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Zein as a source of functional colloidal nano- and microstructures

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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

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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].

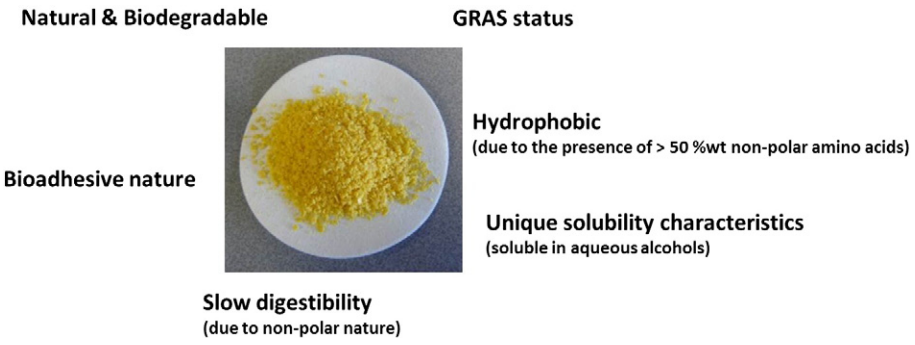

Fig. 1. Properties of zein that makes it a suitable material for development of functional particles (GRAS = Generally Recognized As Safe).

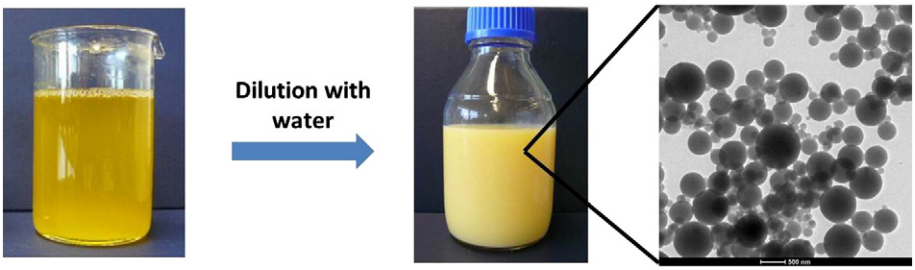

Fig. 2. Molecular solution of zein (in aqueous ethanol) diluted with water to obtain spherical colloidal particles. The spherical morphology of colloidal particles is revealed in the TEM micrograph.
Courtesy of J. de Folter.

Zein was used as a coating material to encapsulate the tomato oleoresin by using spray drying of particle dispersion in zein solution which appears as a versatile method for microencapsulation [38].

3. Encapsulation, stabilisation and controlled release of functional ingredients

Technologies based on colloidal encapsulation have the potential to meet some of the current challenges faced by foods and nutraceuticals industries concerning the stabilisation, controlled release and delivery of functional ingredients such as bioactives, micronutrients, minerals, flavours, antimicrobials and pigments [39–41]. The anticipated impact of these technologies has translated into extensive research to explore edible and natural components as possible carrier materials [42,43]. To find wide spread applications as a carrier material in food and nutraceuticals applications, the materials need to have some or most of these attributes: GRAS status, natural origins, biodegradability, cheap cost, abundant availability and long history of use [41]. Zein as a natural biopolymer fulfils all of these attributes and in addition, properties such as hydrophobicity, low digestion, bioadhesion and potential of cellular uptake makes zein a good candidate as a carrier material for constructing delivery systems of functional ingredients [16,41,44]. Moreover, due to the simplicity of anti-solvent precipitation method, non-polar functional ingredients can be easily encapsulated in zein colloidal particles if they can be co-dissolved in aqueous alcohol stock solutions together with zein [45]. However, zein colloidal particles do have some limitations in their applications since these particles are stabilized due to the surface repulsion (electrostatic stabilisation) they tend to lose physical stability and aggregate in the unfavourable product as well as physiological conditions (neutral/alkaline pH and ionic strength). Another issue that needs to be addressed is the agglomeration of colloidal particles that occur during freeze drying (lyophilization) due to the hydrophobic nature of zein. Electrosteric stabilisation of zein colloidal particles using an oppositely charged protein (sodium caseinate) can be utilized as a solution to overcome these drawbacks. The stabilisation is carried out by precipitation of zein in the presence of sodium caseinate [25]. The presence of sodium caseinate in the precipitating medium results in the surface interaction between positively charged zein particles and negatively charged caseinate. The stabilisation provides protection against aggregation in physiologically relevant conditions and due to the hydrophilic nature of sodium caseinate; the dried colloidal particles can be re-dispersed easily in the aqueous medium without any significant effect on the particle size distribution (Fig. 4). This approach was further utilized by Luo et al. to influence the cellular uptake and transportation of zein nanoparticles [46]. Further applications of caseinate-stabilized zein nanoparticles for preparation of novel antimicrobial films were demonstrated [47–49] and sodium caseinate was also used by Chen et al. to improve dispersibility of spray dried zein particles [50].

