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Perspectives in the design of zein-based polymeric delivery systems with programmed wear down for sustainable agricultural applications

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Design of nanostructured systems for pesticide delivery allows the implementation of a rational and sustainable use of these chemicals. Zein nanoparticles (ZNPs) enable extended foliar adhesion, as well as prolonged and controlled release of the entrapped agrochemical. The present study aimed to investigate degradation of ZNPs with various surface properties under environmentally relevant conditions. Accelerated degradation studies were performed on freeze-dried ZNPs synthesized with different cationic and non-ionic surfactants. These polymeric particles dispersed in alkaline media ($pH = 9$) revealed hydrodynamic diameters ranging between 210.6 and 297.3 nm and negative surface charges from −44.3 mV to −34.56 mV. Degradation profiles of the studied particles were assessed within the pH limits of soils under agricultural exploitation (pH 4 and 9). The time-dependent decrease of the parent protein fractions was monitored by capillary gel electrophoresis, where the rate constants of the hydrolytic degradation allows the estimation of ZNPs' persistence at any temperature of interest. Results showed that in alkaline media promotes a faster chemical degradation of ZNPs (total at 20 °C in ~3918 days at $pH = 4$ vs. 205 days at $pH = 9$), however the nature of the used surfactant may completely reverse the observed trend. Identifying the technological variables that impact their surface chemistry and susceptibility towards hydrolytic degradation, opens new perspectives in the programmed degradation of ZNPs, which may ensure a safer and more sustainable use of these nanodelivery systems. Although chemometric models were able to assess quantitative correlations between recorded physical properties of the ZNPs and the estimated time necessary for their full degradation, further studies are needed to elucidate the causality between the nature of used surfactants and estimated degradation times in aqueous media.

1. Introduction

Population growth and increased demand for food called for intensified development of agriculture [1]. The use of pesticides and artificial fertilizers has increased in order to satisfy these rising demands. On the other hand, excessive and irrational application of agrochemicals led to a significant negative environmental impact [2]. In order to avoid these harmful effects, it is desired to have a more controlled delivery of pesticides. By using designed formulations, the crops are protected with improved efficiency while limiting the harmful exposures of environment and population. Nano-engineered products allow a more targeted and environmentally friendly distribution of agrochemicals through their encapsulation in biodegradable nanocarriers [1]. Nanoparticles (NPs) can be engineered to deliver agrochemicals [1], with the goal of protecting the active load from chemical or photo-degradation, as well as releasing it in a controlled manner for improved

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Abbreviations: GRAS, generally regarded as safe; NPs, nanoparticles; ZNPs, zein nanoparticles; Tris, tris-(hydroxymethyl)-aminomethan; NaOH, sodium hydroxide; HCl, hydrochloric acid; DMAB, didodecyldimethylammonium bromide; NEGMDE, nonaethylene glycol monododecyl ether; DBDM, n-dodecyl β-D-maltoside; CGE, capillary gel electrophoresis; IS, internal standard.

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persistency and efficacy. Polymeric nanoparticles of controlled properties have been previously shown to be suitable for targeted and sustained delivery of drugs [3,4], vitamins and other nutrients [5].

A novel and promising material for nanocarrier synthesis is zein, the main protein from corn endosperm [6]. Particularly rich in proline and glutamine, it demonstrates a unique solubility profile, ascribed to its amino acid sequence with more than half of the units nonpolar, such as proline, leucine, alanine, phenylalanine, isoleucine, valine, etc. [7]. Zein also has a pronounced hydrophobic character and the ability to self-assemble into NPs, as well as being generally regarded as safe (GRAS) for human consumption, biocompatible and biodegradable. Due to these characteristics, it was successfully exploited for the entrapment and delivery of various hydrophobic compounds, drugs [3,8], vitamins [9], and dietary supplements [10].

In agriculture, zein nanoparticles (ZNPs) are considered a suitable delivery vehicle for pesticides, allowing their targeted and sustained delivery to the crops [1,8,11]. By designing the ZNPs with various surface properties, they can adhere to the root system and foliage of plants applied on to [11,12], increasing the localized persistence time of the loaded pesticides. Such advanced pest management practices would limit the waste of agrochemicals occurring in their conventional broadcasting, and improve the efficacy of the treatment, while minimizing the impact of the pesticide pollution on soil and water caused by runoff [13].

Through the application in agriculture as carriers for pesticides, consumers and the environment are intentionally or unintentionally exposed not only to pesticides, but also to NPs. Despite the well-documented toxicity of pesticides, there are some concerns regarding safety of the delivery systems themselves. Ethical, policy and regulatory issues are all rooted in the lack of knowledge about the potential effects of nanomaterials on human and environmental health. Although the literature addressing the fate and impact of NPs on the environment is becoming more robust, there are some key knowledge gaps that remain almost completely unaddressed, especially for polymeric, soft NPs, such as ZNPs [11,12]. Data on interaction, fate and impact of organic NPs on the environment is limited [14]. Because of the potentially wide range of application of ZNPs, it is critical to understand their environmental fate in the event of purposeful exposure through nano-enabled agricultural applications or accidental exposure as a result of runoffs or improper disposal.

