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Magnetic activated release of umbelliferone from lipid matrices

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ABSTRACT

Lipid matrices containing dispersed superparamagnetic iron oxide (SPIO) particles were investigated as a magnetic field-responsive drug delivery system. Lipid matrices were prepared by combining myristyl alcohol, fatty acid coated SPIO particles, and umbelliferone (UMB). With placement of the matrices into the release medium, initial UMB release was fast but fell to zero indicating a burst effect. With application of an alternating magnetic field, additional UMB was released. The rate and extent of magnetic field-stimulated release increased with UMB load but not SPIO content. Differences between oleic and myristic acid coated SPIO appeared to be a result of phase separation. UMB release coincided with matrix melting, which can be controlled by the SPIO content and external magnetic field as shown by theoretical analysis. While significant technological issues remain, the foundation for developing magnetic field-stimulated drug delivery systems has been established.

Superparamagnetic iron oxide (SPIO) particles have many useful properties including controllable size, safety, detection by MRI, and ease of surface modification with ligands (Pankhurst et al., 2003; Duguet et al., 2006; Fortin-Ripoche et al., 2006; Ito et al., 2005; Liong et al., 2008; McCarthy et al., 2007; Shubayev et al., 2009). In addition, they interact with time-varying magnetic fields and undergo heating, which may be exploited for thermal responsive controlled drug release. After the initial work by Widder et al. (1978), several groups have examined magnetic particles for use in stimuli responsive drug delivery systems (Hafeli, 2004) in liposomes (Babincova et al., 2002; Hamaguchi et al., 2003; Kullberg et al., 2005; Matsuoka et al., 2004; Ponce et al., 2006; Sabate et al., 2008; Zhang et al., 2005) and solid lipid particles (Shubayev et al., 2009; Peira et al., 2003; Simon-Deckers et al., 2008; Wang et al., 2007; Gupta et al., 2007; Alexiou et al., 2006).

Magnetic activated release is an indirect process in which an alternating magnetic field causes superparamagnetic particles primarily to undergo physical rotation, which generates heat (Duguet et al., 2006; Hafeli, 2004). The heat in turn increases the temperature, and when sufficient, induces a phase change in the lipid matrix. With melting, drug release can be dramatically increased. Here, the release rate of a fluorescent molecule was determined with lipid matrices containing superparamagnetic particles with different lipid coatings and varying umbelliferone (UMB) loads and SPIO concentrations. The following results are theoretically analyzed by diffusion-controlled release associated with magnetic field induced melting of the lipid matrix.

SPIOs particles were synthesized by coprecipitation of $FeCl_2$ and $FeCl_3$ and coated with oleic or myristic acid as described in detail previously (Massart, 1981; Xie et al., in press). Differential scanning calorimetry of pure lipid and SPIO/lipid mixtures had equivalent melting points with no observable peaks associated with the SPIO particles regardless of the coating. Coated particles dispersed in cyclohexane had a mean diameter near 22 nm and a saturation magnetization of 54 emu/g as measured by a vibrating sample magnometer (Xie et al., in press). Matrices were prepared by combining myristyl alcohol, SPIO coated particles, and UMB in a vial. The mixture was melted and vortexed, and then a dialysis membrane (1k cutoff) was attached to the opening of the vial. After melting again, the vial was inverted to allow the molten material to come into contact with the dialysis membrane. The dialysis membrane covering the vial was then placed just into contact with 30 ml of water that was contained in a 30 ml beaker, which in turn was placed in a double walled beaker recirculated with water at 37 °C (Fig. 1). The alternating magnetic field was generated using a nominal current of 250 A and a frequency of 190 kHz (1 kW Hotshot, Ameritherm Inc., NY) through a three turn copper coil (Zeng et al., 2009).

The release was measured by taking 200 μl aliquots, which were placed into a 96-well plate. The fluorescent intensity was determined with a plate reader using excitation and emission wavelengths of 400 nm and 530 nm and converting to concentration using appropriate standard curves. The mass released in unit area was calculated from the observed concentration, corrected for aliquot removal and replacement. Separate measurements of the temperature change with application of the alternating magnetic field were made as described previously (Zeng et al., 2009).

Placement of the matrices into the release media resulted in an initial rapid release of UMB (Fig. 2). However, the release slowed

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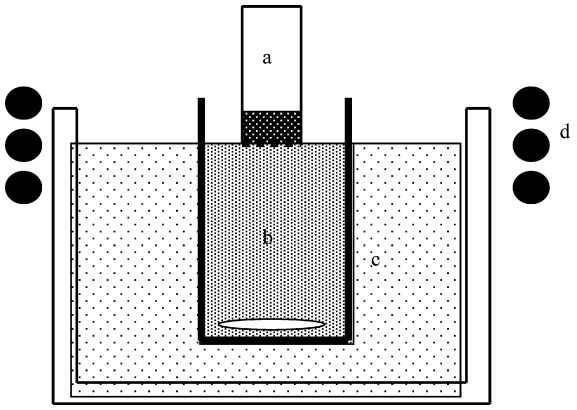

Fig. 1. Schematic diagram for experimental setup for release study with (a) vial containing myristyl alcohol matrix with SPIO particles, and UMB separated from release media by a dialysis membrane, (b) vial containing 30 ml of water, (c) double walled beaker thermostated to 37 °C, and (d) copper coils perfused with water at 5 °C, which generated the alternating magnetic field.

