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# Correlation between emulsifier concentration and emulsion droplet size in oil-in-water emulsion stabilized by zein nanoparticles

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ABSTRACT

Pickering emulsification, a very important technique that was found as early as the 1900s, is still a very valuable tool that is used by many researchers because it provides high stability and reduced coalescence. Scientists have recently realized the usefulness of zein, corn protein, as an emulsifier in making Pickering emulsions and research into this corn protein has been growing rapidly since the beginning of the 21st century. Therefore, an investigation of the basic properties of zein would not only have great value, but would also open up many possible future studies on zein. The aim of this study was to determine the correlation between emulsifier concentration and emulsion droplet size in oil-in-water emulsion systems stabilized by zein nanoparticles. The emulsion system was made through two-stage liquid-liquid dispersion. Five different concentrations of zein nanoparticle emulsifier (0.5%, 0.6%, 0.8%, 1% and 2% w/v) were used for the experiment and samples were analyzed using ImageJ software. From the correlation, the effectiveness of the stabilizing interactions at the molecular level was deduced. Results of the experiment revealed a positive correlation in a linear relationship between the diameter of the droplets and the inverse of the concentration of zein.

## INTRODUCTION

Chemically, water and oil are immiscible. Liquid-in-liquid dispersions such as emulsions can be stabilized by adding surface-active particles, or emulsifiers (Binks & Horozov 2006). Emulsifiers help stabilize two immiscible liquids' interface by means of the reduction of interfacial tension through surface adsorption, which leads to further droplet disruption (Vignati et al. 2003). The phenomenon of solid particles acting as emulsifiers in emulsions was firstly noticed as early as the 1900s (Pickering 1907; Ramsden 1903), but active research in the field did not take place until a few decades ago. Many different substances were studied as potential emulsifiers to be used for various purposes, but the biggest challenge was to find a food-grade emulsifier that could remain insoluble in both oil and aqueous phases of the dispersion while maintaining a stable system over a long period of time (Dickinson 2010). Emulsifiers such as zein colloidal nanoparticles, a class of prolamin protein found in corn, are used to create oil-in-water (o/w) emulsions to be used in foods, pharmaceutical products, and cosmetics (Shukl 2001). In order to maximize the efficacy of the use of zein nanoparticles in different emulsions, it is important to understand the correlation between their concentrations and the droplet size of the emulsion droplets, from which we can deduce the effectiveness of the stabilizing interactions at the molecular level (Osborn et al. 2004). Previous research has revealed that zein nanoparticles are relatively hydrophobic due to amino acids such as leucine, proline, and alanine, which are hydrophobic (Pomes 1971). Researchers realized that due to zein's low solubility in both water and oil, anti-solvent precipitation could be used to control the creation of zein colloidal nanoparticles.

Past studies have conducted surfactant-free experiments to observe the effects of particle concentration, pH, and ionic strength on emulsions, (de Folter et al. 2012) while others explored a novel approach by surface modification with sodium caseinate (NaCas) to create Pickering emulsions (Feng & Lee 2016). This study focused on the correlation between emulsifier concentration and emulsion droplet size in a surfactant-free emulsion created with zein colloidal nanoparticles and the final goal was to propose a mathematical model of the correlation between zein concentration and emulsion droplet size.

# MATERIALS AND METHODS

## Materials

Zein (Z3625) and pure water (320072) were obtained from Sigma Aldrich Inc. (St. Louis, MO, USA). Canola oil (Crisco In., Orrville, OH, USA) was purchased from a local store. Ethanol (anhydrous denatured 200 proof) was obtained from Cole-Parmer Inc. (Vernon Hills, IL, USA).

## Preparation of zein colloid nanoparticles

The first step of this experiment was to create zein colloid nanoparticles, which acted as the emulsifier that stabilized the oil-water interface. Six grams of zein was dissolved into 100 ml of 70% v/v ethanol solution to form a stock solution. The solution was then sonicated using a sonicator (Ultrasonic processor Q55, Qsonica, Newtown, CT, USA) for one minute at 50 amplitudes in order to dissociate aggregated zein molecules into zein monomers. Next the stock solution was dispersed to 150 ml of distilled water under a speed of 12,000 rpm homogenization (IKA-ULTRA-TURRAX T25 basic, IKA Works, Inc., Wilmington, NC, USA). Afterwards, large zein particles were removed by centrifugation (model IEC CENTRA CL2, Thermo Scientific Inc., Waltham, MA, USA). This step caused the zein molecules to form a homogenous suspension. Then, the dispersion was placed in a rotary evaporator (HaakeBuchler Instruments Inc., Saddle Brook, NJ, USA) for 30 minutes in order to evaporate excessive ethanol and water, and a concentrated stock solution was created. Finally, 1 ml of the stock solution was placed into an oven at around 60°C until all the water was evaporated and the concentration of zein in the stock solution was calculated in grams of zein per 1 ml of stock solution. This stock solution was diluted into five different concentrations of solid zein in water (0.5%, 0.6%, 0.8%, 1% and 2% w/v).

