Article

Effect of African Catfish Mucilage Concentration on Stability of Nanoemulsion Using D-Optimal Mixture Design

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Featured Application: African catfish mucus stabilized oil in water nanoemulsions can be used as a means of incorporating functional components into for cosmetics, drug delivery, and personal care products which are applicable in biomedical and personal care industries.

Abstract: Nanoemulsions are alternative means of incorporating functional components into systems. This research aims to model the effect of African catfish mucilage (ACM) concentrations on the particle size (PS) of stable oil-in-water (O/W) nanoemulsions formulated by ultrasonication. A D-optimal mixture design was used to study the influence of three mixture components (MCs) ACM, oil, and water on PS. Morphology and PS were determined with Cryo-TEM and Zetasizer. Findings show that the higher the ACM–emulsifier oil ratio, the higher the nanoemulsion stability as depicted by lower PS. ACM concentration was the factor that had the most dominant effect on the dependent variable (DV) PS. Morphology studies revealed that structural stability was a result of ACM which encapsulated the nanoemulsion by mucoadhesion. The model’s lack of fit ($F [0.17, 0.11] = 0.3104$; $p = 1.49$) was not significant, and the predicted R-squared value was 0.9977 and adequate precision was 104.158 indicating a model with adequate goodness-of-fit. The model was adequate to determine the effects of the three MCs on the precise stability parameter for the investigated dependent variable particle size. Therefore, ACM could be used as a natural stabilizer in oil-in-water nanoemulsions that are applicable in biomedical and personal care industries.

Keywords: African catfish mucilage; D-optimal mixture; emulsifier–oil ratio; encapsulated; particle size

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1. Introduction

Oil-in-water (O/W) emulsions are basic constituents of many products in industries such as beverages, cosmetics, drug delivery, foods, nutraceuticals, and personal care products [1–3]. Kinetic instability of O/W emulsions can be ascribed to the presence of positive free energy made available when dispersions are formed that allows colloids to separate through creaming, coalescence, flocculation, and sedimentation [2,4]. Emulsifiers are materials that kinetically stabilize emulsions by coating the O/W interface by generating repulsive forces that hinder oil globules aggregation [2,5]. The food industry has legally approved the use of several food-grade emulsifiers, however, costs, ease of use, and functionality make it difficult for food scientists to select the most proper emulsifier for an application [2,6,7]. The kinetic stability of O/W emulsion has been increased by adding emulsifiers, stabilizers, and biopolymers [2,8,9].

Food scientists have been faced with the challenge of formulating biologically nutritious semi-solid foods either as colloids, emulsions, gels, or simple solutions for individuals facing health challenges such as diabetes, obesity, or allergies [10–12]. These challenges

have been complicated because the biologically nutritious components tend to be immiscible with water [11,12]. Researchers have therefore shifted focus to emulsifying methods that apply high-energy emulsification methods to deliver emulsions [2,13]. Authors have reported the use of micro-emulsions and nanoemulsions to deliver different emulsion components [4,14]. However, nanoemulsions are preferred in comparison to microemulsions because nanoemulsions can be stabilized with low emulsifier concentrations [4,14]. Emulsifying methods apply systems that require high-pressure, microfluidics, rotors, or ultrasonic equipment [2,13,15]. These high-energy emulsifying methods use machine-driven devices that generate strong disruptive forces that fragment the dispersed phase and result in small oil globules [2,16]. Authors have proposed ultra-sonication which involves shearing the interface between oil and water globules to form stable dispersions at low frequencies [10–12].

Authors have reported that an emulsion can attain stability when its particle size is reduced [8,17]. The literature established that the lower the particle size the more stable an emulsion becomes [8,18,19]. Response surface methodology (RSM) a multivariate statistical method has been commonly used to optimize food processing techniques [20,21]. RSM is a regression-based analysis that fits mathematical models, such as linear, square, or quadratic polynomial functions, and other models, to the experimental response observed in the experiment by applying multivariate statistical analysis [20,22,23]. The RSM strategy establishes relationships between input factors and responses with the aid of a mathematical model, deals with hindrances of optimizing with sole parameters, and provides an efficacious way to formulate emulsions in a mixture design [13,23]. Mixture designs are statistical experimental designs derived from mixture components [24] where constraints are placed on the concentration of the dependent variables used [25–27].

