

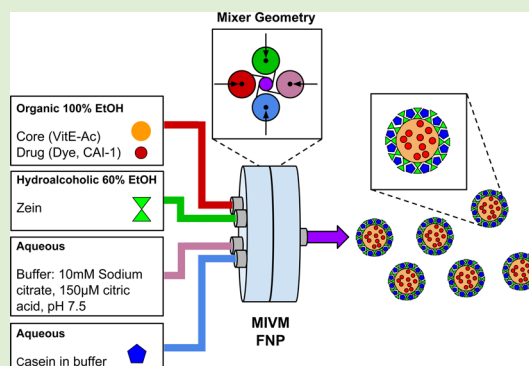
# Nanocarriers from GRAS Zein Proteins to Encapsulate Hydrophobic Actives

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## S Supporting Information

**ABSTRACT:** One factor limiting the expansion of nanomedicines has been the high cost of the materials and processes required for their production. We present a continuous, scalable, low cost nano-encapsulation process, Flash Nanoprecipitation (FNP) that enables the production of nanocarriers (NCs) with a narrow size distribution using zein corn proteins. Zein is a low cost, GRAS protein (having the FDA status of "Generally Regarded as Safe") currently used in food applications, which acts as an effective encapsulant for hydrophobic compounds using FNP. The four-stream FNP configuration allows the encapsulation of very hydrophobic compounds in a way that is not possible with previous precipitation processes. We present the encapsulation of several model active compounds with as high as 45 wt % drug loading with respect to zein concentration into ~100 nm nanocarriers. Three examples are presented: (1) the pro-drug antioxidant, vitamin E-acetate, (2) an anticholera quorum-sensing modulator CAI-1 ((S)-3-hydroxytridecan-4-one; CAI-1 that reduces *Vibrio cholerae* virulence by modulating cellular communication), and (3) hydrophobic fluorescent dyes with a range of hydrophobicities. The specific interaction between zein and the milk protein, sodium caseinate, provides stabilization of the NCs in PBS, LB medium, and in pH 2 solutions. The stability and size changes in the three media provide information on the mechanism of assembly of the zein/active/casein NC.



## INTRODUCTION

**Nanomedicines.** Nanotechnology has been an area of intense research commitment over that last two decades, and nanocarriers (NCs) for the delivery of therapeutics has been one of the successful areas in biomedical nanotechnology. Most successes in NC delivery have been in oncology, where the high value of treatment has allowed the application of relatively expensive formulations. Formulations most often employ block copolymers with polyethylene glycol (PEG) blocks to provide biocompatible surface properties.<sup>1,2</sup> NCs for oral administration, most often, have as their aim increased bioavailability of very hydrophobic drug compounds.<sup>3</sup> Oral formulations must balance the advantage of increased bioavailability against the increased costs of NC processing and of the excipients required for NC formation. Using materials that are accepted by regulatory agencies as GRAS (Generally Regarded as Safe) are preferred in oral delivery applications, as they reduce the complexity of regulatory approval.

Our interest is in the encapsulation of hydrophobic compounds for oral delivery using low-cost, GRAS excipients. Biodegradable protein polymers, such as albumin, casein, gelatin, and chitosan, have been investigated as all-natural low-cost encapsulants.<sup>4</sup> However, these soluble proteins generally provide poor coupling with hydrophobic compounds and, therefore, result in low loading and low encapsulation

efficiency. Excellent work by the Johnston and Elder's group on direct precipitation of hydrophobic drugs with hydroxypropyl methylcellulose (HPMC) has demonstrated significant increases in drug supersaturation upon dissolution of the resulting powders.<sup>5–8</sup> Direct precipitations rely upon highly hydrophobic compounds to achieve high nucleation rates, and encapsulation by HPMC relies solely on hydrophobic interactions between the polymer and compounds. Both hydrophobic and electrostatic interactions between zein and the compounds being encapsulated provides increased flexibility in compounds that can be processed into NC form.

In this paper we present the new process for the encapsulation of hydrophobic actives with zein proteins using the kinetically controlled, rapid precipitation process Flash NanoPrecipitation (FNP) using a Multi-inlet Vortex Mixer (MIVM). The MIVM, with multiple inlet streams enables the encapsulation of actives at higher loading, control of size, and narrow size distributions than have been reported for alternate NC formation processes. The specific interaction between zein and the milk protein casein provides effective stabilization of the NCs. We provide three examples to show the generality of

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