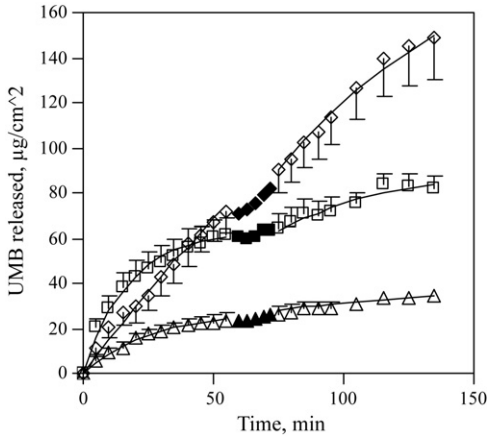

Fig. 2. UMB mass released per unit area as a function of time for a system consisting of (△) 1%, (□) 2% and (◊) 4.8% UMB and 20% SPIO, and the balance of myristyl alcohol with filled symbols indicating when the magnetic field was engaged and open symbols indicating when the magnetic field was off. The solid lines represent best fits to first order or zero order equations.

and appeared to reach a plateau. The magnetic field was then turned on at 60 min as shown by the filled symbols, and following a lag time, UMB was again released. This increase in the rate of release corresponded to the time when the matrix visibly melted. The field was then discontinued as reflected by the open symbols, and the UMB release rate slowed and appeared to approach a plateau.

For the lipid matrix with 2% UMB, the plateau value and associated rate constant were 66 μg/cm^2 and 0.0008 s^{-1}. When the field is engaged, the lag time was estimated to be less than 3 min and release rate was 0.4 μg/cm/s. The ending plateau value, corrected for the initial release, and an associated first order rate constant were 33 μg/cm^2 and 0.00040 s^{-1}, respectively.

Also in Fig. 2, the initial burst phase for the 1% load approached a plateau value of 20 μg/cm^2, which is lower than that observed for the 2% load. The amount released from the 4.8% matrix was comparable to that observed with the 2%, but the plot of the data had relatively little curvature. This distinct appearance suggests a change in the state of the UMB. It may be that the initial release was limited by the solubility for the 4.8% loading, which would cause the rate constant for release to be lower than expected After the initial 60 min of release, the value continued to rise at approximately a constant rate. Overall, the release rate increased with increasing UMB load during application of the field.

The release profiles for the 10% SPIO concentration were similar to that observed for the 20% SPIO matrix (Table 1). Thus, in contrast to UMB load, SPIO load did not appear to affect the release profile. Exchanging myristic acid coated SPIO for oleic acid coated particles caused a decrease in the rate constant associated with the initial plateau (Table 1). The release rate with engaging the magnetic field was also significantly less. However, a white layer formed at the top of the black lipid matrix revealing phase separation of the myrsityl alcohol from the iron particles.

With application of the field, a rapid rise in temperature was evident for all three systems of 10, 15, and 20% SPIO myristic acid coated particles (Fig. 3). This clearly demonstrates the magnetic particles remain superparamagnetic within the matrix. Following the initial rise, the 10% SPIO appeared to reach a plateau, whereas the 15 and 20% systems continued to increase in temperature.

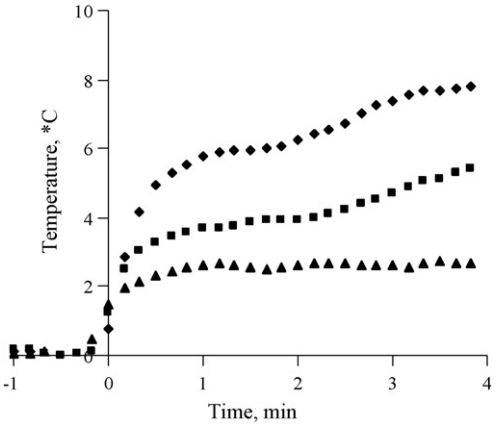

Fig. 3. Temperature change as a function of time for systems containing (▲) 10%, (■) 15%, and (♦) 20% SPIO when heated with application of the alternating magnetic field taken as time zero.

Table 1
Fitted parameters of the release profiles including the first order rate constant and plateau of the burst effect and zero order release rate constant observed with magnetic stimulations. Values were obtained at two different coatings (oleic, OA, and myristic acid, MA), three different UMB loads (1, 2, and 4.8%, w/w), and two different SPIO concentrations of 10 and 20 wt%. Each value represents the mean and standard deviation of three measurements.