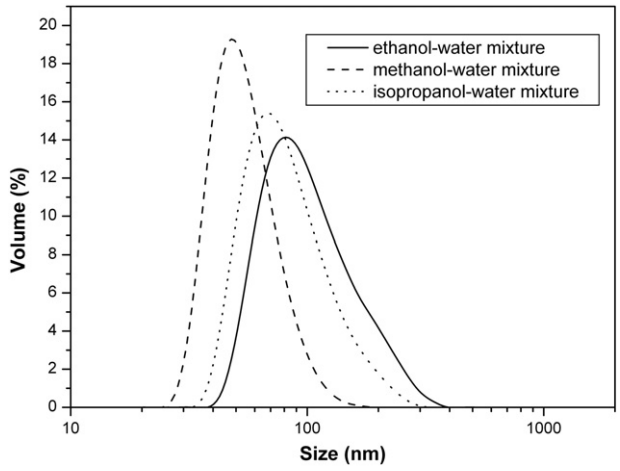

Fig. 3. Volume averaged particle size distribution of zein colloidal particles loaded with curcumin prepared using ethanol, methanol and isopropyl alcohol as the alcohol component (80 wt.%) of binary mixtures with water [23].
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3.1. Stabilisation of polyphenols

Polyphenols are most abundantly distributed group of compounds in phytochemical bearing plants. Their potent anti-oxidant activity is the reason behind their well-known health benefits in human beings. The recent studies on polyphenols have generated increased interest from both food as well as nutraceutical manufacturers with a view of incorporating them in consumer products [51]. However, their clinical efficiency is severely restricted due to their instability in the physiological conditions of intestine where they undergo rapid degradation due to pH and enzymatic attacks [52,53]. Polyphenols are known to strongly interact with proline rich proteins via non-covalent interactions such as H-bonding and hydrophobic interactions [54,55,56]. Based on this preferential binding phenomenon of polyphenols, it was hypothesized that polyphenols could be loaded onto the zein colloidal particles especially because of the high proportion of proline residues. Moreover, polyphenols have excellent solubility in lower alcohols thus they can be encapsulated in zein colloidal particles using the process of anti-solvent precipitation. Some of the examples of polyphenol loaded zein colloidal particles are listed below.

3.1.1. Curcumin loaded zein particles

Curcumin (diferuloylmethane) is a natural polyphenol obtained from the rhizome of turmeric (*Curcuma longa*). It is a very powerful anti-oxidant but the formulation and delivery of curcumin in oral products is a very challenging task due to the combination of factors including low solubility in aqueous medium, photo-degradation and instability in alkaline intestinal conditions. Encapsulation of curcumin in zein colloidal particles was carried out by co-precipitating different zein:curcumin ratios (50:1 to 5:1 wt/wt) in the presence of sodium caseinate as a stabilizer. The mean particle size of colloidal particles was influenced by the ratio of zein and curcumin used in the stock solution.

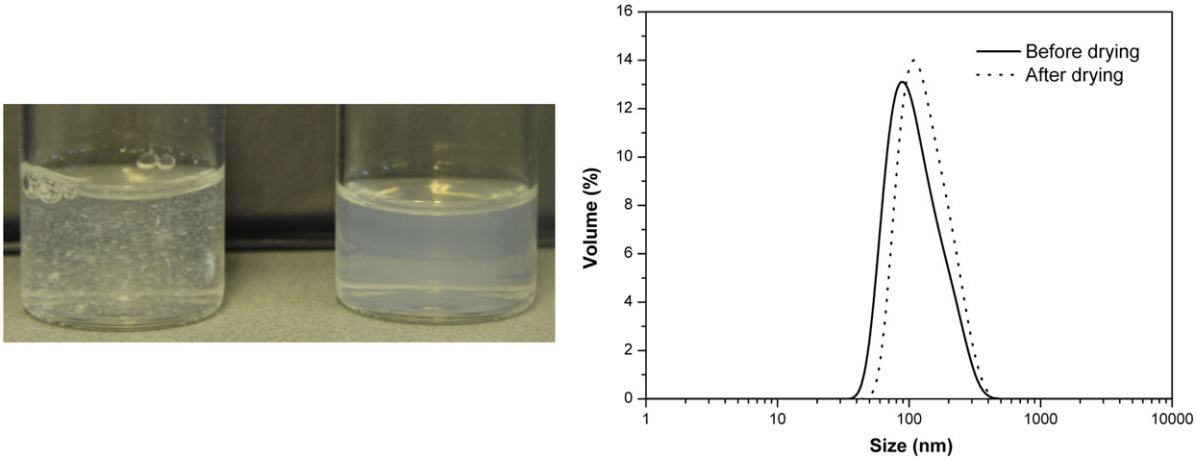

Fig. 4. (On the left) Photograph showing the instant aggregation of zein colloidal particles when diluted with phosphate buffer (7.4) whilst the caseinate stabilized zein particles remains stable on dilution; (On the right) Volume-averaged particle size distribution graph of sodium caseinate stabilized zein colloidal dispersion before and after drying and re-dispersion [25]. Published with permission of ACS.

