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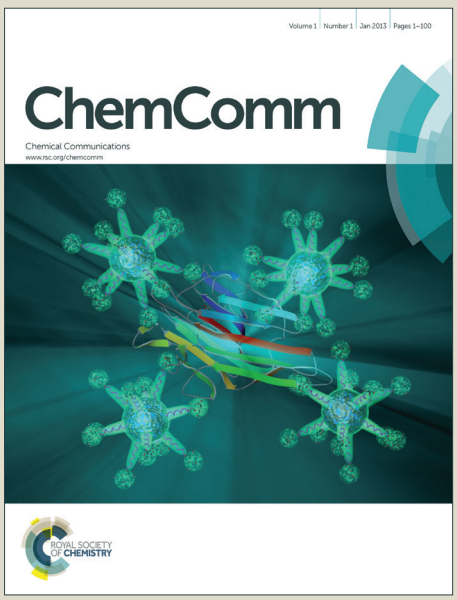

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Controlled Release from Protein Particles Encapsulated by Molecular Layer Deposition

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Molecular layer deposition (MLD) was used to coat micron-sized protein particles in a fluidized bed reactor. Our results show that the dissolution rate of particles coated with MLD rapidly decreases with the increase in number of coating cycles, while the uncoated particles dissolve instantaneously.

In the last two decades, much effort has been put in towards the development of drug delivery systems which overcome the shortcomings of the traditional methods such as toxic effects due to unpredictable concentration levels, degradation of drugs in digestive tract before entering the bloodstream, non-personalized nature etc.[1–3] With this development, proteins and peptides have become the natural choice for drugs due to their incredible specificity and bioactivity.[4,5] However, their administration has been mostly limited to parental route due their low bioavailability.[6,7] Encapsulation of the active pharmaceutical ingredient inside a shell is an attractive way to control the temporal and spatial release, by either varying the thickness or composition of the coating.[8] To this end, a number of novel and efficient drug delivery systems has been developed based on encapsulation methods.[9] For certain applications, it will be attractive to have full control over the coating thickness at nm scale, while the coating is conformal.[10,11] Such a method has a huge scope for extensibility to coating of bio-organic nanoparticles.[12] With molecular layer deposition (MLD) such a precise and well-controlled coating can be achieved.

MLD is a thin-film growth technique developed during the early 1990s for the deposition of molecular fragments on the surface of an active material,[13] and has been an attractive method for the deposition of a variety of organic polymers[14] and more recently hybrid organic-inorganic polymers.[15] In a typical MLD process, molecular fragments of the bi-functional precursors are deposited on the surface of an active substrate. This process involves two alternating reactions. It is the self limiting nature of these reactions which enables the deposition of ultra thin layers on the surface of the substrate.[13] In this paper, we show that controlled release of the active material can be achieved by MLD coating. To our knowledge the work presented here is the first adaptation of MLD process to encapsulate protein particles to study their controlled release property.

To demonstrate the concept, we use protein particles as the substrate and the precursors used are malonyl chloride and 1,2-butanediol. An illustration of the reactions involved is shown in Fig. 1. The amine groups on the surface of the protein particles acts as the active group for reaction with the acyl chloride group of malonyl chloride during the initiating reaction (reaction 0). In the next reaction step (labelled as reaction 1), the unreacted acyl chloride group of malonyl chlo-

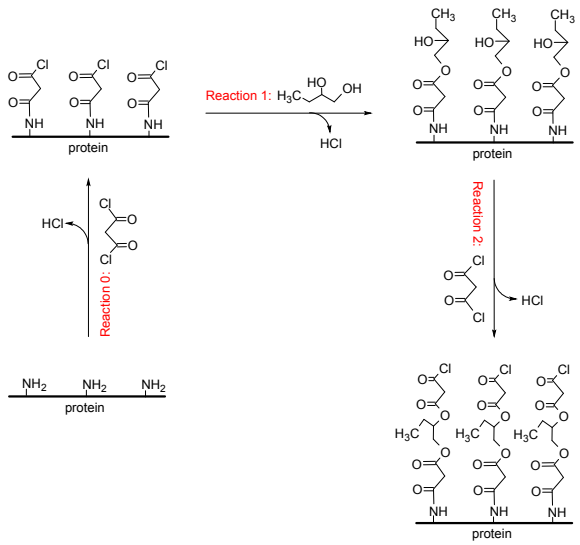

Fig. 1 An illustration of the reactions involved in an MLD process.

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ride which is now attached to the surface of the substrate acts as the active site for the reaction with the hydroxyl group of 1,2-butanediol. In 1,2-butanediol, the hydroxyl group on position 1 is the most reactive (less sterically hindered) in ester formation. In the subsequent reaction step (reaction 2), the unreacted hydroxyl group of 1,2-butanediol reacts with the acyl chloride group of malonyl chloride. Only the first coating cycle involves reaction step 0 and 1, while subsequent coating cycles involve reaction step 1 and 2.

