaction of doxorubicin
366   with oleate ions: Fluorescence spectroscopy and liquid-liquid extraction study, *Chemical*
367   *and Pharmaceutical Bulletin*, 55 (2007) 1006-1010.
- 368   [4] P. Pouponneau, J.C. Leroux, G. Soulez, L. Gaboury, S. Martel, Co-encapsulation of
369   magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue
370   targeting by vascular MRI navigation, *Biomaterials*, 32 (2011) 3481-3486.
- 371   [5] C. Sapet, L. Le Gourriec, U. Schillinger, O. Mykhaylyk, S. Augier, C. Plank, O.
372   Zelphati, Magnetofection: Magnetically assisted & targeted nucleic acids delivery, *Drug*
373   *Delivery Technology*, 10 (2010) 24-29.
- 374   [6] A.L. Coates, Guiding Aerosol Deposition in the Lung, *New England Journal of*
375   *Medicine*, 358 (2008) 304-305.
- 376   [7] J. Ally, B. Martin, M. Behrad Khamesee, W. Roa, A. Amirfazli, Magnetic targeting of
377   aerosol particles for cancer therapy, *Journal of Magnetism and Magnetic Materials*, 293
378   (2005) 442-449.
- 379   [8] D. Upadhyay, S. Scalia, R. Vogel, N. Wheate, R.O. Salama, P.M. Young, D. Traini,
380   W. Chrzanowski, Magnetised thermo responsive lipid vehicles for targeted and controlled
381   lung drug delivery, *Pharmaceutical Research*, 29 (2012) 2456-2467.
- 382   [9] P. Dames, B. Gleich, A. Flemmer, K. Hajek, N. Seidl, F. Wiekhorst, D. Eberbeck, I.
383   Bittmann, C. Bergemann, T. Weyh, L. Trahms, J. Rosenecker, C. Rudolph, Targeted
384   delivery of magnetic aerosol droplets to the lung, *Nature Nanotechnology*, 2 (2007) 495-
385   499.
- 386   [10] N. Laurent, C. Sapet, L. Le Gourriec, E. Bertosio, O. Zelphati, Nucleic acid
387   delivery using magnetic nanoparticles: The Magnetofection™ technology, *Therapeutic*
388   *Delivery*, 2 (2011) 471-482.
- 389   [11] N. Tsapis, D. Bennett, B. Jackson, D.A. Weitz, D.A. Edwards, Trojan particles:
390   Large porous carriers of nanoparticles for drug delivery, *Proceedings of the National*
391   *Academy of Sciences of the United States of America*, 99 (2002) 12001-12005.

392 [12] D.A. Edwards, J. Hanes, G. Caponetti, J. Hrkach, A. Ben-Jebria, M.L. Eskew, J.
393 Mintzes, D. Deaver, N. Lotan, R. Langer, Large porous particles for pulmonary drug
394 delivery, *Science*, 276 (1997) 1868-1871.

395 [13] D.E. Geller, J. Weers, S. Heuerding, Development of an inhaled dry-powder
396 formulation of tobramycin using PulmoSphere technology, *J Aerosol Med Pulm Drug*
397 *Deliv*, 24 (2011) 175-182.

398 [14] A.M. Healy, B.F. McDonald, L. Tajber, O.I. Corrigan, Characterisation of excipient-
399 free nanoporous microparticles (NPMs) of bendroflumethiazide, *European Journal of*
400 *Pharmaceutics and Biopharmaceutics*, 69 (2008) 1182-1186.

401 [15] L.M. Nolan, L. Tajber, B.F. McDonald, A.S. Barham, O.I. Corrigan, A.M. Healy,
402 Excipient-free nanoporous microparticles of budesonide for pulmonary delivery,
403 *European Journal of Pharmaceutical Sciences*, 37 (2009) 593-602.

404 [16] L.M. Nolan, J. Li, L. Tajber, O.I. Corrigan, A.M. Healy, Particle engineering of
405 materials for oral inhalation by dry powder inhalers. II - Sodium cromoglicate,
406 *International Journal of Pharmaceutics*, 405 (2011) 36-46.