The mean particle size of colloidal particles with a zein:curcumin ratio of 5:1 wt/wt was found to be around 110 nm with a positive zeta potential (+35.6 mV, measured at pH 4.0). Curcumin in colloidal particles showed enhanced water dispersibility, better photo-stability (Fig. 5) and increased stability in the intestinal conditions [23].

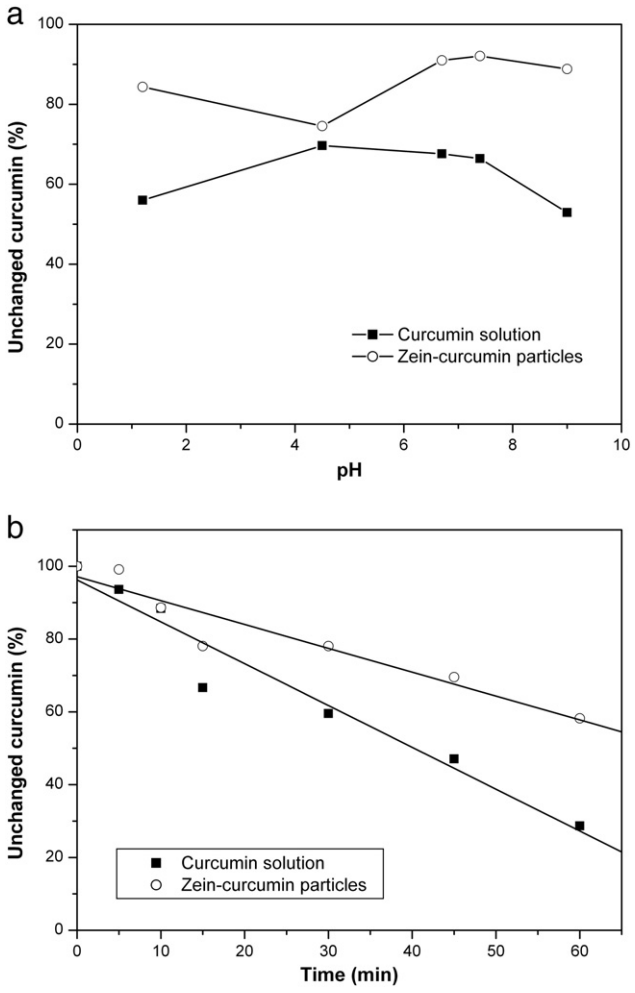

Fig. 5. a) pH stability of curcumin and in zein–curcumin composite colloidal particles and b) Percent unchanged curcumin during UV irradiation as a function of time for curcumin and zein–curcumin composite colloidal particles. For both these studies, curcumin samples were prepared in water-DMSO mixtures and the colloidal particles were prepared at zein:curcumin ratio of 5:1 wt/wt and stabilized with 2 wt.% sodium caseinate [23]. Published with permission of RSC.

3.1.2. Quercetin loaded zein particles

Quercetin (3,3′,4′,5′,-7-penta-hydroxy flavone), is another well-researched natural polyphenol known to possess wide range of physiological benefits in humans, including antioxidant, anti-cancer and anti-viral activities. However, low aqueous solubility and degradation in physiological conditions limits its oral bioavailability [57,58]. Quercetin is practically insoluble in water but dissolves in low molecular weight alcohols such as ethanol [59,60]. This solubility characteristic of quercetin was utilized to prepare zein–quercetin composite colloidal particles [26]. The precipitation of quercetin form an organic solvent generally results in the formation of needle like crystals. Incorporation of quercetin in zein matrix resulted in the formation of spherical particles with complete disappearance of needle like particles at a zein:quercetin ratio of 25:1 wt/wt suggesting effective encapsulation of quercetin (Fig. 6). The positive effect of encapsulation was successfully demonstrated by comparing the anti-oxidant activity of quercetin in alkaline medium.

