O2-2	**Curdlan: a new hydrophobic platform for copolymer drug delivery systems**	

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INTRODUCTION

Curdlan is a hydrophobic, linear 1,3-β-Glucan consisting of glycosidic linkages between the 1st and 3rd carbons on gluclose monomers. Like all 1,3-β-Glucans, it is capable of interfacing with human leukocytes and lymphocytes to generate a localized, non-specific immune response consisting of increased phagocytic activity, increased cytokine production, and respiratory burst and a specific immune response that activates Complement Receptor 3 (CR3) to respond to iC3b opsonins (Vetvicka, 1996). The opsonin, iC3b, is the proteolytically deactivated form of C3b that is present on many systems *in vivo* including healthy cells, tumour cells, fungal spores, and bacteria. Under normal circumstances, C3b is deactivated to iC3b to avoid immune recognition of self-cells but fungal and bacterial pathogens are specifically targeted via this opsonin due to the presence of 1,3-β-Glucans in fungal cell walls and lipopolysaccharide layers respectively, priming CR3 to recognize and eliminate these pathogens. Local delivery of 1,3-β-Glucan to tumour sites has been shown to effectively inhibit tumour growth (Ohno, 2001). Current research trends use 1,3-β-Glucans as adjuvants in combination with other immunotherapeutic approaches such as monoclonal antibodies (Liu, 2009).

When placed in polar solvents, 1,3-β-Glucans form triple helical structures held together by a network of hydrogen bonds that form between secondary hydroxyl groups along the backbone of the polymer. Curdlan in particular forms helical structures that have been very well characterized in the literature (McIntire, 1996; Ikeda, 2005). These unique helical structures have been postulated to be advantageous for their potential association with hydrophobic drugs. This, and the tendency for Curdlan to form viscous gels above 55 °C due to the incorporation of water into the hydrogen bonding structure has inspired research into drug delivery platforms based on thickened gel suppositories (Kanke, 1995).

Curdlan has become particularly popular due the ease of functionalization. Curdlan sulfates have been of increasing interest due to their anti-HIV activity (Yoshida, 1995). The immune activating capacity of Curdlan, and other 1,3-β-Glucans, has led to research into the formation of water soluble derivatives such as carboxymethylcurdlan for direct delivery (Zhang, 2006).

The development of copolymer systems based on the combination of hydrophobic curdlan with hydrophilic polymers or hydrophilic curdlan derivatives with hydrophobic polymers has been of growing interest due to the ability to incorporate immune stimulating activity into the structure of a nanocapsule as opposed to in the form of a separate adjuvant. Literature precedent exists for such systems but often their application is not for drug delivery applications (Yoshida, 1996) or the helical structure forming capacity of curdlan is not taken advantage of (Na, 2000).

Despite the interest in the use of curdlan for drug delivery applications, to the knowledge of the author, there have been no reported findings demonstrating the inherent ability of curdlan to incorporate hydrophobic drugs into its helical pockets. The objective of this work was to demonstrate, using a hydrophobic drug model, Vitamin A, that curdlan is capable of associating with hydrophobic drugs with significant mass yields.

EXPERIMENTAL METHODS

Materials

Raw curdlan was obtained from Sigma Aldrich Chemical Corporation and fractionated using a precipitation based procedure. Briefly, a 10 mL mixture of ~40 mg/mL of raw curdlan in 0.05 g/mL LiCl in DMSO was prepared and stirred vigorously to make a partially solubilised mixture. The mixture was added dropwise into 50 mL of stirring water with a mild vortex. After 30 minutes of stirring, the aqueous suspension was separated and diluted to 5% DMSO with additional DI water and concentrated through 10 000 MWCO Amicon Centrifugation Units at 4000 RPM. Finally, the retenate was resuspended in 2 mL of DI water and freeze dried.

Nanoprecipitation

Six solutions of drug to curdlan ratios (0%, 2%, 4%, 10%, 20%, and 40%) were created with total volumes of 2.2 mL created from stock solutions of curdlan (15 mg/mL) and retinol (6 mg/mL). Standard nanoprecipitation methods were used to form nanoparticles of Curdlan and Retinol that were concentrated and solvent exchanged to DMSO.