The mucilage secreted from the fish skin when fish are stressed is a waste that contains elements such as carbohydrates, enzymes, lipids, metabolites, proteins, and water [24,28,29]. Several authors have established that vital enzymes and proteins have been identified in fish skin mucilage which is responsible for its natural defense [28–30]. These include antimicrobial peptides (AMP), glycoproteins, immunoglobulin, lectins, lysozyme, mucins, complement proteins, proteases, transferrins, and various other antibacterial proteins and peptides [28–30]. The skin of fish contains goblet and paneth cells which play a focal role in defending the fish by producing humoral immune factors and mucins [28–30]. The humoral immune factors include antimicrobial peptides, cytokines, immune-globulins, and lectins [29,30]. Mucins present in mucilage are glycoproteins joined to considerable amounts of high molecular weight sugars that play a vital role in fish defense [29,30]. Glycoproteins are structurally intricate chemical compounds that comprise proteins and carbohydrates [31,32]. Authors have reported that oligosaccharide chains of glycoproteins are covalently attached to the polypeptide side-chains and proteins secreted from fish are glycosylated [31,32]. Authors have reported that stabilized oil in water emulsions have been formulated with amphiphilic mucilage such as hagfish or saliva mucins which have the potential to form cross-linked stable structures [33]. These emulsions were also shown to adhere to pig mucus in the buccal cavity, which confirms that amphiphilic mucilage can be used to formulate mucoadhesive oil-in-water (O/W) emulsions [33]. Hagfish mucilage has been used as an emulsifier because of its mucoadhesive properties and the authors concluded that hagfish mucilage could emulsify soy milk emulsions [34]. Authors have proposed that the mucilage from fish, insects, and pigs could be used as vehicles for cosmetics, drug delivery, and personal care products which are applicable in biomedical and personal care industries for animals and humans [35–37]. Hence, African catfish mucilage (ACM) could be a potential emulsifier. The literature claims that the catfish mucilage contains about 2.3% ash, 1.1% fat 2.0% fiber, 7.4% protein, 10.9% moisture, 76.3% carbohydrates [38]. The mucin extract from fish has been reported to have exceptional antimicrobial, lubricating, and wound healing properties. A wavelength peak of 1136 cm^{-1} was recorded in the raw infra-red spectra of African catfish mucilage which could be attributed to the anti-symmetric glycoside bond [19]. Several authors have established that

the presence of the glycosidic bond in glycoproteins is the reason a glycan can covalently attach to polypeptide side-chains and form stable structures that are suitable for biological processes [39,40]. It can be inferred that the raw spectrum of ACM has glycosidic linkages similar to other glycoproteins which can encapsulate emulsions and confer stability [19]. A study has proposed that it could be applied in stabilizing emulsions that could be used as vehicles for nutritious nutraceutical, cosmetic and drug-delivery systems for animals and humans [19].

This study aims to model the effect of African catfish mucilage concentrations on the particle size of stable oil in water nanoemulsions by ultrasonication using the D-optimal mixture design.

2. Materials and Methods

2.1. Collection and Preservation of African Catfish Mucilage

The African catfish mucilage (ACM) was collected using the Guidelines for Ethical Conduct in the care and use of nonhuman animals in research, and ethical considerations for field research on fishes [41,42]. Special care was taken to adhere to animal ethical issues on handling the African catfish, treating the fish with respect, and the implications of feeling pain [41,42]. The African catfish was put under anesthesia using 100 mg per liter methanesulfonate before collection [30]. The anesthetized African catfish were blotted dry on a dissection tray, then placed in a bowl and a fish stimulation gadget (HPG1, Velleman Instruments, 18 V, 80 Hz) was used to enhance mucus discharge aseptically from mucus glands by placing the gadget on the ventral lateral section of the catfish [43]. After the ACM was collected, the anesthetized African catfish was resuscitated by conveying them to a recuperation bath. This ACM was instantly stored at 4 °C for measurements that involved ACM mucins. Proteolysis was avoided by adding 1 mL of protease inhibitor cocktail (Sigmafast, P8340 Sigma Aldrich, USA) to every 100 mL of citrate/PIPES buffer. ACM was stabilized in two ways. The ACM stabilized in MCT could be used throughout the day that it was collected, while ACM stabilized in high osmolarity citrate/PIPES buffer (HOC/PIPES) could only be used for measurements immediately, as skeins stopped untangling when exposed to (HOC/PIPES) for too long [44]. Part of the retained ACM was frozen in liquid nitrogen and freeze-dried for storage at −80 °C.