Similarly, other polyphenols such as Tangeretin (5,6,7,8,4′-pentamethoxyflavone, PMF) and Cranberry procyanidins have also been encapsulated in zein colloidal particles to enhance their stability as well as improve their bioavailability through controlled in vivo delivery [61,62]. The limited solubility of Tangeretin in water severely limits their use in food applications thus, Tangeretin loaded zein nanoparticles were prepared using β-lactoglobulin as the stabilizer in order to obtain freeze dried powder which could be re-dispersed in water with ease. The delivery system could potentially deliver hard-to-formulate Tangeretin in aqueous based food products [61]. In the case of Cranberry procyanidins, the high binding affinity of procyanidins with proline-rich proteins was exploited to generate Cranberry procyanidins-zein nanoparticles with a mean particle size of less than 450 nm. The preliminary evaluation suggested that the primary interactions between the procyanidins and zein were mediated by H-bonding and hydrophobic interactions [62].

3.2. Encapsulation of essential oils

Essential oils are hydrophobic, volatile liquids obtained from plants. They are mainly used as flavouring agents for food and pharmaceutical applications along with aroma imparting agents in cosmetics, textiles and paints products. More recently, the use of essential oils as anti-microbial agents have generated wide interest among researchers [63]. Due to their sensitive nature, essential oils can easily degrade under the action of oxygen, light and moderate to high temperatures.

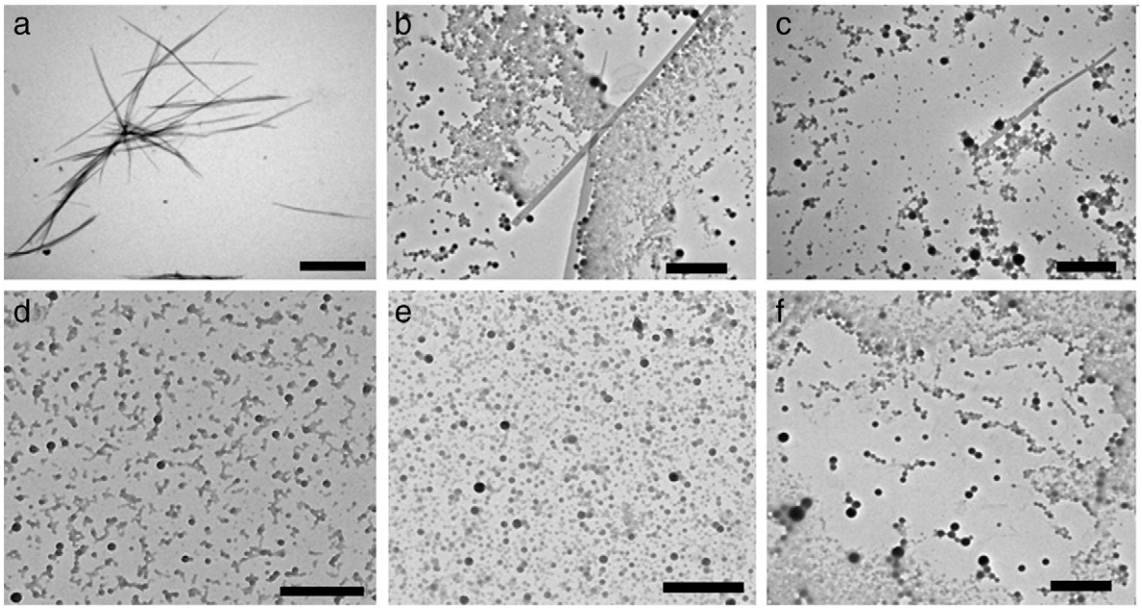

Fig. 6. TEM images of (a) Needle-like crystals of quercetin obtained by precipitating its alcoholic solution in water (Scale bar = 5 μm); Colloidal particles prepared at different zein:quercetin ratios of (b) 5:1 wt/wt, (c) 10:1 wt/wt; (d) 25:1 wt/wt; (e) 50:1 wt/wt (Scale bar = 1 μm) and (f) blank zein colloidal particles (Scale bar = 1 μm) [26]. Published with permission of Elsevier.

Moreover, for some applications (such as preservative, anti-bacterial and anti-oxidant effects), controlled release is required to achieve the desired results. Therefore, the common goals in the development of essential oil formulations are to protect the essential oil from degradation or from losses by evaporation, to facilitate handling and achieve controlled release for sophisticated applications [64]. Encapsulation of essential oils such as oregano and cassia oil, thymol and red thyme in zein colloidal particles was first reported by Parris et al. [17]. Encapsulates were prepared by anti-solvent method wherein essential oils and zein dissolved in 85 wt.% aqueous ethanol were precipitated in water containing a small amount of dispersant (0.01% silicon fluid). Based on the in-vitro release studies, the encapsulates showed limited digestibility in gastric fluid (due to the acid resistant nature of zein), a slow release of oil in the small intestine and a more rapid release in the large intestine. In a very recent study, reported by Wu. et al., thymol and carvacrol were encapsulated in zein colloidal particles to enhance their water dispersibility [65]. The better dispersibility of encapsulated oil resulted in the enhancement of their anti-oxidant and anti-bacterial properties. In another study, thymol encapsulated in zein particles stabilized by caseinate and chitosan hydrochloride double layers sustained stronger anti-microbial effect for a longer time [66].