407 [17] F. Tewes, K.J. Paluch, L. Tajber, K. Gulati, D. Kalantri, C. Ehrhardt, A.M. Healy,
408 Steroid/mucokinetic hybrid nanoporous microparticles for pulmonary drug delivery,
409 *European Journal of Pharmaceutics and Biopharmaceutics*, (2013).

410 [18] F. Tewes, O.L. Gobbo, M.I. Amaro, L. Tajber, O.I. Corrigan, C. Ehrhardt, A.M.
411 Healy, Evaluation of HP β CD-PEG microparticles for salmon calcitonin administration
412 via pulmonary delivery, *Molecular Pharmaceutics*, 8 (2011) 1887-1898.

413 [19] F. Tewes, L. Tajber, O.I. Corrigan, C. Ehrhardt, A.M. Healy, Development and
414 characterisation of soluble polymeric particles for pulmonary peptide delivery, *European*
415 *Journal of Pharmaceutical Sciences*, 41 (2010) 337-352.

416 [20] J. Schultz, L. Lavielle, C. Martin, The role of the interface in carbon-fiber epoxy
417 composites, *J Adhes*, 23 (1987) 45-60.

418 [21] G.A. Sotiriou, E. Diaz, M.S. Long, J. Godleski, J. Brain, S.E. Pratsinis, P.
419 Demokritou, A novel platform for pulmonary and cardiovascular toxicological
420 characterization of inhaled engineered nanomaterials, *Nanotoxicology*, 6 (2012) 680-690.

421 [22] R. Vehring, Pharmaceutical particle engineering via spray drying, *Pharmaceutical*
422 *Research*, 25 (2008) 999-1022.

423 [23] B. Shekunov, P. Chattopadhyay, H.Y. Tong, A.L. Chow, Particle Size Analysis in
424 Pharmaceutics: Principles, Methods and Applications, *Pharmaceutical Research*, 24
425 (2007) 203-227.

- 426 [24] P.F. DeCarlo, J.G. Slowik, D.R. Worsnop, P. Davidovits, J.L. Jimenez, Particle
427 Morphology and Density Characterization by Combined Mobility and Aerodynamic
428 Diameter Measurements. Part 1: Theory, *Aerosol Science and Technology*, 38 (2004)
429 1185-1205.
- 430 [25] D. Traini, P. Young, P. Rogueda, R. Price, In Vitro Investigation of Drug
431 Particulates Interactions and Aerosol Performance of Pressurised Metered Dose Inhalers,
432 *Pharmaceutical Research*, 24 (2007) 125-135.
- 433 [26] V. Swaminathan, J. Cobb, I. Saracovan, Measurement of the surface energy of
434 lubricated pharmaceutical powders by inverse gas chromatography, *International Journal*
435 *of Pharmaceutics*, 312 (2006) 158-165.
- 436 [27] Q.T. Zhou, L. Qu, I. Larson, P.J. Stewart, D.A.V. Morton, Improving aerosolization
437 of drug powders by reducing powder intrinsic cohesion via a mechanical dry coating
438 approach, *International Journal of Pharmaceutics*, 394 (2010) 50-59.
- 439 [28] L.N. Okassa, H. Marchais, L. Douziech-Eyrolles, K. Hervé, S. Cohen-Jonathan, E.
440 Munnier, M. Soucé, C. Linassier, P. Dubois, I. Chourpa, Optimization of iron oxide
441 nanoparticles encapsulation within poly(d,l-lactide-co-glycolide) sub-micron particles,
442 *European Journal of Pharmaceutics and Biopharmaceutics*, 67 (2007) 31-38.
- 443 [29] O.R. Walton, Review of Adhesion Fundamentals for Micron-Scale Particles, *KONA*
444 *Powder and Particle Journal*, 26 (2008) 129-141.
- 445 [30] D.B. Buxton, Nanomedicine for the management of lung and blood diseases,
446 *Nanomedicine*, 4 (2009) 331-339.
- 447 [31] W. Moller, K. Hauszinger, L. Ziegler-Heitbrock, J. Heyder, Mucociliary and long-
448 term particle clearance in airways of patients with immotile cilia, *Respiratory Research*, 7
449 (2006) 10.
- 450 [32] M.T. Zhu, W.Y. Feng, Y. Wang, B. Wang, M. Wang, H. Ouyang, Y.L. Zhao, Z.F.
451 Chai, Particokinetics and extrapulmonary translocation of intratracheally instilled ferric
452 oxide nanoparticles in rats and the potential health risk assessment, *Toxicol Sci.*, 107
453 (2009) 342-351. Epub 2008 Nov 2020.
- 454 [33] I.M. El-Sherbiny, H.D. Smyth, Controlled release pulmonary administration of
455 curcumin using swellable biocompatible microparticles, *Mol Pharm*, 9 (2012) 269-280.
- 456 [34] I.M. El-Sherbiny, S. McGill, H.D. Smyth, Swellable microparticles as carriers for
457 sustained pulmonary drug delivery, *J Pharm Sci*, 99 (2010) 2343-2356.
- 458 [35] C.J. Fee, Size comparison between proteins PEGylated with branched and linear
459 poly(ethylene glycol) molecules, *Biotechnology and Bioengineering*, 98 (2007) 725-731.