## Preparation of emulsions

Five different emulsions were prepared using the five different concentrations of zein colloid nanoparticles (0.5%, 0.6%, 0.8%, 1% and 2% w/v). 20 ml of canola oil was slowly added to 20 ml of zein colloidal dispersion while mixing at 12,000 rpm using a high-speed homogenizer (IKA-ULTRA-TURRAX T25 basic, IKA Works, Inc., Wilmington, NC, USA). After all the oil was added, the sample was homogenized for an additional 2 minutes. A drop test was conducted by adding a drop of each emulsion sample to pure canola oil. O/w emulsion was confirmed when emulsion droplets remained intact in the oil phase.

## Imaging and calculating droplet size

Digital images of each emulsion were taken at 20X magnification using an optical microscope equipped with a camera (Axio Imager A1, Zeiss, Germany). The Sauter mean diameter ($d_{3,2}$) was calculated using the following equation:

$$D_{3,2} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \qquad \text{Eq. 1}$$

where $n_i$ is the total number of droplets with diameter $d_i$. Figure 1 shows how ImageJ software was utilized to measure the Sauter mean diameter of the emulsified oil droplets.

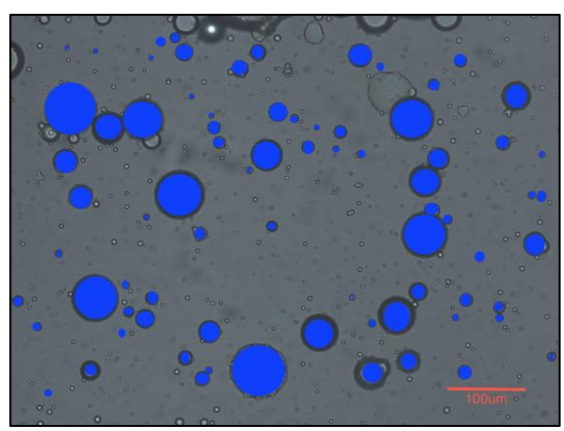


**Figure 1**. Representative image of emulsified oil droplets in ImageJ software, used to measure the Sauter mean diameter.

Data analysis, including calculating the analysis of variance (ANOVA) and the least significant difference (LSD), was done using Microsoft Excel 2011 (Microsoft Inc., Bellevue, WA, USA).

## RESULTS AND DISCUSSION

Figure 2 depicts optical microscope images of emulsified oil droplets at different zein concentrations. The images show that as the concentration of zein increases (from 0.5% to 2%), the diameter/size of the oil droplets decreases.

A further statistical analysis, including the ANOVA and the least significant difference, was done to find any relationships and correlations among different concentrations.

The ANOVA value is a tool in statistics that compares all the statistical data and gives a possibility that the data would be considered similar enough. Alpha (α) is a threshold value that below which treatments will be considered as significantly different. Herein, we use $\alpha = 0.05$, as it is a scientific convention. The calculation shows that there is only a 0.6% chance ($p = 0.006$) that the data for different types of zein concentrations is similar. Therefore, such a low p-value indicates that my null hypothesis that all the Sauter mean diameters of the emulsified droplets are the same will be rejected. Therefore, a further analysis using the least significant difference is needed.

