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Zein as a source of functional colloidal nano- and microstructures

Ashok R. Patel [a,*], Krassimir P. Velikov [b,c,**]

[a] Vandemoortele Centre for Lipid Science and Technology, Laboratory of Food Technology & Engineering, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Gent, Belgium

[b] Soft Condensed Matter Group, Debye Institute for NanoMaterials Science, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands

[c] Unilever R&D Vlaardingen, Olivier van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands

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ABSTRACT

The application of colloidal particles from natural materials for purposes ranging from the delivery of bioactives to interfacial stabilisation and bulk structuring have recently gained a lot of interest for applications in the field of fast moving consumer goods, nutraceuticals, agricultural formulations and medicine. Zein-a proline rich water insoluble protein obtained from natural and sustainable source has been recently researched to generate colloidal structures that can find a wide range of applications. In this paper, we review the recent progress in the preparation of colloidal structures and their further application as functional materials in the field of delivery of functional ingredients and structuring of bulk phases and interfaces.

1. Introduction

Scientific community working at the interface of chemistry and biology is always on the lookout for biopolymers from natural and sustainable sources to generate newer structures which could be used for applications ranging from product structuring to the in vivo delivery of bioactives. Since, most of the biopolymers approved and been used for food and pharmaceutical applications (such as gelatin, casein, dextran, etc.) are water soluble in nature; it becomes necessary to involve steps of physical and chemical alterations like cross-linking and hydrophobic modifications in order to generate colloidal particles from these materials [1–6]. Apart from the elaborate synthetic efforts, the removal of residual crosslinking agents makes these processes time consuming and expensive. Moreover, as a hydrophilic polymeric system, these colloidal particles have difficulties to achieve controlled release of encapsulated functional ingredient leading to a rapid diffusion in aqueous environment [5–8].

Zein, which is the main storage protein of maize seeds (*Zea mays* L.) has long been a subject of research for scientific interest as well as industrial applications (as material used in production of coatings, fibres and printing ink) [9,10]. Zein is one of the few hydrophobic water insoluble biopolymers which have been approved for oral use by Food and Drug Administration. The hydrophobicity of zein is attributed to the high percentage of non-polar amino acids (Leucine, Alanine and Proline), which together makes up more than 50% of its total amino acid content (Table 1) [10,11]. Due to this inherent hydrophobicity, zein can be easily constructed into colloidal particles by simply changing the solubilizing capacity of the primary solvent through dilution with a non-solvent, the process commonly known as anti-solvent precipitation method. Zein is also known for its resistance to digestive enzymes resulting in a slower digestibility in the gastrointestinal tract which could be exploited to have a controlled release of functional components loaded in zein colloidal particles. These properties taken together make zein an attractive starting material for the generation of functional colloidal particles (Fig. 1). This paper presents a review of the preparation of colloidal structures from zein and their further application as functional materials in the field of foods and nutraceuticals. The current review will not cover the progress in the area of zein based nanofibers or the application of zein in the field of drug delivery (which is covered in the recently published work of Luo. et.al.) [12].

2. Preparation of colloidal structures from zein

The earliest study on the solubility of zein in binary solvent consisting of water and a lower aliphatic alcohol was carried out in the 1890s [13]. It was reported that zein was freely soluble in aqueous methanol, ethanol and propanol. The differential solubility of zein depending on the concentration of alcohol in the binary mixture has been covered in literature and has been described in terms of a phase diagram. At lower (<40%) and higher (>90%) concentrations of ethanol, two liquid phases appear, containing zein, water and ethanol. This

* Correspondence to: A.R. Patel, Coupure Links 653, 9000 Gent, Belgium. Tel.: +32 9 264 6209; fax: +32 9 264 6218.

** Correspondence to: K.P. Velikov, Soft Condensed Matter Goup, Debye Institute for Nanomaterials Science, Utrecht University, Princetonplein 5, 3584 CC Utrecht, The Netherlands.

E-mail addresses: Patel.Ashok@Ugent.be (A.R. Patel), K.P.Velikov@uu.nl (K.P. Velikov).

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Table 1
Properties of commercial zein [10,11].

Properties	Characteristics
Appearance	Amorphous light yellow-colored powder
Molecular weight	~35,000
Composition	Major non-polar amino acids: Leucine (19.3 g/100 g zein), Proline (9.0 g/100 g zein) and Alanine (8.3 g/100 g zein) Major polar amino acids: Glutamic acid (22.9 g/100 g zein), Serine (5.7 g/100 g zein) and Tyrosine (5.1 g/100 g zein)
Iso-electric point	pH 6.2
Glass transition temperature	165 °C
Thermal degradation point	320 °C
Solubility	Primary solvents (capable of forming at least 10 wt.% solution) belong to the class of glycols, glycol-ethers, amino-alcohols, nitro-alcohol acids, amides, and amines Secondary solvents: Water along with lower aliphatic alcohols and ketones

phenomenon has been referred to as the appearance of a 'taffy' layer and is widely used to recover zein after extraction. It corresponds to a transition state between complete solubilisation and precipitation of zein [13]. This solubility characteristic was first exploited to generate discrete microspheres by controlling the precipitation of zein from a binary mixture of water and alcohol [14]. Approaches based on similar principles were further used by other researchers to generate aqueous coating dispersions for pharmaceutical purposes [15], microspheres for drug encapsulation [6,16] and nanospheres for encapsulation of essential oils [17]. This method to prepare colloidal particles is categorised as anti-solvent precipitation, where the precipitation of particles is caused by the change in the solvent quality of the fluid phase, in which the material to be precipitated is initially present. This is achieved by mixing the initial fluid phase with a second fluid phase (usually water) which is miscible with the organic phase but which is not a good solvent for the dissolved material [18]. The method has obvious advantages in terms of simplicity and technical convenience over other methods used for the preparation of zein microspheres as described by Matsuda et al. [19] and Demchak et al. [20,21].