- 460 [36] J.M. Harris, R.B. Chess, Effect of pegylation on pharmaceuticals, *Nat Rev Drug*
461 *Discov*, 2 (2003) 214-221.
- 462 [37] J. Pauluhn, Subchronic inhalation toxicity of iron oxide (magnetite, Fe₃O₄) in rats:
463 pulmonary toxicity is determined by the particle kinetics typical of poorly soluble
464 particles, *Journal of Applied Toxicology*, 32 (2012) 488-504.
- 465 [38] K. Müller, J.N. Skepper, M. Posfai, R. Trivedi, S. Howarth, C. Corot, E. Lancelot,
466 P.W. Thompson, A.P. Brown, J.H. Gillard, Effect of ultrasmall superparamagnetic iron
467 oxide nanoparticles (Ferumoxtran-10) on human monocyte-macrophages in vitro,
468 *Biomaterials*, 28 (2007) 1629-1642.
- 469 [39] C. Rudolph, B. Gleich, A.W. Flemmer, Magnetic aerosol targeting of nanoparticles
470 to cancer: nanomagnetosols, *Methods in molecular biology* (Clifton, N.J.), 624 (2010)
471 267-280.
- 472

Figure 1: Powder X-ray diffractograms of hybrid SPION loaded microparticles

Figure 2: SEM micrographs of trojan hybrid SPION loaded microparticles: A) 25P-75H-5F B) 25P-75H-30F C) 100P-50F, D) 50P-50H-50F.

Figure 3: Geometric particles size distribution of hybrid SPION loaded microparticles. A: 100P-50F; B: 100P-50F-CO₃; C: 50P-50H-50F; D: 25P-75H-30F; E: 25P-75H-5F, F: 100HP-50F.

Figure 4: SEM micrographs of hybrid SPION loaded microparticles formulated with $(\text{NH}_4)_2\text{CO}_3$: A) 100P-50F- CO_3 , B) 50P-50H-50F- CO_3 , SEM micrographs of hybrid SPION loaded microparticles formulated with Mg-ST: C) 50P-50H-50F-ST, D) 50P-50H-50F-ST- CO_3 .

Figure 5: Mass percentage of the recovered total dose of the trojan microparticles , i.e. the total amount of powder collected from the device, capsule and impactor recovered on each NGI stage, with and without a magnetic field applied on stage 2. Two-way ANOVA, *P < 0.05; ** P<0.01; *** P<0.001.

Figure 5: Water sorption isotherms. A) SPION alone, B) 100HP-50F, C) 100P-50F, D) 100P-50F-CO₃, E) 50P-50H-50F, F) 50P-50H-50F-CO₃-ST