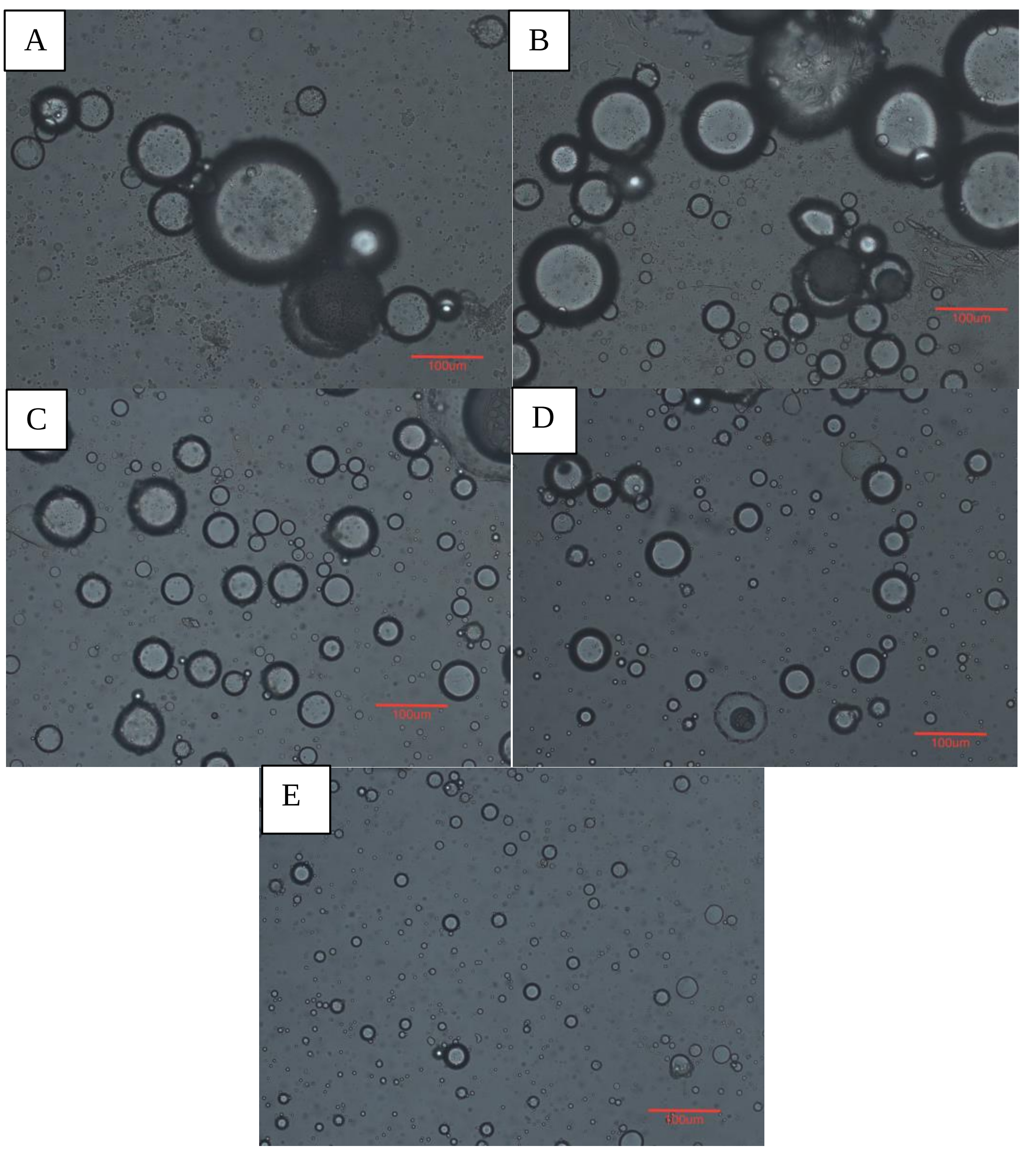


**Figure 2.** Optical microscope images of oil droplets emulsified with water in (A) 0.5% (B) 0.6% (C) 0.8% (D) 1% (E) 2% zein concentrations.

The least significant difference (LSD) value is very similar to the ANOVA value; however, the ANOVA value compares the data as a whole while the least significance difference is able to compare two specific data points. For instance, the probability of 0.5% zein concentration droplet size and 0.6% zein concentration droplet size being similar is 52.31%. The alpha value of 5% is a tool that is used in statistics that sets the baseline which determines whether two data points will be considered similar or not. Because 50% is more than 5%, we can group the two statistics in the same least significant group, A (Table 1). The same LSD groupings mean that there is not enough of a distinguishable difference between the emulsion droplet sizes. Therefore in future studies, if one would like to have a big enough of a difference in zein droplet sizes, one would have to choose the zein concentrations for which the LSD grouping does not overlap.

**Table 1.** Relationship between the Sauter mean diameter and the inverse of zein concentration.

| Zein Concentration (w/w%) | Inverse of Concen-tration | Average Sauter mean diameter ($\mu$m) | Standard Deviation |
|---|---|---|---|
| 0.5 | 200 | 103.21[a] | 36.47 |
| 0.6 | 167 | 88.44[ab] | 21.06 |
| 0.8 | 125 | 58.45[bc] | 1.27 |
| 1 | 100 | 53.60[bc] | 14.24 |
| 2 | 50 | 23.03[c] | 1.44 |

The Sauter mean diameter was plotted against the inverse of zein concentration in order to quantitatively determine the relationship between the two properties (Figure 3). The correlation showed a strong linear model, with a coefficient of determination ($R^2$) of 0.98862. As the inverse of zein concentration increases, the Sauter mean diameter of the droplets increases. This trend occurs because as the concentration of zein increases, more zein particles are available to adsorb at the oil-water interface. In other words, the higher the concentration of the emulsifier, the larger the surface area of oil the zein nanoparticles will be able to cover and stabilize.