3.3. Controlled release of functional micronutrients

Formulating functional micronutrients (such as vitamins, minerals, antioxidants etc.) into controlled release colloidal systems can improve their bioactivity by increasing their bioavailability, minimizing in-vivo degradation and targeted delivery [36,67,68]. Zein in combination with another biopolymer (chitosan and its derivatives) was used to encapsulate hydrophobic functional micronutrients including α-tocopherol and vitamin D3 into colloidal nanoparticles. The physicochemical properties of these colloidal particles, including encapsulation efficiency and controlled release profile were significantly enhanced relative to those of colloidal particles prepared from single polymer (i.e. either zein or chitosan) [69,70]. A two-step method was used to generate these composite colloidal particles. Firstly, the functional active was encapsulated in zein colloidal particles followed by addition of second polymer to cause phase separation induced by surface interactions. Tween 80 and calcium were used as stabilisers to obtain discrete particles in the sub-micron size range. The zein–chitosan colloidal particles showed biphasic release kinetics in simulated intestinal conditions including an initial burst release in first two hours followed by a sustained release for next 5–7 h depending on the proportion of zein and chitosan used during the preparation of colloidal particles [69,70]. Controlled release of another micronutrient, lutein (a derivative of β-carotene) was also achieved by preparing lutein–zein nanoparticles using 'solution enhanced dispersion by supercritical fluids' (SEDS) technique [71]. It has been reported that lutein supplementation can have many beneficial effects in humans such as decreasing the risk of age-related macular degeneration and prevention of some cardiovascular disorders [72,73]. However, the heat and light sensitivity of lutein coupled with its low water solubility results its poor adsorption and bioavailability [74]. The encapsulation of lutein in zein particles resulted in a decreased burst release in the initial 40 min with a zero-order release profile followed by around 90% release at the end of 120 min [71]. The encapsulation of mineral in the form of insoluble mineral salts was demonstrated using anti-solvent precipitation. Ferric pyrophosphate and calcium carbonate were used as sources of iron and calcium, respectively [36]. The chemical reactivity of iron ions from zein-coated ferric pyrophosphate was compared to pure ferric pyrophosphate colloidal particles. This is an important development that can help improve stability of iron fortified products.

3.4. Antimicrobial loaded colloidal particles

Several studies report the use of zein as a preferred material to generate films loaded with food grade antimicrobials including lysozyme, thymol and nisin [75–77]. Loading antimicrobials in colloidal particles could facilitate incorporation of these particulate antimicrobial delivery systems in food matrixes and also enhance the long term effectiveness of antimicrobials due to their controlled and sustained release [78,79]. Loading of antimicrobial-lysozyme in zein particles via processes such as supercritical anti solvent technique and spray drying have been reported where the loaded lysozyme showed sustained release of antimicrobial over weeks [80]. A food grade antimicrobial delivery system was developed by Xiao et al. wherein a combination of antimicrobials including thymol and nisin were co-encapsulated in zein microcapsules [78]. The developed delivery systems showed sustained release of both the encapsulated antimicrobials and led to enhanced anti-listerial properties. Recently, zein was also used to prepare composite particles

with silver in order to enhance its growth inhibitory property and at the same time dramatically improve its hemocompatibility [81].

3.5. Other encapsulation applications

Due to its various advantages, zein has been used as a biopolymeric matrix to load other functional ingredients including pigments, fixed oils and proteins. Hydrophobic food colouring agent such as curcumin (Fig. 7) was loaded in zein matrix [23], the enhanced colouring property of curcumin loaded in zein particles through the process of electro hydrodynamic atomization was further successfully evaluated by Gomez et al. in semi-skimmed milk samples [82]. The preliminary results indicated that the addition of encapsulated colorants made it possible to obtain aqueous dairy products with different shades and chromaticities. Zein fibres in micro/nano range produced by electrospinning were also used to encapsulate β-carotene which is a food colorant and an antioxidant. The encapsulation in zein matrix resulted in a significant increase in light stability of β-carotene [83]. Recently, an interesting colloidal approach was reported by our group where different shades of green colour (Fig. 8) were obtained by co-encapsulating yellow and blue pigments in composite colloidal particles of zein [84]. Green is a very desirable colour for food applications because as a colour, it carries the promise of freshness. However, it is also a notorious colour, it easily fades under the influence of light and at acidic pH of most food beverages. Currently, chlorophylls and molecular complexes of chlorophylls are the only known natural green colorants approved for food applications. The weak aqueous stability of chlorophylls has meant that the green colours have only been sparingly used in food products. It was recently identified that aqueous dispersible, acid and photo stable green colour could be easily obtained by loading food colorants curcumin (CUR) and indigocarmine (IC) in composite zein particles through dye–protein interactions. The co-precipitation of crystalline colorants in zein matrix resulted in amorphous spherical particles in the size range of 175–300 nm as confirmed by X-ray diffraction studies and transmission electron microscopy [84].