2.2. Experimental Design

The mixture design methodology was used to study the influence of the ACM, sunflower oil, and MilliQ water (Millipore, Merck) concentrations on stability. The sunflower seed oil from *Helianthus annuus* was procured from Merck. A D-optimal experimental mixture design was used with the constraints African catfish mucus (A or ACM) + sunflower oil (B or Oil) + MilliQ water (C) = 100%. The lower and high limits for ACM, oil, and water were (1 to 5%), (3 to 10%), and (85 to 96%). The stability responses analyzed using mixture design surface interface was Sauter mean diameter ($D_{(3,2)}$) as a function of particle size [11]. Design Expert (version 10.0: Stat Ease, Inc., Minneapolis, MN, USA) was used to generate the D-optimal mixture design experimental plan. The mixture studied was a three-component system: a mixture of ACM emulsifier (A), sunflower oil (B), and MilliQ water (C). The total sum of the mixture was 100% (100 mL). The optimal combinations of ingredients proportions and levels of components for active oil water-type (O/W-type) nanoemulsions stabilized with free-dried ACM were determined by D-optimal mixture design using Design-Expert software (version 10, Stat-Ease, Inc.). The coded low and high for L_Pseudo Coding for ACM was (+0 ↔ 1 and +0.3636 ↔ 5), that of oil was (+0 ↔ 3 and +0.6365 ↔ 10) while that of water was (+0 ↔ 85 and +1 ↔ 96).

The trace plot and the 3D plot were used to compare the component effects on the design space. L-pseudo phase diagrams were constructed to predict and establish the regions where stable O/W-type nanoemulsions were formulated [45,46]. D-optimal mixture design was set up to enable the definition of the quadratic model to quantify the effect of concentration of ACM emulsifier on emulsion stability property Sauter mean

diameter ($D_{(3,2)}$). Model equations were calculated after converting the actual values of emulsifier concentration into l-pseudo levels.

2.3. African Catfish Mucilage Stabilized O/W-Type Nanoemulsion Formulation

The ACM stabilized O/W-type nanoemulsions were formulated using a modification of the ‘premixing, sonication, and rest method’ developed by [47]. It involved cavitating of the oil flocs in the MilliQ water aqueous medium with the aid of an ultrasonic processor (Sonics and Materials, Inc., Newtown, CT, USA). Sunflower oil and MilliQ water were premixed for 5 min at a temperature set to 10 ± 2 °C. Then, ACM was added to the sunflower and MilliQ water and the mixture was stirred with an acoustic amplitude (ζ) of 40% for 2 min in periods of 0.5 min of stirring and 0.5 min of rest using a 20-kHz Vibracel processor, 750 W Sonics, a tip diameter of 13 mm, and a temperature set to 10 ± 2 °C [47]. Oil–water mixtures containing 85–96 mL MilliQ water, and 3–10 mL sunflower oil were homogenized without ACM with the aid of an ultrasonic processor as control (Sonics and Materials, Inc., Newtown, CT, USA).

2.4. Particle Size Measurements

The particle size was measured using the principle of direct light scattering with the aid of a Particle electrophoresis Nano series (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK). The ACM stabilized O/W-type nanoemulsions’ particle size was analyzed in three replicates at 25 °C.

