ONE-POT SYNTHESIS AND ENCAPSULATION OF HYDROPHILIC DRUG IN UNIFORM MICROPARTICLES FOR CONTROLLED RELEASE

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ABSTRACT
The route for single step synthesis and encapsulation of a model hydrophilic drug onto homogeneous microparticles for controlled release applications is described. The microfluidic spray drying technique, able to handle complex fluids, was used to produce uniform particles with adjustable functionality. Here, an aqueous polymeric dispersion of Eudragit® NE was used as the controlled release matrix, with no organic solvent required. This study investigated the effects of additives in the form of lactose and silica nanoparticles on the release properties of the microparticles. Depending on precursor composition, the evaporation-induced self-assembly during drying resulted in a heterogeneous spatial distribution of drug and matrix materials. Accordingly, these particulate systems demonstrated intricate release kinetics compared with the behavior of a single material system. We demonstrated that the release properties, whether immediate or sustained, could be directly tuned using this route as an effective approach to design new spray-dried pharmaceutical systems.

INTRODUCTION
Controlled release technology is one of the most rapidly advancing areas of science contributing to human health care (Uhrich et al, 1999). Particle-based drug carriers offer several advantages, including the capability to incorporate both hydrophilic and hydrophobic drugs, low risk of dose dumping, and enhanced stability (Dey et al, 2008; Pancholi et al, 2009). For the design of controlled release systems, regulating the speed of drug liberation and ensuring that the desired dose is maintained are the most critical factors determining their efficacy (Pancholi et al, 2009). In the case of polymeric-based systems, the drug release behavior is dependent on the physicochemical properties of the matrix materials used, as well as the morphological features such as particle size and shape, since non-uniform characteristics could lead to sub-optimal drug loading and poor reproducibility of release behaviors (Berkland et al, 2003; Champion et al, 2007). It has become imperative to develop a reproducible method for generating monodisperse drug carriers with easily tunable release kinetics (Huang et al, 2009).

The introduction of water-based encapsulation materials has brought new insights into the formulation of controlled release drug delivery systems (Singh & Khan, 1997). Polymeric aqueous dispersions have gained popularity as they are more biocompatible and ecologically friendly than solvent-based systems (Harris & Ghebre-Sellassie; Singh & Khan, 1997). Eudragit® NE 30D is the aqueous dispersion of a neutral copolymer based on ethyl acrylate and methyl methacrylate. It is swellable rather than soluble in the physiological pH range (Bajdik et al, 2003) and is a potentially useful excipient for
controlled release formulations due to its stabilities and relatively low cost (El-Malah & Nazzal, 2008). Herein, we observed the drug release characteristics from uniform Eudragit® NE-based microparticles with vitamin B\textsubscript{12} as the hydrophilic model drug, assembled in a single step via a microfluidic drying technique (Wu et al, 2011a; Wu et al, 2011b). The synthesis route avoided the use of organic solvents and the need for post-processing, while the properties of the particles could be directly modified from the composition of the precursors with controlled drying conditions. The effects of different hydrophilic dopants of lactose and silica nanoparticles on the drug release characteristics were investigated, with possible drug release mechanisms proposed.

**MATERIALS AND METHODS**

**Materials**

Eudragit® NE 30D (30% aqueous dispersion) was kindly provided by Evonik Degussa Industries (Australia). Vitamin B\textsubscript{12} (VB\textsubscript{12}), alpha-D-lactose monohydrate, Ludox® SM-30 colloidal silica (30% solid suspension), and phosphate buffered saline (PBS, pH = 7.4, consisting of 0.138M NaCl and 0.0027M KCl) were purchased from Sigma-Aldrich (Australia). Deionized water (Milli-Q) was used for all precursor preparation.