Due to the property of zein to form hydrophobic barrier around the encapsulated active, it was used as a shell material to encapsulate fish oil in colloidal particles ranging from 350 to 500 nm. The encapsulation of fish oil in zein particles resulted in an increase in the oxidative stability of fish oil [45]. Similarly, flax oil (which is rich in polyunsaturated fatty acids and hence susceptible to oxidation) was stabilized by encapsulation in zein microparticles prepared by spray drying and lyophilisation [85]. Zein forms complexes with pectin based on electrostatic interactions; these interactions also forms basis for development of hydrogel particles, the pH dependent swelling behaviour of these particles was utilized to control the release of protein molecules for probiotic related applications [86,87].

3.6. Zein-based bioadhesive colloidal particles

Bioadhesive polymers are known to form entanglements and physical bonds with mucin, the main component of mucosa which covers the gastrointestinal lining [88,89]. The interactions of bioadhesive polymers with mucin has been used as a strategy to improve the oral bioavailability of bioactive molecules by developing bioadhesive colloidal systems that keeps the bioactive molecules in close proximity to the cell membrane for longer duration thereby enhancing their absorption across the membrane.

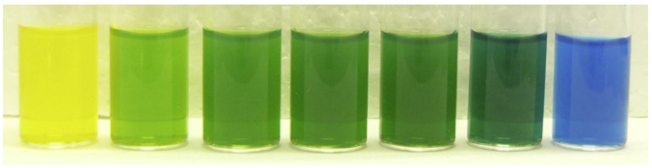

Fig. 8. Colloidal dispersions with a tuneable yellow–green–blue colour containing (from left to right) zein:Cur:IC (20:1:0; 20:1:0.25; 20:1:0.5; 20:1:1; 20:0.5:1, 20:0.25:1 and 20:0:1). The ratios are expressed on weight basis and the total concentration of zein in all cases was 1 wt.% [84].
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Zein has been classified as a natural bioadhesive polymer based on its property to bind with biological surfaces based on hydrogen bonding and hydrophobic interactions [90,91]. Bioadhesive nature of curcumin loaded zein colloidal particles was studied using two different methods including in-vitro washed-off method with agarose gel acting as mucomimetic layer and mucin association study on coated Caco-2 cells [23,92]. The binding of colloidal particles to agarose gel and mucin coated Caco-2 cells was studied with respect to the retention and association of curcumin. The enhanced residence time (>60% after 150 min), faster kinetics and higher saturation of binding (Fig. 9) were indicative of the bioadhesive properties of zein colloidal particles. The speculated reasons for the bioadhesion of zein colloidal particles were physical entanglements of particles with the mucin (mucomimetic) layer and possible involvement of the amino and carboxylic groups of zein in hydrogen bonding with mucin/mucomimetic layer [23,92].

4. Controlling the microstructure of food

Zein colloidal structures by virtue of their intrinsic surface activity can stabilise oil–water and air–water interfaces and thus can be utilised for controlling the food colloid microstructures such emulsions and foams. In a recent study, generation of Pickering emulsions using spherical zein colloidal particles as interfacial stabilisers was reported [93]. Zein particles used in this study were prepared via anti-solvent precipitation of zein (from 80% aqueous ethanol as described earlier) with an average particle size of 82 ± 16 nm. The average diameter of the obtained particles was controlled to get particles that were not too large (because particles close to 500 nm can trigger immediate sedimentation) or too small (in order to have sufficient energy for attachment at the oil–water interface). Emulsions were prepared by simply adding oil to zein dispersion under vigorous mixing (Fig. 10). It was found that stable oil-in-water emulsions at pH above and below the zein isoelectric point, and for low to moderate ionic strength could be prepared using zein colloid as a novel class of natural particle stabilisers. Based on the preliminary experiments (drop test) it was speculated that zein colloid could also be used to create oil in water in oil emulsions as well as water in oil emulsions [93]. Juttulapa et al. studied emulsifying of pectin as an emulsifier and zein as