2.5. Cryogenic-Transmission Electron Microscopy and Particle Size Analysis

The structure of ACM stabilized sunflower O/W-type nanoemulsion was analyzed with the aid of an FEI/Tecna F20 Cryogenic transmission electron microscope (Cryo-TEM) and a charged coupled device camera size 4096×4096 Gatan UK Milton Park Innovation Centre Abingdon OX14 4RY United Kingdom Gatan, the USA [48]. The Sauter mean diameter ($D_{(3,2)}$) was calculated from the particle size distribution using Equations (1)–(3) using methods developed by [49,50]. The particle size distribution of ACM stabilized emulsions was elucidated by calculating Sauter mean diameter ($D_{(3,2)}$) using Equation (1) while the standard deviation i.e., value of the width of the milk goblets distribution in the ACM stabilized emulsion was calculated using Equation (2). The average droplet size is given by Equation (3) where n_i is the number of goblets in each size class, d_i is the particle diameter, $\sum n_i$ is the total number of droplets, and d_n is the average droplet size [50].

$$D_{(3,2)} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

$$\sigma = \frac{\sum n_i (d_i - d_n)^2}{\sum n_i} \quad (2)$$

$$d_n = \frac{\sum n_i d_i}{\sum n_i} \quad (3)$$

Source: [50].

2.6. Data Optimization and Statistical Analysis

In determining the optimum ACM stabilized O/W-type nanoemulsion formulation, a D-optimal mixture design was chosen as the desirable design type based on the number and constraints of the selected mixture components (MCs). The 3 mixture constituents were (A) ACM, (B) oil, and (C) water. Each constituent was constrained with lower and upper limits; the 3 components in real scale amounted to 100% (1–5% for ACM, 3–10% for oil, and 85–96% for water), respectively. The design consisted of 16 formulations which comprised 11 different runs, and 5 runs had two replicates. The quadratic equation generated using Design expert software 10 established the relationship between the formulation variables

and responses. Each of the measured responses (dependent variables) were fitted to a quadratic model (Equation (4)).

$$Y = \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_4x_1x_2 + \beta_5x_1x_3 + \beta_6x_2x_3 \quad (4)$$

where β_1 – β_6 represent the regression coefficients. The values of these coefficients were calculated based on the synergy between the response and formulation variables. The terms x_1 , x_2 , and x_3 represent the coded formulation variables, Y represents the measured response. Analysis of variance was used to assess both models' level of significance. p -value and lack-of-fit of the selected models should be either significant ($p < 0.05$) or not significant ($p > 0.05$), respectively [51]. The trace and contour plot was used to examine the role of mixture parameters ACM, oil, and water on the studied stability properties Sauter mean diameter ($D_{(3,2)}$). The Piepel trace plot was used to assess the effect of changing each variable of the mixture on the studied responses, along with a trace imaginary line from a reference blend to the vertex, while the proportion between the other components was kept constant [46,52]. Trace plots ascertain the independent variable that influences and has a dominant effect on the studied responses [45,46]. The selected independent variables from plots were used to construct the contour plots. The contour plot method provides a meaningful way to visualize the changes in a response that has various levels of constituents [46]. The response surface in a contour plot connects all points that have the same responses within a two-dimensional plane [46].

3. Results and Discussions

3.1. Stability of ACM Stabilized O/W-Type Nanoemulsion

The particle size data measurements from Cryo-TEM imaging studies were identical to the measurements of particle size made on the Zetasizer. The Sauter mean diameter ($D_{(3,2)}$) was calculated from the particle size data using Equations (1)–(3) using methods developed by [49,50]. Table 1 shows the constituents used in formulating the sets as determined by the three-component D-optimal mixture design and the stability as a function of ($D_{(3,2)}$). Higher concentrations of ACM led to a decrease in $D_{(3,2)}$ of oil globules for all investigated ACM stabilized O/W-type nanoemulsions. When ACM concentration was highest regardless of oil concentration the $D_{(3,2)}$ of emulsions was low. For instance, the average $D_{(3,2)}$ of stabilized O/W nanoemulsions containing an ACM–emulsifier oil ratio (EOR) of 5:3.0, 5:4.6, 5:7.3 and 5:10.0% were 6.6 ± 0.1 , 6.9 ± 0.1 , 7.0 ± 0.1 , and 7.4 ± 0.2 nm, respectively (Table 1). Overall, the trend was higher the EOR the lower the $D_{(3,2)}$ (particle size) i.e., higher stability as seen in Table 1. The literature supports this claim as revealed by a study conducted by [40] which investigated the key elements that influenced the formulation of the formulation of non-ionic surfactants-stabilized fish oil nanoemulsions using high-intensity ultrasound. The authors observed that when the EOR and HLB were high the particle size was low and concluded that EOR and HLB influenced particle size [53]. Hence, the results obtained on the ($D_{(3,2)}$) of ACM stabilized nanoemulsions are consistent with results reported by [53] that the higher the EOR the lower the particle size of non-ionic surfactants-stabilized fish oil nanoemulsions.