**Preparation of uniform microparticles**

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>Eudragit® NE (w/v)</th>
<th>Lactose (w/v)</th>
<th>Colloidal silica</th>
<th>Vitamin B\textsubscript{12} (w/w)</th>
<th>APS of the resultant particles Size (µm) ± SD\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>E4</td>
<td>4%</td>
<td>0%</td>
<td>/</td>
<td>5%</td>
<td>100.6±4.7</td>
</tr>
<tr>
<td>E3L1</td>
<td>3%</td>
<td>1%</td>
<td>/</td>
<td>5%</td>
<td>85.5±3.4</td>
</tr>
<tr>
<td>E2L2</td>
<td>2%</td>
<td>2%</td>
<td>/</td>
<td>5%</td>
<td>77.9±3.5</td>
</tr>
<tr>
<td>E1L3</td>
<td>1%</td>
<td>3%</td>
<td>/</td>
<td>5%</td>
<td>70.7±4.5</td>
</tr>
<tr>
<td>E4S0.1</td>
<td>4%</td>
<td>/</td>
<td>0.1%</td>
<td>5%</td>
<td>98.1±4.8</td>
</tr>
<tr>
<td>E4S0.5</td>
<td>4%</td>
<td>/</td>
<td>0.5%</td>
<td>5%</td>
<td>114.8±4.7</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The amount of Vitamin B\textsubscript{12} was added according to the drug to excipients ratio.

\textsuperscript{b} APS i.e. average particle size; SD i.e. standard deviation of the particle size.

The precursors (compositions as shown in Tab. 1) were fed into a 1.5L stainless steel reservoir and atomized by a specially designed micro-fluidic aerosol nozzle. Dehumidified instrument air was used to force the precursor in the reservoir to jet through the orifice of the nozzle. A piezoelectric pulse was exerted to break the liquid jet into droplets. The liquid flow rate and the frequency of piezoelectric pulse applied were adjusted to best achieve a monodisperse droplet formation (Wu et al, 2011b). The droplet generation mode was monitored by a digital SLR camera (Nikon, D90) with a speed-light (Nikon SB-400) and a micro-lens (AF Micro-Nikkor 60mm f/2.8D). The droplets formed were air-dispersed and dried in the chamber of a micro-fluidic jet spray
dryer (Wu et al., 2011a). The temperature set point for drying process was maintained at 180 °C and all other conditions were kept constant to exclude the influence of drying conditions on the resulting particles. The detailed modelling work about the particle residence time in this specific spray dryer has been done in our group (Woo et al., 2011). Briefly, the particle residence time varies with its own particle size. For instance, for particles with a diameter of 150 µm, the residence time is around 10 s.

**Characterization of microparticles**

The morphology and structure of microparticles before and after drug release test were characterized by scanning electron microscopy (SEM, JEOL 7001F, Japan). Images of microparticles were also recorded by light microscopy (Motic B1-223A, UK). Particle size and size distribution were analyzed using the software package Motic Images Plus 2.0 ML and ImageJ. The average particle size ($\bar{d}$) was defined as $\bar{d} = \frac{1}{N} \sum d_i$, and the standard deviation of particle size was described as $SD=\sqrt{\frac{\sum (d_i - \bar{d})^2}{(N-1)}}$, where $d_i$ is the diameter of each particle, N is the total number of particles counted (a minimum of 500 microparticles was analyzed for each sample).

**In vitro drug release test**

The release profiles of VB$_{12}$ from spray-dried microparticles were studied in phosphate buffer saline (PBS, pH 7.4) at 37°C using a shaking incubator (100 rpm). In a typical experiment, the drug-loaded microparticles (50 mg) were added into a 100 mL conical flask, and 50 mL of PBS release medium was transferred into the flask. At certain time intervals, 1 mL of sample was removed and replaced with an equivalent quantity of the release medium. Collected samples were transferred into 1.7 mL microtubes and subjected to assay immediately. The content of VB$_{12}$ in the release medium was measured by a microplate reader (SpectraMax M2e, Molecular devices) at the wavelength of maximum absorbance (361 nm). The release data presented for each data point was the average of three test trials.