The results in this study were supported by various similar studies in the past. One research group investigated the effect of different concentrations of whey protein on the diameter of the emulsified oil droplets (Sun & Gunasekaran 2009). Their results suggested that an increased concentration of whey protein allowed higher surface area coverage of oil droplets, causing the droplet size to decrease. Another similar investigation studied various factors that affected the particle size in the formation of nano-emulsions using different types of proteins and small-molecule surfactants (Qian & McClements 2011). They found that the concentration of the emulsifier was one of many factors that had a

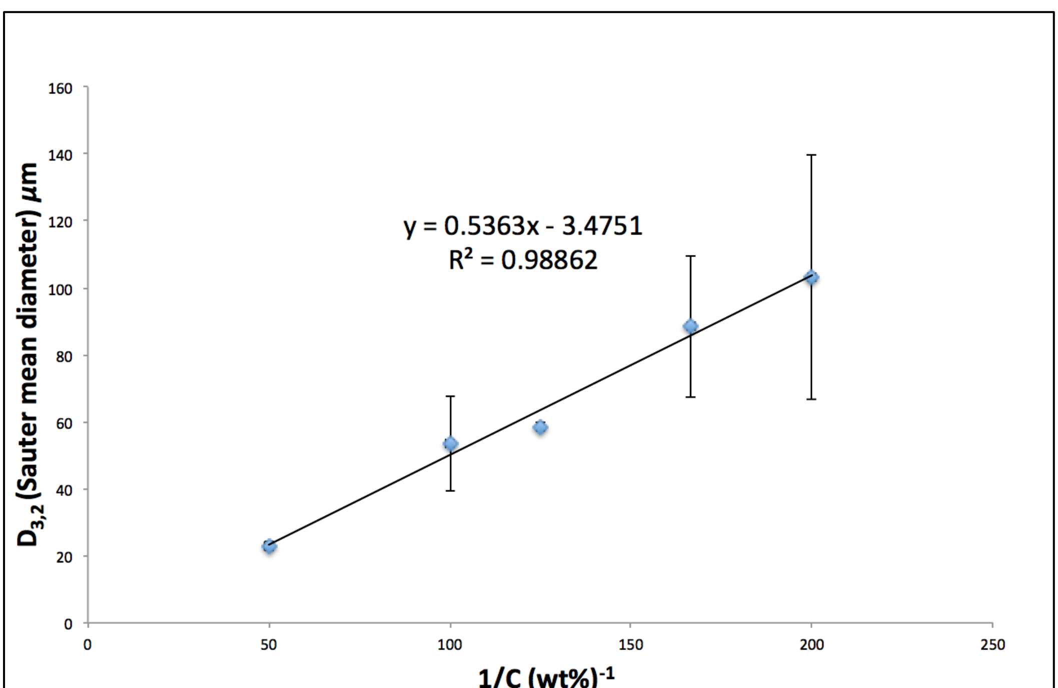


**Figure 3.** Effect of the inverse of zein concentration on the Sauter mean diameter.

significant effect on emulsified particle sizes and concluded that generally, the diameter of the droplets decreased with an increase in the emulsifier concentration. They suggested that the results occurred because there would be more emulsifier present to surround the droplets that were produced during high-pressure homogenization. Although the emulsions in past studies were stabilized by different protein materials, both results showed a very similar correlation between emulsifier concentration and the diameter of emulsified droplets when compared with the results found in this study.

## CONCLUSIONS

In conclusion, a linear relationship between the Sauter mean diameter of the emulsion droplets and the inverse of emulsifier concentration was found. The correlation revealed that high zein concentration yielded smaller emulsion droplets. An extension to this project would involve investigating the relationship between emulsifier concentration and droplet size at higher concentrations of zein, including 5% or even 10%. This could allow for determination of the minimum size of emulsified oil droplets produced using this method.

## FUTURE RESEARCH

A possible future study would be to investigate the effects of redispersion. The first step would require the emulsified solution to be spray dried or freeze dried in order to evaporate all the water out and only allow solids to be formed. Then, one could redisperse the encapsulated material into the water and study what aspects, such as the diameter of the oil droplets, of the emulsion were affected due to redispersion.

## ACKNOWLEDGMENTS

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