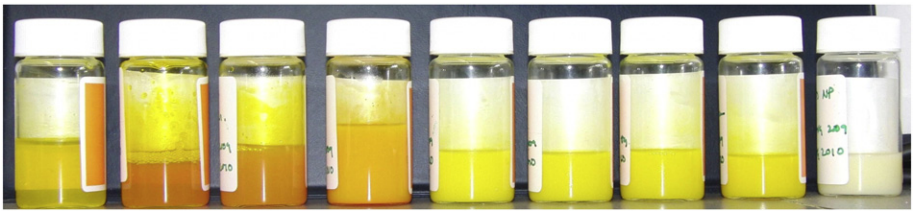

Fig. 7. Different shades of orange–yellow colour obtained by preparing composite colloidal particles at varying zein:curcumin (Z:C) ratios. (Starting from left: pure curcumin; 1:1; 2:1; 4:1; 5:1; 10:1; 25:1; 50:1 and pure zein) [23].
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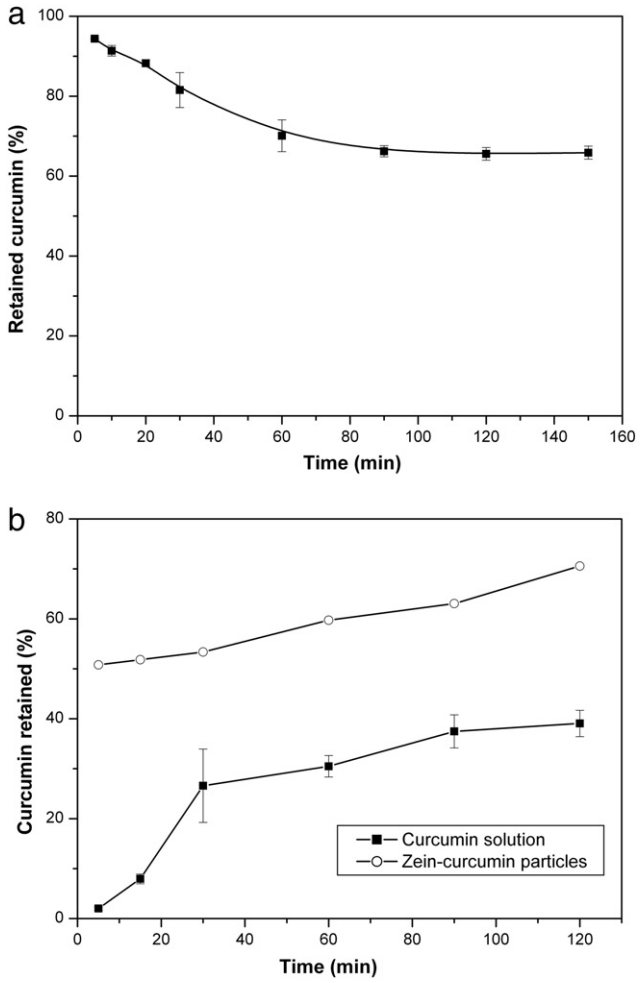

Fig. 9. a) Percent curcumin retention as a function of time for zein–curcumin composite colloidal particles and b) Percent curcumin bound as a function of time for curcumin solution and zein–curcumin composite colloidal particles. Colloidal particles for both these studies were prepared at zein:curcumin ratio of 5:1 wt/wt and stabilized with 2 wt.% sodium caseinate [23].
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an auxiliary emulsifier [94]. At pH 7, where both pectin and zein are negatively charged, less stable emulsions were formed. The results demonstrated that emulsions prepared at pH 4 were smaller in size and more stable. The stabilizing effect in the presence of zein, at pH 4, where zein and pectin have opposite charges, is attributed to a zein–pectin complex formation at the interface. Gao and co-authors explored another approach for Pickering stabilisation of emulsions using zein colloidal particles and small molecular weight surfactant complexes [95]. In particular, sodium stearate to control interfacial properties and loading of the particles at oil–water interfaces. The resulting emulsions show good stability against both coalescence and creaming. The partial unfolding of zein particles modified by the sodium stearate triggered aggregation and close packing of the particles at the oil–water interface providing a steric barrier against coalescence. Moreover, they demonstrated zein-based oil gels without oil leakage by a one-step freeze-drying of a zein-stabilized Pickering emulsions. Although expensive, the process is claimed to provide a viable strategy for structuring liquid oils into semisolid fats without the use of saturated or *trans* fats.

By the virtue of its inherent hydrophobicity, zein colloidal particles can be potentially used to structure food products as most of the food matrices are hydrophilic in nature. Zein colloidal particles added to representative food matrices such as 0.5 wt.% carboxy methyl cellulose and semi-skimmed milk did not show any dramatic negative effects such as aggregation or sedimentation based on electrostatic interactions [22,82]. Recently Filippidi et al. demonstrated the use of zein to form all natural microcapsules containing a liquid lipid core encapsulated by zein [96]. The thickness of the zein shell can be controlled by the amount of precipitated protein (Fig. 11) and was shown to control the lipid hydrolysis and microcapsule biodegradability. Such microcapsules can be used as alternative to particles based stabilisation of oil-in-water emulsions against coalesce and for encapsulation and release of various lipophilic compounds.