Table 1. Composition and observed responses from randomized runs for D-optimal mixture design.

Formulation/Run	Independent Variable			Dependent Variable
	A: ACM ^b (%)	B: Oil ^b (%)	C: Water ^b (%)	D _(3,2) ^b
1	1.0	9.6	89.4	31.9 ± 0.6 ^a
2	1.0	9.6	89.4	32.4 ± 0.6 ^a
3	1.0	3.0	96.0	10.2 ± 0.3 ^a
4	5.0	7.3	87.7	7.0 ± 0.2 ^a
5	1.0	5.7	93.3	23.2 ± 0.6 ^a
6	5.0	3.0	92.0	6.7 ± 0.1 ^a
7	5.0	3.0	92.0	6.6 ± 0.1 ^a
8	5.0	10	85.0	7.4 ± 0.2 ^a
9	2.3	10	87.7	15.4 ± 0.4 ^a
10	1.0	3.0	96.0	10.4 ± 0.4 ^a
11	2.9	3.1	93.9	15.9 ± 0.5 ^a
12	3.3	6.2	90.5	13.8 ± 0.4 ^a
13	5.0	10.0	85.0	7.4 ± 0.2 ^a
14	1.0	8.0	91.0	29.1 ± 0.5 ^a
15	5.0	4.6	90.4	7.0 ± 0.2 ^a
16	3.3	6.2	91.0	13.6 ± 0.4 ^a

^a Indicated values are reported as means ± standard deviation (n = 5), ^b ACM—African catfish mucilage, oil—sunflower oil, and water—MilliQ water, and D_(3,2)—Sauter mean diameter.

The particle size distribution of emulsions stabilized with 5% ACM was between 0.1 and 0.2 nm while that of emulsions stabilized with 1% was between 0.5 and 0.6 nm (Table 1). This signifies that as the ACM–emulsifier oil ratio (EOR) increased the particle size distribution of ACM stabilized nanoemulsions (ASE) decreased (Table 1). Hence, the higher the ACM-EOR the lower the D_(3,2) and PSD and the higher the stability of the ASE. Authors have reported that increases in emulsifier concentration octenyl succinic anhydride concentration lead to a decrease in D_(3,2) and PSD [49,50]. Hence, the higher the EOR the lower the D_(3,2) and PSD i.e., the higher the stability of the emulsion [49,50]. Thus, the lower particle size D_(3,2); and PSD enhance the structural stability of the nanoemulsions as the ACM is better able to adsorb at the oil/water interface of the nanoemulsion. This implies that the nanoemulsion formulated by encapsulation with ACM could be an alternative means of incorporating functional components into food-grade, personal care, and drug delivery products for health benefits.

3.2. Model Fit and Adequacy for the Stability of ACM Stabilized O/W-Type Nanoemulsion

The precise stability parameters for D_(3,2) obtained from the mixture design for determining the ACM stabilized O/W-type nanoemulsion formulation is presented in Table 1. The summary of the model statistics is shown in Table 2. The quadratic mixture model for D_(3,2) was significant (F [235.71, 0.13] = 1805.99; *p*-value < 0.0001) in describing the component effects (ACM, oil, and water) on D_(3,2) stability parameter (Table 2). There was only a 0.01% chance that a model F-value this large could occur due to noise. The model's lack of fit (F [0.17, 0.11] = 0.3104; *p* = 1.49) was not significant, and the predicted R-squared value was 0.9977 indicating a model with adequate goodness-of-fit (Table 2). All variables were fitted to a quadratic model, and the residual errors were calculated to determine the goodness of the model fit. All variables were fitted to a polynomial quadratic model, and the residual errors were calculated to determine the goodness of model fit. The normality of data was confirmed with the normality plot of the residuals adequacy tool. The straight line obtained from the plot indicated a normal distribution of residuals and showed that changes in transformation would not lead to any improvement of the analyzed data. Studies have shown that the Box-Cox plot, defined as “the natural log of the sum of the squares of the residuals against lambda,” indicates whether lambda should be transformed [8,18].