**RESULTS AND DISCUSSION**

**Fabrication of uniform microparticles**

By atomizing the precursor into monodisperse droplets (Fig.1) that were then dried into individual, non-agglomerated microparticles, uniform size and morphology could be achieved by this synthesis technique (Fig.2). The mean particle size and size distribution were summarized in Tab.1, all indicating narrow size distributions. The homogenous properties indicated that each droplet experienced the same drying process, thus ensuring consistent functionalities of the microparticles. The microparticles exhibited different shapes depending on the composition of the precursors. Microparticles of E4 showed a bowl-like shape, while with the incorporation of lactose (E3L1 to E1L3) induced more spherical shapes. On the other hand, the addition of silica nanoparticles (E4S0.1 and E4S0.5) resulted in increasing deformation of the shapes.
Fig. 1: Photographs of monodisperse droplets generation: A. E4, B. E3L1, C. E2L2, D. E1L3, E. E4S0.1, F. E4S0.5

Fig. 2: SEM photographs of the spray-dried microparticles: A. E4, B. E3L1, C. E2L2, D. E1L3, E. E4S0.1, F. E4S0.5 (inset scale bar: 20 µm)
The different morphologies of assembled microparticles were the direct result of their physicochemical properties upon drying (Velev et al., 2000). Eudragit® NE exists in the form of a polymeric dispersion, thus their drying behavior is more complex than soluble polymer systems due to the existence of nanoparticles in the droplets (Nimkulrat et al., 2004). The morphological transition of the dried polymeric particles proceeds via buckling of the initial spherical droplets when deformation forces overcome stability forces, leading to various non-spherical morphologies such as doughnut and mushroom (Sen et al., 2009). On the other hand, spray-dried lactose microparticles generally display spherical shapes (Takeuchi et al., 1998). As a low molecular weight sugar, droplets of lactose solution have little tendency to fold their surfaces during the drying process (Alexander & King, 1985), as the lactose molecules are more mobile than the dispersed Eudragit® NE polymer granules. Thus they could replace the water molecules to some extent during evaporation and reduce shrinkage (Fäldt & Bergenståhl, 1995). With the addition of silica nanoparticles, the shapes become buckled since the nanoparticles facilitated early skin formation that was subjected to capillary forces with further drying (Iskandar et al., 2003; Wu et al., 2011).

**Effect of lactose on release profile**

![Drug release profiles of the spray-dried microparticles with different Eudragit® NE: lactose ratio](image)

Fig. 3: Drug release profiles of the spray-dried microparticles with different Eudragit® NE: lactose ratio.

Fig. 3 showed the *in vitro* drug release profiles of spray-dried microparticles with different Eudragit® NE: lactose ratio. These microparticles possessed very distinct drug release properties, with increasing lactose content resulted in faster drug release. Microparticles with the highest amount of lactose (E1L3) released almost all VB₁₂ in 2 hours, whereas in the absence of lactose (E4), only about 40% of total VB₁₂ was released in 32 hours. Due to water insolubility and low permeability of Eudragit® NE, it was expected that E4 microparticles containing only the polymer displayed...
comparatively slow release, while the dense structure of these microparticles (Fig.4) could also be a contributing factor. With partial displacement of water-soluble lactose in the matrix, the hydrophilicity of the microparticles increased, resulting in enhanced water permeability. The increased water infiltration would facilitate the dissolution of lactose and the swelling of the microparticles. Both effects would result in more contact between the encapsulated drug and the release medium, thus posing reduced barrier for drug diffusion and accelerating the release rate.

Fig.4: The inner structure of E4 spray dried microparticles

Fig.5: Microscopy photographs of the spray-dried microparticles after drug release test:
A. E4L0, B. E3L1, C. E2L2, D. E1L3

Fig.6: SEM photographs of the spray-dried microparticles after drug release test: A. E4L0, B. E3L1, C. E2L2, D. E1L3
Fig. 5 and 6 showed the optical images of the particles in phosphate buffer and the SEM photographs of dry microparticles after release tests, respectively. Upon contact with the release medium, the shape of E4 microparticles (Fig. 5A) did not show visible change from the as-dried particles (Fig. 2A) or from the shape after the release test (Fig. 6A). The low permeability of Eudragit® NE should prevent further water penetration into the microparticles. With less contact with the release medium, the encapsulated drug that was located away from the particle surface would be difficult to release, consistent with the incomplete release profile. On the other hand, the microparticles of E3L1, E2L2, and E1L3 swelled into spherical shapes upon contact with water (Fig. 5B, C, D), confirming the increased hydrophilicity and water penetration into the microparticles. From SEM photographs of the particles after release tests (Fig. 6B, C, D), the microparticles of E3L1, E2L2, and E1L3 showed increasingly collapsed structures (to varying degrees) in comparison to the original particles (Fig. 2B, C, D) due to the removal of lactose from the matrix.