Therefore, it is essential to have a better knowledge of the wear down of the zein-based delivery systems under environmentally relevant conditions. Identifying the technological variables (type of surfactant, surface charge, polydispersity, etc) that impact their surface chemistry and susceptibility towards hydrolytic degradation, opens new perspectives in the programmed degradation of the pesticide-loaded ZNPs. This may ensure a safer and more sustainable use of these delivery systems for agricultural applications.

The main objectives of this study were to assess the influence of the used surfactants (cationic, anionic, neutral) on the hydrolytic degradation of zein nanocarriers under environmentally relevant conditions (different pHs and temperatures), as well as identifying potential correlations between the physico-chemical properties of ZNPs with different surface chemistries and their estimated theoretical environmental persistence.

2. Materials and methods

2.1. Reagents and instrumentation

All reagents used were of analytical grade purity and were purchased as follows: sodium dodecyl sulfate (SDS, >99% purity), tris-(hydroxymethyl)-aminomethan (Tris) from Redox Biovet (Romania), hydrochloric acid (HCl) from Chemical Company (Romania), sodium hydroxide (NaOH) from Lach-Ner (Czech Republic), citric acid from Merck (Germany), pyromellitic acid (96% purity) from Aldrich (Germany). Zein powder, acetone, trehalose, didodecyldimethylammonium bromide (DMAB), and polysorbate 80 (Tween 80) were purchased from Sigma Aldrich (USA). Nonaethylene glycol monododecyl ether (NEGMDE), and n-dodecyl β-D-maltoside (DBDM) were purchased from Fisher Scientific (USA). SDS-Gel Separation Buffer, SDS Sample Buffer, 100 mM Tris-HCl (pH 9.0 with 1% SDS), SDS Protein Sizing Standard (10–225 kDa, 16 mg/ml) were purchased from Beckman Coulter Inc. (USA).

Buffer solutions were prepared using ultrapure water (18.2 MΩ, Barnstead, EASYpure® RoDi), adjusting the required value of pH with 0.1 M NaOH or HCl solutions. Samples were homogenized through sonication (Elmasonic, Germany). The controlled degradation studies were performed using an ECOCELL 110 convection oven (BMT, USA). Throughout sample processing quick and gentle solvent removal was achieved through vacuum concentration at room temperature using Concentrator Plus (Eppendorf, Germany). All electrophoretic separations were carried out on an Agilent G1600 capillary electrophoretic system (Agilent Technologies, Germany). Multivariate data analysis (Principal Component Analysis and Orthogonal Projections to Latent Structures) has been performed using Simca-p+v. 13.0.3 software (MKS Data Analytics Solutions, Sweden).

2.2. Zein nanoparticle synthesis

ZNPs were prepared by nanoprecipitation using an adapted version of a previously published synthesis protocol [12]. Briefly, the organic phase was formed by adding 600 mg of zein in 20 ml of acetone-water (80:20 (v/v)) solution under strong stirring at room temperature. The aqueous phase was formed by dissolving 150 mg of the surfactant DMAB in 220 ml of deionized water at room temperature. Next, the organic phase was added to the aqueous phase under strong stirring at room temperature. The mixture was then passed three times through a microfluidizer (Microfluidics M-110P, Westwood, MA) at 25,000 psi. Finally, acetone was evaporated with a rotary evaporator (Rotavapor R-300 Buchi Analytical Inc., New Castle, DE), under vacuum. The resulting suspension was freeze-dried (Labconco Freezone 2.5, 12, Kansas City, MO) for 2 days, after addition of trehalose (3:1, w/w) as cryoprotectant. ZNPs synthesized with other surfactants followed the above described protocol, but the surfactant concentrations in the aqueous phase were changed to 3.2 mg/ml of polysorbate 80, 1.6 mg/ml of NEGMDE, and 3.2 mg/ml of DBDM. The resulting ZNPs are to be referred to as Z-DMAB, Z-polysorbate 80, Z-NEGMDE, and Z-DBDM.

2.3. Characterization of freeze-dried zein nanoparticles

Hydrodynamic size, dispersity and surface charge of the freeze-dried ZNPs was assessed by dynamic light scattering (Zetasizer ZS, Malvern Instruments Ltd.) in the same medium in which degradation studies took place. Accurately weighed samples of zein particles were redispersed by sonication for 1 min at a concentration of 0.05 mg/mL each, in distilled water; 25 mM sodium citrate buffer pH = 4; and 25 mM Tris buffer, pH = 9. All measurements were carried out at 20 °C using three replicates, considering a mono-modal distribution.

2.4. Molecular weight determination

For the determination of the molecular weight of the emerging peptide fractions of the protein-based NPs, a linearized calibration