Table 2. Analysis of variance for the effect of the three variables of Sauter mean diameter $D_{(3,2)}$.

Source	Sum of Squares	df	Mean Square	F Value	p-Value Prob > F	Significance Level
Model	1178.55	5	235.71	1805.99	<0.0001	Significant
I_L	909.19	2	454.6	3483.07	<0.0001	
AB	32.44	1	32.44	248.53	<0.0001	
AC	11.06	1	11.06	84.76	<0.0001	
BC	26.97	1	26.97	206.65	<0.0001	
Residual	0.91	7	0.13			Not significant
Lack of fit	0.34	2	0.17	1.49	0.3104	
Pure error	0.57	5	0.11			
Cor total	1179.46	12				
R-squared	0.9992					
Adjusted R-squared	0.9987					
Predicted R-squared	0.9977					
Adequate precision	104.158					

In this case, the statistical tool did not recommend any transformation for the $D_{(3,2)}$ response variables of ACM stabilized O/W-type nanoemulsions. Hence, the model was found adequate to determine the effects of the three ingredients (ACM, oil, and water) on the precise stability parameters for particle size as a function of Sauter mean diameter $D_{(3,2)}$. Based on these values, the quadratic mixture model was found to be adequate in determining the effects of the three ingredients. The final equation in terms of actual components for $D_{(3,2)}$ is given by Equation (5).

$$\gamma_1 \text{ (BS flux\%)} = -267.43 * (\text{ACM}) + 6.48 * (\text{Oil}) + 0.64 * (\text{Water}) + 3.55 * (\text{ACM} * \text{Oil}) + 2.86 * (\text{ACM} * \text{Water}) - 0.12 * (\text{Oil} * \text{Water}) \quad (5)$$

The R^2 value for the $D_{(3,2)}$ model was 0.9992 and the closeness of this value to 1 makes it represent a good fit.

3.3. Effects of Mixture Components on Stability as a Function of Sauter Mean Diameter of ACM Stabilized O/W-Type Nanoemulsion

Figure 1 shows the Piepel trace and response surface plot for the effect of 3 components (A: ACM, B: oil, and C: water) on $D_{(3,2)}$. The observation of dominance of ACM concentration in the stability ($D_{(3,2)}$) as evidenced by higher RC values in the interactions of oil and water with ACM was confirmed in the Piepel trace plots as the stability (low $D_{(3,2)}$) was greatly influenced by ACM (Figure 1a). A curvature was observed in the Piepel trace plots for all independent variables ACM, oil, and water (Figure 1a). It was observed that as the concentration of ACM increased, the stability increased as $D_{(3,2)}$ values became lower also, a sharp curvature was observed as the concentration of ACM increased. This means that stability (low $D_{(3,2)}$) can be achieved at a certain concentration of mixture constituents and subsequent increases in the concentration of ACM would continually lower $D_{(3,2)}$ and increase stability as shown in Figure 1a. For the influence of water, the curvature was more pronounced forming a parabola shape curvature. This means that higher concentrations of water did not negatively impact the stability because the $D_{(3,2)}$ was low. This occurrence was confirmed with the high RC interaction value of ACM and water and is in line with the theory that water-solubility enhances HLB values and ensures the stability of O/W-type nanoemulsions. The curvature for oil was slight, hence, as the concentration of oil increased, the stability decreased as evidenced by high $D_{(3,2)}$ values. An extremely sensitive response by ACM (mucilage) shown by a sharp curvature effect on stability as a function of $D_{(3,2)}$ was the most dominant effect among the three mixture constituents consistent with [25]. The observation of dominance of ACM concentration in

