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Low pressure mediated enhancement of nanoparticle and macromolecule loading into porous silicon structures

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Abstract

Ensuring drug loading efficiency and consistency is one of the most critical stages in engineering drug delivery vectors based on porous materials. Here we propose a technique to significantly enhance the efficiency of loading by employing simple and widely available methods: applying low pressure with and without centrifugation. Our results point toward the advantages the proposed method over the passive loading, especially where the size difference of loaded materials and the pore size of the porous silicon particles is smaller, an increase up to 20-fold can be observed. The technique described in the study can be used for efficient and reproducible loading of porous materials with therapeutic molecules, nanoparticles and contrast imaging agents for biomedical application.

Porous particles and materials are frequently being investigated and used for various biological applications such as tissue engineering, catalysis, analytical separation, drug delivery and imaging (Arcos et al., 2009; Prestidge et al., 2007; Salonen et al., 2005). As an example, porous silicon particles have been extensively studied as delivery nanovectors for drugs and contrast agents (Godin et al., 2012; Salonen et al., 2005). The porosity, surface chemistry and geometry of porous silicon particles can be easily and precisely tailored to accommodate loading of macromolecules and nanoparticles (NP) using microfabrication methods (Vallet-Regí et al., 2007). Thus, porous silicon particles with pores ranging from 5 to 100 nm are frequently employed for impregnation with various biologically active nanoparticles or macromolecules. porous silicon or porous silica particles can be fabricated

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by top-down approach via photolithography and electrochemical etching or by bottom-up approach via assembly of silicate with polymeric template (Anglin et al., 2008; Vallet-Regi et al., 2001). In both cases, the active components are loaded to the porous silicon particles after the fabrication process is completed. Additionally, modifying the surface of the pores with charged moieties can facilitate the embedding of both negatively or positively charged molecules and nanoparticles. Biodegradability and biocompatibility of pSiP has been established in numerous studies (Bimbo et al., 2010; Low et al., 2006; Tanaka et al., 2010). These characteristics allow the formation of multifunctional systems, which can be administered intravenously or orally. Porous silicon particles utilized in this study (pSiP) have been well studied and a greater control over drug loading and release kinetics can be attained by tuning particle geometry, size and pore size distribution (Chiappini et al., 2010; Godin et al., 2011) as well as by adjusting pore surface modifications (Godin et al., 2010). The pSiP has been shown to not only allow the extended multistage delivery of the active agents, but also increased the efficiency of targeting and improved the protection against the uptake by the reticulo-endothelial system (Tasciotti et al., 2008).

The loading efficiency of mesoporous materials post-fabrication typically relies on factors such as electrostatic interactions between the carrier and the loaded substance, surface tension and pH of the solute, concentration of loaded compound, time, temperature and the sizes of the pores (Liu et al., 2013; Salonen et al., 2005; Serda et al., 2011). For the multistage pSiP we used in this study, loading was previously performed using a passive incipient wetness method, namely, by introducing a concentrated solution or nanoparticulate dispersion into a dry pellet of lyophilized particles to allow for passive capillary force to pull the liquid into pores (Serda et al., 2011). This method mostly led to inconsistent results and low efficacy of loading. Other possible loading procedures generally involve exposure of the loaded materials to organic solvents, including slow evaporation, melting, spray drying or rotary evaporation (Limnell et al., 2011). These techniques, especially if used with bioactive compounds, such as genetic materials and proteins, were reported to interfere with the solubility, stability or activity of the loaded molecule (Mattos and Ringe, 2001; Zendlová et al., 2007). Therefore, there is an obvious need for process optimization and improvement.

In this report, we describe a simple technique to increase efficiency and reproducibility of loading by employing a combination of methods available in any research lab, such as applying low pressure with or without centrifugation to enhance the power of capillary forces. Using the proposed technique, a general increase in loading efficiency is attained and the material can be easily stored in the same tubes.

Here we report the data for four combinations of pSiP (1 μ m diameter X 0.4 μ m thickness) loading differing in its porosity/zeta potential and size and charge of the loaded substances. Namely, three systems are based on pSiP with pore size of up to 120 nm (Giant pores-GP) having positive or negative zeta potential impregnated with: 1) negatively charged iron oxide nanoparticles (NP) 60 nm in diameter (loaded in positive GP); 2) positively charged silica NP about 40 nm in diameter (loaded in negative GP); and 3) fluorescein isothiocyanate-bovine serum albumin (FITC-BSA) molecules with 10 nm hydrodynamic size (loaded in positive GP). One more system consisted of a positively charged pSiP with 10–20nm pores (Small pores-SP) encapsulating FITC-BSA molecules. Active loading was

carried out by either subjecting the particles to low pressure conditions or speedvac concentrator. The passive loading was performed as previously described by pipetting the liquid with the substance to be loaded onto the pellet of the lyophilized particles.