2 g of protein particles with an average diameter of 200 μm is suspended in an upward gas flow of pure N_2, this is called a fluidized bed. N_2 acts as the carrier gas for feeding the precursors into the fluidized bed reactor (FBR). A schematic diagram of the experimental setup is shown in Fig. 2.

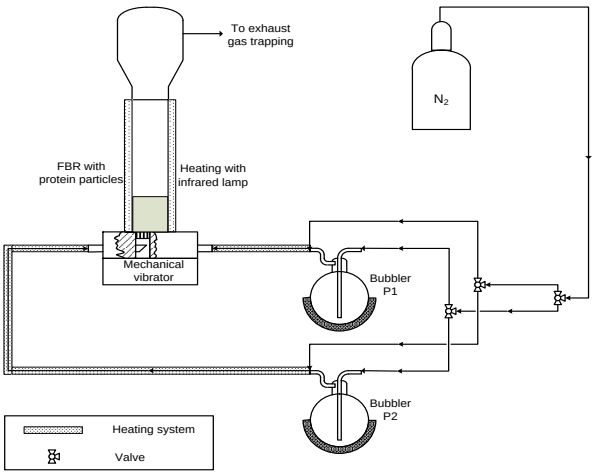

Fig. 2 Schematic diagram of the experimental setup used for the MLD process.

The FBR primarily consists of a vertical glass tube with an inner diameter of 2.6 cm and length of 40 cm with thermocouples inserted at the entrance and exit. The FBR is maintained at a temperature between 40° C and 45° C using an infrared lamp. It is vital to maintain considerably low temperatures because the protein used is found to denature at a temperature above 55° C. The denaturation temperature of the protein to a great extent limited the choice of precursors for the MLD process. Because of the low vapour pressures of malonyl chloride and 1,2-butanediol at room temperature, they are preheated in bubblers to 40° C and 115° C, respectively. A distributor plate is provided at the entrance of the FBR to ensure uniform distribution of the inlet gas stream mixture. By measuring the particle bed height variation with flow rate, the minimum velocity required to keep the particles afloat in N_2 gas is determined to be $2.7 \times 10^{-2}\,\mathrm{m\,s^{-1}}$. This velocity is often referred to as the minimum fluidization velocity. The unreacted precursor and by-product of the reaction HCl is trapped using a mineral oil cold trap at the exit of the FBR. We employ two methods to improve the fluidization of the particles: mechanical vibration of the FBR at a frequency of 50 Hz, and a microjet of 100 μm. The microjet is inserted into the FBR from the top and the mechanical vibrator is fixed at the bottom of the FBR. The microjet ensures good fluidization by breaking the agglomerates formed during the coating process.

Particles coated to different number of cycles are prepared. A typical coating cycle consists of four steps: 30 s dosage of malonyl chloride; minimum 2 min of purging with pure N_2 to remove the unreacted precursor; 30 s dosage of 1,2-butanediol and finally purging with pure N_2 for at least 2 min. The dosage times are relatively long (i.e., much excess reactant is fed) to maximize completion of each cycle; this does not harm since the reaction is self-limiting. The purge times are chosen long ($\gg$residence time) to prevent the unreacted compounds or products from staying behind in the tube system or on the particle surface. We observed a tendency for particle agglomeration during the reaction steps in a coating cycle which aggravates with increase in number of cycles. This increased agglomeration affects the fluidzation of the particles and in certain cases the fluidization is completely lost. Purging the FBR with high flow rate N_2 gas for long duration of time reinforced fluidization. Hence, for later cycles the reactor was purged until the fludization was completely re-established. Particles were coated with 2, 6 and 10 MLD cycles. The particles obtained an increasingly intense yellow/orange colour with increasing number of cycles.

Fourier transform infrared (FTIR) spectroscopy has been used to characterize the coating of protein particles. FTIR spectra are obtained using a Nicolet 8700 FTIR spectrometer (Thermo Electron Corporation) operating with a liquid N_2 cooled KBr/DLaTGS D301 detector. FTIR spectra of the coated protein particles are obtained by pressing the sample onto KBr salts and the data is collected with a resolution of $4\,\mathrm{cm^{-1}}$ averaged over 128 scans. The FTIR spectra of the coated particles shown in Fig. 3 are subtracted results from the spectra of the uncoated particle.

In Fig. 3, two distinct peaks are observed very close to $1736\,\mathrm{cm^{-1}}$ and $2971\,\mathrm{cm^{-1}}$; these correspond respectively to the stretching of [−COO−] and [CH_3−] groups. Increase in the absorbance peak due to [−COO−] and [CH_3−] stretching indicates the increase in coating thickness with number of cycles.