The working principle of this method is covered in detail by Zhong et al. [22]. When a stock solution of zein is sheared into bulk deionised water, the stock solution is sheared to small droplets. Due to the excellent miscibility of ethanol and water, ethanol in the dispersed droplets partitions into the bulk water. Zein becomes insoluble and precipitates to form spherical colloidal particles when the ethanol concentration in the dispersed droplets decreases below the solubilisation limit (Fig. 2).

Effects of various parameters on zein colloidal particle formation using anti-solvent precipitation method were evaluated and it was reported that the concentrations of zein and ethanol in the stock solution are the major factor influencing the formation of colloidal particles by this process. A higher concentration of ethanol leads to the formation of smaller particles and a high concentration of zein results in increased particle sizes [22]. At lower ethanol concentration, the solubility limit for zein (e.g., <50% ethanol) is reached in a shorter time, and zein in the dispersed droplets solidifies quickly before the droplets can be sheared into smaller sizes, resulting in the formation of bigger particles from a stock solution with a lower ethanol concentration. It is not mandatory to use ethanol as the lower alcohol component in the binary mixture, other primary solvents such as methanol or isopropyl alcohol can also result in colloidal particles in the similar size ranges (Fig. 3) [10,23]. The dependence of the resulting particle size on zein concentration has been explained on the basis of the increased viscosity of the dispersed phase which impacts the rate of solvent diffusion from the dispersed to the continuous phase whereas, the concentration of ethanol in the stock solution influences the solubility characteristics of zein and therefore the rate of precipitation [24]. Ideal results (in terms of narrow size distribution) are obtained when the concentration of zein in the stock solution is around 2–5 wt.% [22,23,25,26]. When zein is precipitated from its molecular solution into a diluting aqueous medium, usually spherical shaped particles are obtained. The formation of spherical shaped polymeric particles obtained by anti-solvent precipitation method has been studied extensively [27–29]. The mechanism of particle formation has been explained by interfacial turbulences between the two liquid phases which are governed by the Marangoni effect [27,28,30]. The particle formation and growth is influenced by nucleation and diffusion controlled Ostwald ripening [31,32]. The anti-solvent precipitation method can however also be used to generate particles with non-spherical morphology by simply changing the viscosity of the precipitating medium [33]. Recently, a continuous technique based on the antisolvent precipitation was developed by Li et al. for facile production of zein colloidal particles with controlled particle sizes on a large scale [34].

Core–shell structures are of general interest for encapsulation purposes in the pharmaceutical, food and cosmetics industries. Core–shell structures formed by citral–zein and lime–zein were demonstrated by Wang et al. [35]. Ferric pyrophosphate, a compound use for delivery of iron in food products, coated with zein using anti-solvent precipitation was also demonstrated by van Leuuwen et al. [36]. Another interesting modification of anti-solvent precipitation method was recently reported by Xu et al. to generate core-shell, hollow zein nanoparticles by using calcium carbonate cores as sacrificial templates [37]. The process involves precipitation of zein from its molecular solution in the presence of a calcium carbonate dispersion which leads to the precipitation of zein on calcium carbonate particles, the subsequent dissolution of calcium carbonate cores gives rise to hollow nanoparticles of zein. The use of these novel hollow nanoparticles was demonstrated in terms of controlled release and direct cell delivery of encapsulated drug molecules [37].

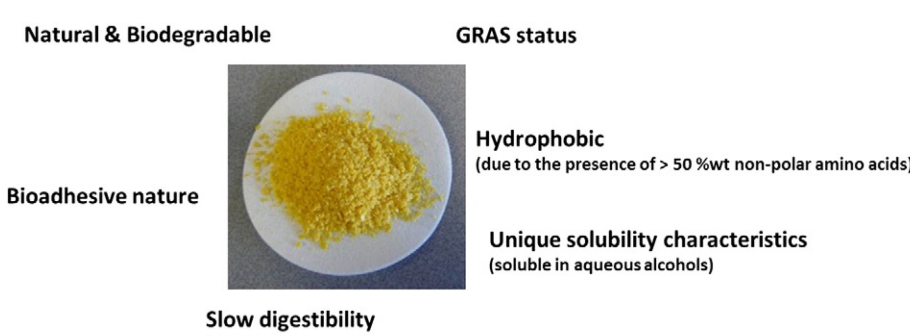

Fig. 1. Properties of zein that makes it a suitable material for development of functional particles (GRAS = Generally Recognized As Safe).