Discoidal pSiP were fabricated using photolithography and electrochemical etching and modified with 10% of HNO_3 to obtain negatively charged particles. The weight of the particles was 20 μg /108 particles with 80% porosity, as measured by Brunauer–Emmett–Teller (BET) surface area measurement. To obtain particles with positive zeta potential and to enhance the loading of the negatively charged nanoparticles, the particles were modified with 3-amino-propyltriethoxysilane (APTES) as previously described (Godin et al., 2012; Srinivasan et al., 2013). 10^8 particles were then aliquoted and lyophilized before further use. 10 μl of aqueous suspensions of secondary nanoparticles were used for loading, sufficient to wet the particles. Each containing either 5 μg of plain silicon oxide nanospheres (Corpuscular Inc, Cold Springs, NY), 10 μg of iron oxide NP (Ocean NanoTech, Springdale, AR) or 50 μg of FITC-BSA. Loading procedures were conducted in three different methods: passive loading, low pressure loading and Speedvac loading. The first step for all methods was similar: introducing the nanoparticle dispersion directly onto the lyophilized pSiP followed by 10 seconds sonication. In the passive loading method required no further processing after the sonication step, while in low pressure loading, this step was immediately followed by inserting the particles into steriflip filtration tubes (Millipore Corporation, Billerica, MA) and connecting to the in-house low pressure system (~130 mbar) for an overnight incubation. In speedvac method, the samples were dried in the speedvac concentrator, a centrifuge coupled with vacuum system for gentle evaporation of the solvents. Loading efficiency was determined via indirect loading measurement of the non-loaded NPs or macromolecules. This was conducted by re-suspending particle suspension or dry cake in deionized water followed by washing via centrifugation at 21,000 $\times g$ for 15 minutes. The pellets were washed three times in total and the supernatants were collected for indirect loading efficiency measurement. Determinations of loaded iron and silica contents were conducted using Varian 720-ES Ion Coupled Plasma Optical Emission Spectrometer. Silicon content was detected at the following wavelengths 250.69, 251.43, 251.61 and 288.158 nm, and iron at 238.20, 234.35 and 259.94nm. Released silica content was subtracted from the baseline value of non-loaded pSiP to calculate the values from the silica NPs. Centrifugation effect on the NPs was also studied and was found to be negligible for the experiments. Measurement of loaded FITC-BSA was conducted via analysis of fluorescence at 488nm excitation and 530 nm emission using Synergy H4 Hybrid reader (Biotek, Winooski, US). Hydrodynamic size and surface potential of the particles were measured with Dynamic Light Scattering (DLS) Zetasizer (Malvern, Worcestershire, UK).

The hydrodynamic sizes of the macromolecules/nanoparticles loaded into the pSiP as determined by DLS measurements were: $34.1 \pm 10.6 \text{ nm}$ for silica NP, $65 \pm 5.9 \text{ nm}$ for iron oxide NP and $10.3 \pm 2.7 \text{ nm}$ for FITC-BSA. Active loading by both methods showed a significant increase in loading efficiency (Table 1). Iron oxide NP loading was increased by 20 times just by applying the low pressure conditions, and about by 18 times by using speedvac. For silicon oxide nanoparticles the enhancement in loading efficiency was not so dramatic, however it was possible to attain about 30% higher loading by the speedvac method and about 20% higher loading by the low pressure method. No significant difference

was obtained from the loading of FITC-BSA into the GP particles regardless of the methods and improvement via active loading can only be obtained when they were loaded into SP particles, with the loading increased by 8-fold and 6.5-fold by using speedvac and low pressure method respectively (table 1). Remarkable effect seen in the case of loading iron oxide NP to GP particles and FITC-BSA to SP particles can be attributed to their size in proximity to the pores' size, which prevents them from being efficiently sucked into pores solely by capillary forces.

The results were verified by scanning electronic microscopy (SEM) images of respective samples (Figure 1). While no very significant difference was observed in the exterior of porous particles loaded with silica NP by various methods, noticeable changes were seen in the case of iron oxide NP. Images taken after active loading showed clusters of loaded materials extending and clogging the pores in the particles. FITC-BSA seemed to be layering the silicon structures, clogging the pores of SP particles after low pressure and speedvac loading methods, while the passive loading resulted in thinner layer of FITC-BSA with visible structures of pore walls in SP particles.

The increased efficacy of the low pressure loading methods for GP pSiP could be observed in both positively charged silica NP and negatively charged iron oxide NP, but not with FITC-BSA. SP pSiP with smaller pore sizes were effectively loaded with FITC-BSA under a low pressure. Thus, we suggest that the increase in loading efficiency is correlated to the size difference to the secondary particles to be loaded. The smaller the size difference between the particle and the pore, the greater the loading efficiency amplification achieved by active loading. We hypothesize that low pressure induces evaporation of the solvent, simultaneously creating more concentrated solution, changing the surface tension of the loaded solution and increasing the forces acting in the capillary effect. All these cause an accumulation of the dispersed/dissolved content in the pores of the porous particles. The content uniformity and the loading efficiency reproducibility via active loading may also provide an additional advantage. These parameters are important when aiming to transform a promising experimental delivery system to an established drug dosage form. It should be noted, that although the speedvac method is very effective in increasing loading efficiency, dispersion of particles embedded by this method in water may provide a challenge. Therefore, we conclude that the low pressure method is the preferred one in terms of significant increase in loading efficiency of mesoporous particles, accompanied by simplicity and a milder effect on the product.

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Figure 1. Scanning electron microscope images of pSiP loaded with iron oxide NP (GP particles), silica NP (GP), FITC-BSA (SP), empty GP particle and empty SP particle. Images represent nanoparticles loading conducted via speedvac method, low pressure method and passive loading.

Table 1

Loading efficiency of nanoparticles loading into second stage porous particles via various loading methods. Silicon oxide, iron oxide nanoparticles and FITC-BSA were loaded into giant pores particles (GP, pore sizes: 60–120 nm) and additional FITC-BSA loading into small pores particles (SP, pore sizes: 10–20nm).

Methods of loading	GP particles			SP Particles FITC-BSA (%)Hydrodynamic size=10.3nm
	SiO ₂ NP (%)Hydrodynamic size=34.1nm	FeO ₂ NP (%)Hydrodynamic size=65nm	FITC-BSA (%)size=10.3nm	
Speedvac	72.6±9.9	79.1±6.8	41.3±4.7	67.2±2.2
Low pressure	69.7±16.4	90.4±4.9	45.0±5.2	53.9±5.9
Passive	57.3±8.06	4.4±3.8	50.9±9.1	8.4±4.7