Dissolution experiments have been performed to study the controlled release of the coated protein samples. It is expected that during dissolution the ester bonds of the coating are stepwise hydrolysed. All the dissolution experiments have been performed at room temperature and pressure. 0.15 g of a coated particle sample is dissolved in 150 ml of deionized water. The resulting mixture was stirred with a magnetic stir-

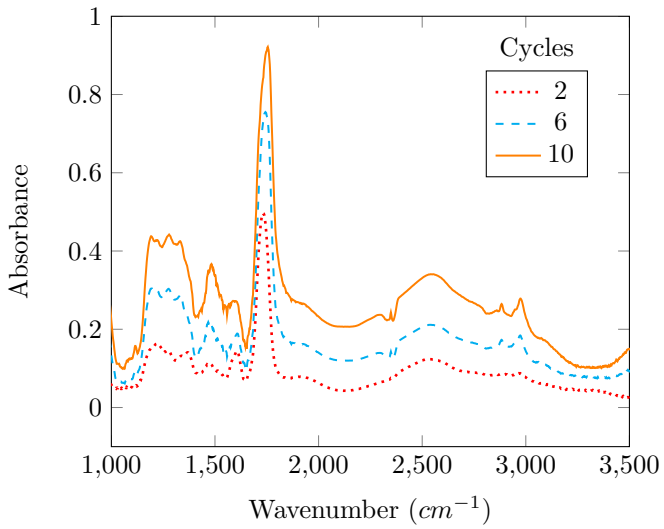

Fig. 3 FTIR spectra for 2, 6 and 10 cycles sample. The spectral data shown are subtracted results of coated samples from the uncoated sample.

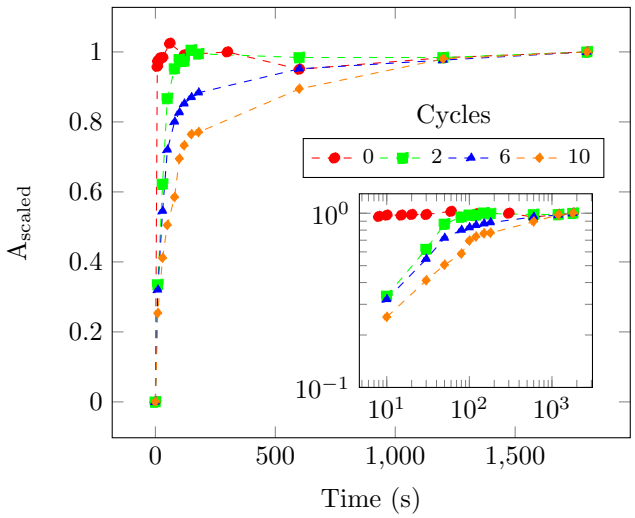

Fig. 4 Dissolution results obtained from UV-vis spectroscopy for different number of cycles.

rer to ensure uniform dispersion of the particles in deionized water. However, due to their low density most of the coated particles remain on the surface of the solution. Samples have been collected at regular intervals for a time period of 30 min. The collected samples are immediately filtered through a 0.45 μm pore size polyvinylidene difluoride membrane (Millex®) to avoid further dissolution of protein particles. After a time period of 30 min, undissolved protein particles denatured to form strands in the solution.

UV-vis spectroscopic measurements (UV-1800, Shimadzu) were performed on the collected dissolution experiment samples of uncoated and coated protein particles at a wavelength of 260 nm. The results are shown in Fig. 4.

In Fig. 4, A_{scaled} the scaled absorbance is defined as $(A(t)-A(t=0))/(A(t=1800s)-A(t=0))$, where $A(t)$ is the absorbance at time t and $A(t=0)$ is assumed to be zero. Here, scaled absorbance gives a measure of scaled concentration because absorbance scales linearly with concentration. The solution containing uncoated sample attains maximum concentration in about 10 s, while the coated samples dissolve at a much slower rate. In the inset of Fig. 4, the UV-vis test results are plotted on a log-log scale. Two distinct regions are observed: initial short time scale $\sim$ 30 s corresponding to a fast release of the coated protein and the long time scale $\sim$ 1000 s corresponding to a slow release of the coated protein.

Dissolution in controlled release applications is often described by a power law;[16] we used this approach too. The fast and slow regions are fitted individually to a power law function which scales with time as t^{α}. The values of α_{fast} for 2, 6 and 10 cycles sample, are respectively 0.482 ± 0.166, 0.425 ± 0.121 and 0.422 ± 0.059. α_{fast} values obtained are close to 0.5 which is observed in diffusion governed dissolution mechanism models.[16,17] We suspect the close resemblance of the fast release exponent to that of diffusion governed drug release mechanism models could be due to the presence of protein particles whose surface area is not completely coated. These particles are formed as a result of continuous breakage and formation of agglomerates, respectively, during purging and precursor dosage periods. For the 2 cycles sample, $\alpha_{slow} \approx 0$ indicating that the maximum concentration has been attained after a time period of 100 s. However, for 6 and 10 cycles sample, α_{slow} is found to be 0.058 ± 0.008 and 0.117 ± 0.013, respectively. These exponents likely correspond to dissolution after de-esterification of the film.

In conclusion, we found that with an increasing number of coating cycles of MLD the thickness of coating increases as shown by FTIR. We also demonstrate experimentally that controlled release of protein particles can be realized by MLD. The controlled-release behaviour is validated through dissolution experiment of coated particles wherein the decrease in the rate of dissolution is observed with increase in the number of coating cycles. This proof-of-principle demonstrates that MLD of fluidized particles is an attractive way to give protein materials tunable controlled-release properties.

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