Composition (coating–% SPIO–%UMB)	Rate constant (s^{-1})	Plateau value (μg/cm^2)	Zero order slope (μg/s/cm^2)
MA–10–4.8[a]	0.000389 ± 0.00029	106 ± 34	0.67 ± 0.23
MA–20–1.0[b]	0.0011 ± 0.00020	23 ± 11	0.152 ± 0.038
MA–20–2.0[b,c]	0.000804 ± 0.00019	66 ± 12	0.40 ± 0.16
MA–20–4.8[a,b]	0.000303 ± 0.0000812	72 ± 31	0.64 ± 0.43
OA–20–2.0[c]	0.000581 ± 0.000055	31.4 ± 6.9	0.22 ± 0.10

[a] No significant difference ($p < 0.05$) in rate constant, plateau value or zero order slope from SPIO content.
[b] Significant difference ($p < 0.05$) in rate constant, plateau value and zero order slope between UMB load of 1.0 and 2.0%, but no difference with these parameters between 2.0 and 4.8%.
[c] Significant difference ($p < 0.05$) in rate constant, plateau value or zero order slope between myristic acid (MA) and oleic acid (OA) coating.

Hsu and Su (2008) developed a model to describe drug release from solid lipid nanoparticles containing SPIO, which emphasized diffusive transport. Here, the dependence of the phase transition on heat flow is examined assuming a uniform matrix of specified composition (SPIO and solute in lipid) and geometry. The change in temperature with time can be estimated by considering the energy balance, inflow versus outflow,

$$\frac{dT}{dt} = \left(\frac{1}{c_s m_s}\right)(q_p - q_d)$$

where c_s is the heat capacity of the delivery system (ca. 0.00637 J/s cm K), m_s is the mass, q_p is the heat production rate and q_d is the heat dissipation rate, which in accordance with Fourier's law is given as

$$q_d = \frac{\kappa A(T - T_b)}{h}$$

where κ is the thermal conductivity of the medium (6.37 mW/cm K for water at 320 K), and A, $(T - T_b)$, and h are the area, difference in temperature and boundary layer thickness, respectively, between the matrix and medium. Integrating from T to T_∞, the resulting expression is

$$\frac{T - T_b}{T_\infty - T_b} = 1 - \exp\left[\frac{\kappa A}{h c_s m_s t}\right]$$

where the temperature at infinity is

$$T_\infty = \frac{q_p m_s h}{\kappa A}$$

In actuality, the temperature will rise to the melting point of the matrix, which will then undergo melting during which time the temperature of the matrix will remain constant (assuming first order phase transition) (Zeng et al., 2009). After melting is complete, the temperature will again rise. The duration of melting, t_m, will be given by

$$t_m = \frac{\Delta H_m m_s}{q_p - q_{d(T=T_m)}}$$

where ΔH_m is the heat of fusion on a mass basis and the value of the heat dissipation is specified when the temperature of matrix is equal to the melting point, T_m.

For a cylindrical shaped matrix in thermal contact with the release medium at one circular surface and neglecting the heat loss through the surfaces in contact with air, the temperature increase of the matrix at steady state may be estimated as

$$\Delta T = \frac{12\,(\mathrm{J/s/g}) \times 0.02\,\mathrm{g} \times 0.03\,\mathrm{cm}}{0.00637\,(\mathrm{J/s\,cm\,K}) \times 0.55\,\mathrm{cm}^2} = 2.1\,\mathrm{K}$$

using a boundary layer thickness of 300 μm. Since the steady state temperature change is proportional to SPIO concentration, the temperature difference at 15 and 20% would be 3.1 and 4.1 K, which is in reasonable agreement with that seen in Fig. 3. The half-life for the temperature change is about 1 s which also corresponds well with the observed temperature change and the independence of the rate on SPIO content.

Following melting, the release rate of solute at steady state is described by the following:

$$\frac{\Delta m}{\Delta t} = \frac{DAC_s}{\Delta l}$$

where D is the effective diffusion coefficient of the solute in the dialysis membrane separating the lipid matrix from the dissolution medium, A is the surface area as above, C_s is the total amount of the solute in solution in the dissolution medium, and Δl is the membrane thickness. To a reasonable approximation, the release was zero order and increasing the UMB load gave rise to a greater release rate. There did not appear to be any effect of SPIO concentration, which suggests the small temperature difference did not appreciably affect the diffusion coefficient or solubility. While the profile was fit as a zero process, there was clear evidence of a reduction in rate with time. It may be that with depletion of the drug exposed to the aqueous medium, dissolution/diffusion within the molten matrix is no longer negligible as is implicitly assumed in the above equation. Such issues as well as the evident phase separation of the oleic acid coated SPIO pose technical difficulties that need to be addressed in developing a practically useful drug delivery system.

Overall, we have demonstrated that the release of a solute contained in a thermal sensitive matrix can be stimulated by an alternating magnetic field. The temperature change was related to the SPIO content and heat dissipation in a predictable manner. The rate of the release was affected by solute load, area, and matrix material, consistent with diffusion-controlled release. Based on these results, the feasibility can be evaluated with information on the therapeutic index and pharmacokinetic clearance of the drug.

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