Determination of Encapsulation Efficiency and Mass Yield of the Different Drug Loading Levels

Three hundred microlitres of each solution was added in triplicate to a microplate where the absorbance of retinol was measured at 325 nm to determine the concentration. Encapsulation Efficiency was calculated by dividing the actual drug concentration by the ideal drug concentration through all processing steps. The mass yield, the percentage of drug to polymer in the final product, was calculated by multiplying the initial drug loading percentage by the encapsulation efficiency.

RESULTS AND DISCUSSION

The encapsulation of retinol in raw curdlan was demonstrated by nanoprecipitation and quantified by absorbance at 325 nm to obtain the encapsulation efficiencies depicted below in Figure 1.

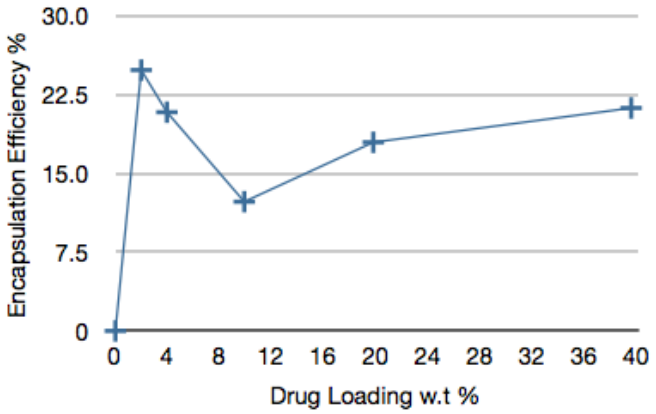

Figure 1 : Encapsulation Efficiency of Raw Curdlan

These encapsulation results suggest that regardless of the initial drug loading percentage, the encapsulation efficiency appears to be limited to the size of the helical pockets formed in solvent.

The mass yield is the parameter of highest interest in drug encapsulation experiments because it represents the final percentage of drug to polymer in the particles that are formed. Figure 2 illustrates the mass yields obtained for the encapsulation of retinol with raw curdlan.

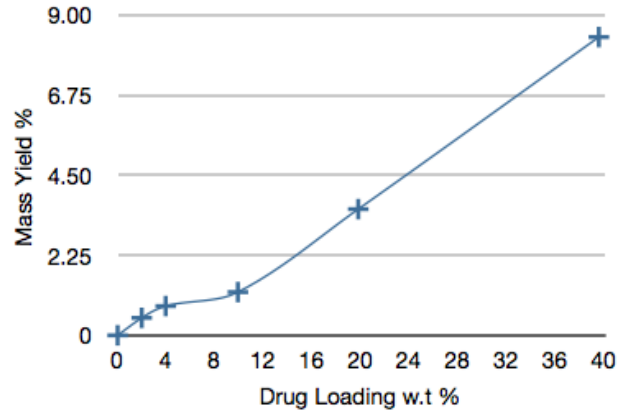

Figure 2 : Mass Yield of Retinol in Raw Curdlan

The results obtained with raw curdlan are on the order of results obtained for some complete drug delivery systems, suggesting that the helical forming capacity of curdlan could position it as an ideal candidate for the hydrophobic components of copolymer systems for drug delivery.

Dynamic light scattering (DLS) results were in accordance with initial results showing fractionated curdlan forms ~20 nm particles. These particles are postulated to be helical aggregations of curdlan. At the drug loading levels that showed significant mass yield, the particle sizes increased to 400-700 nm, suggesting the formation of drug-curdlan aggregations. These particle size results provide significant rationale for pursuing the creation of copolymer systems with curdlan, using hydrophilic groups to lower the particle size.

CONCLUSION

The preliminary data recorded herein provides the baseline evidence showing that immune stimulating curdlan is capable of associating with hydrophobic drugs with mass yields significant for drug delivery applications. More study is needed to test the encapsulation of different drugs and to quantify the release characteristics.

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