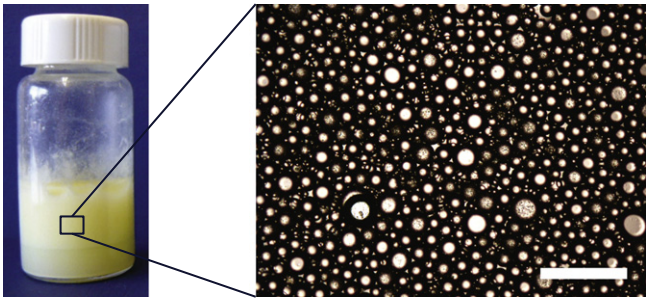

Fig. 10. Photograph of oil-in-water Pickering emulsion prepared at 50 wt.% of oil using spherical zein colloidal particles (at 1 wt.% zein) as stabilisers [93]. Insert: Microscopy image of emulsion, scale bar = 500 μm.
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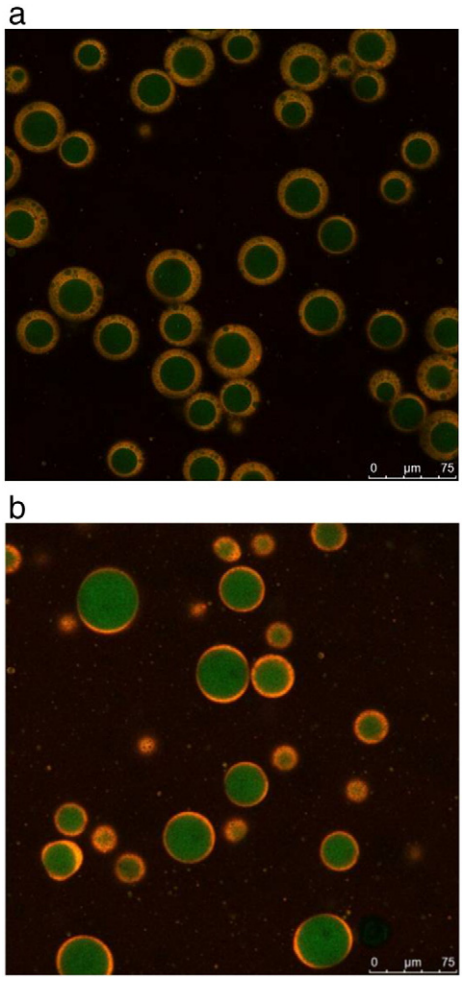

Fig. 11. Confocal micrographs of oil-core zein-shell microcapsules in water formed at different oil-to-zein ratios: (a, 1.45; b, 4.50).
Courtesy of P. Voudouris.

5. Conclusions and outlook

Zein has long been used in pharmaceutical and fast moving goods sectors mainly for coating applications. Recently, however, the use of zein as a fully natural, biodegradable and food-grade source for generation of functional colloidal structures have received wide spread interest. The simplicity of generating zein colloidal particles coupled with the ease of loading them with functional ingredients makes it an attractive system to work with. The hydrophobic nature of zein provides a barrier to the encapsulated actives improving their storage stability in product conditions as well as effectively reducing their degradation in the gastro intestinal tract. The inherent slow digestion of zein in the intestine lends an interesting aspect to zein colloidal particles for controlling the release of encapsulated actives. This area can be explored to control the delivery of various nutrients and micronutrients as well as for controlling the rate of energy intake (e.g. from starches and oils). The possibility of drying these particles into solid powder form which could be re-dispersed when required opens up further opportunities of using zein colloidal particles in dry product formats. The additional functionality of zein particles to interact and bind to the mucosa and direct internalization into cells makes them well-suited for broader and sophisticated applications especially in the area of functional foods with smart properties and in the area of drug delivery. Already, zein has been utilized extensively for encapsulation of functional actives ranging from essential oils to natural anti-oxidants, all of which are frequently used ingredients in food products. Another interesting application of encapsulation can be extended to controlling the optical properties of pigments and colorants and subsequent development of responsive or structural colours. Other than encapsulation and delivery applications (which is the dominantly researched area) of zein colloidal particles, studies on structuring and stabilisation of (food) colloid microstructure have also emerged recently. Future developments are expected in the field of design of more complex colloidal structures such as core-shell and shape anisotropic particles, which can further improve the control on particle surface activity in their ability to stabilise fluid-in-fluid dispersions such as foams and emulsions. Finally, future developments may further include the utilisation of other water insoluble proteins (e.g. gliadin) or mixture of them. Even larger opportunities can be expected by combining prolamins with other biopolymers or synthetic polymers which will allow a further control on their biodegradability.

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