**Effect of colloidal silica on release profile**

The drug release profiles of spray-dried microparticles with different amount of silica nanoparticles were compared in Fig. 7. Incorporation of nanoparticles significantly accelerated VB₁₂ release. With the addition of 0.5% silica, 90% VB₁₂ was released in 0.5 hour. By only adding 0.1% silica, the drug release was considerably enhanced, with a 55% release in one hour. However, after the initial burst, the release rate of the remaining VB₁₂ was almost negligible.

![Drug release profiles](image)

**Fig. 7: The drug release profiles of the spray-dried microparticles with different amount of colloidal silica.**

The accelerating effect of silica nanoparticles could be ascribed to their strong affinity for polar compound like water, with similar impacts as the hydrophilicity of lactose (Kho & Hadinoto, 2010). However, it was worth noting that the accelerating mode/kinetic was significantly different than the behaviour found with lactose in the
matrix. While the addition of lactose caused the release profile to accelerate evenly, the addition of silica nanoparticles caused an immediate release, followed by an extremely slow release rate of the remaining drug.

To understand the cause of this distinct release effects, the shape and morphology of microparticles after release test were observed (Fig.8). The microparticles did not show visible swelling in the release medium after the release test (Fig.8A₁,B₁). The absence of swelling meant that the incorporation of silica nanoparticles did not increase the permeability and water penetration onto the microparticles, so that the drug located in the inner part of microparticles could not be released, corresponding to the very slow release rate after the initial burst. Accordingly the initial fast release could be due to the high amount of drug on the particle surface (or near to the particle surface). The cobalt atoms in VB₁₂ carry partially positive charge, such that the VB₁₂ molecules have affinity to negatively charged surfaces (Beckmann & Brown, 1976). Since the silica nanoparticles (Ludox SM-30) used in this study were negatively charged (Yoon & Lueptow, 2006), we proposed that the high surface amount of VB₁₂ would be accompanied with enrichment of silica on the surface of the microparticles. To verify this hypothesis, elemental distribution analysis of the cross section of E4S0.5 microparticles was conducted (Fig.9). The data showed that silica was more prolific on the surface of the microparticles which might be caused by the evaporation induced self-assembly during the fast spray drying process (Sen et al, 2010). Fig.8A₂,B₂ showed that although the shapes of the particles after release tests were almost unchanged, both systems containing silica nanoparticles demonstrated noticeably eroded surface characteristics, further illustrating the removal of relatively high amount of silica (and consequently VB₁₂) from the surface that induced the burst in the early stage.

Fig.8: The microscopy (A1, B1) and SEM (A2, B2) photographs of the spray-dried microparticles after drug release test: A. E4S0.1, B. E4S0.5
CONCLUSION

Microfluidic drying technique was used to generate uniform polymeric microparticles for drug encapsulation in a single step, to study the drug release behaviors of blended materials. The morphologies and drug release profiles of the microparticles could be directly modified from the composition of the excipients. Both the hydrophilic dopants investigated here accelerated the release rate, but with significantly distinct release modes due to the drug and/or excipients spatial distribution mode. The evaporation induced self-assembly during drying determined the architectural composition of the dried particles, and consequently their release behaviour. Here, the capability to synthesise uniform microparticles provided the opportunity to systematically study the functionalities of pharmaceutical particles. Future studies will incorporate model drugs with fluorescence properties to ease the tracking of drug distribution within the microparticles to investigate other effects including the change in size on the release behaviors of spray-dried systems.

REFERENCES


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