the stability ($D_{(3,2)}$) as evidenced by higher RC values in the interactions of oil and water with ACM was confirmed in the response surface plots as the stability ($D_{(3,2)}$) was greatly influenced by ACM (Figure 1b). The actual stability- $D_{(3,2)}$ value obtained on the response surface plots (Figure 1b) was 6.6 nm which compares favorably with the value obtained as the average $D_{(3,2)}$ (6.7 ± 0.1 nm). The significance of a low $D_{(3,2)}$ is that the particles of the ACM stabilized O/W-type nanoemulsions are more tightly packed which makes them less susceptible to kinetic instability mechanisms. Hence, with the low $D_{(3,2)}$, the nanoemulsion takes a longer time to coalesce hence they remain stable. Although the $D_{(3,2)}$ is low, as a result of the adhesion of ACM at the oil and water interface, the creaming of the emulsion is avoided hence the nanoemulsion maintains stability. Literature supports the claim that emulsifiers reduce interfacial tension and reduce coalescence in oil droplets [54,55]. The response surface plots (Figure 1b) also showed that as the concentration of ACM increased, the stability increased as $D_{(3,2)}$ values became lower. The results obtained on the oil were that stability ($D_{(3,2)}$) decreased with increasing concentrations of oil (Figure 1b). In the case of water, the stability increased with increasing concentrations of water as $D_{(3,2)}$ values became lower. This means that the water-solubility of ACM was needed to ensure stability (low $D_{(3,2)}$ values). The effects of the three independent variables on stability ($D_{(3,2)}$) as shown in the Piepel trace plot (Figure 1a) agrees with the results obtained from the response surface (Figure 1b), where the stability (low $D_{(3,2)}$ values) is as a result of the concentration of ACM which influenced the ACM–emulsifier oil ratio (EOR). The result that ACM by showing a sharp curvature effect on the effect of three mixture components on stability as a function of $D_{(3,2)}$ values agrees with results reported by [25,56]. Hence, with the low $D_{(3,2)}$, the nanoemulsion takes a longer time to coalesce because the ACM makes it less susceptible to kinetic instability mechanisms hence they remain stable. Additionally, because the $D_{(3,2)}$ is low, as a result of the adhesion of ACM at the oil and water interface, the creaming of the emulsion is avoided hence the nanoemulsion maintains stability. Literature supports the claim that emulsifiers reduce interfacial tension and reduce coalescence in oil droplets [54,55]. The response surface plots (Figure 1b) also showed that as the concentration of ACM increased, the stability increased as $D_{(3,2)}$ values became lower. The results obtained on the oil were that stability ($D_{(3,2)}$) decreased with increasing concentrations of oil (Figure 1b). In the case of water, the stability increased with increasing concentrations of water as $D_{(3,2)}$ values became lower. This means that the water-solubility of ACM was needed to ensure stability (low $D_{(3,2)}$ values). The effects of the three independent variables on stability ($D_{(3,2)}$) as shown in the Piepel trace plot (Figure 1a) agrees with the results obtained from the response surface (Figure 1b), where the stability (low $D_{(3,2)}$ values) is as a result of the concentration of ACM which influenced the emulsifier–ACM oil ratio (EOR). The result that ACM by showing a sharp curvature effect on the effect of three mixture components on stability as a function of $D_{(3,2)}$ values agrees with results reported by [25,56].

Figure 1. (a) Trace (Piepel) plot and (b) response surface plot for the effect of three components (A: ACM/slime, B: oil, and C: water) on Sauter mean diameter.

3.4. Effects of Process Optimization for Stability of ACM Stabilized O/W-Type Nanoemulsion

The stability as a function of $D_{(3,2)}$ of the ACM stabilized O/W-type nanoemulsion obtained from all the observed responses from randomized runs in the D-optimal mixture design runs in the mixture design is given in experimental responses from randomized runs in the D-optimal mixture design (Table 1). The experimental responses (Table 1) were used to calculate the coefficients of the polynomial equation, subject to the degree of fit, predictive power, and robustness of the model, and the derived equation was then used to predict the optimized values of $D_{(3,2)}$ of the nanoemulsions runs in the D-optimal mixture design (Table 1). The experimental responses (Table 1) were used to calculate the coefficients of the polynomial equation, subject to the degree of fit, predictive power, and robustness of the model, and the derived equation was then used to predict the optimized values of $D_{(3,2)}$ of the nanoemulsions. Figure 2 gives the parity plot of predicted and experimental values of Sauter mean diameter of ACM O/W-type stabilized nanoemulsion. The predicted values fitted well with the experimental responses obtained from the RSM design as seen in Figure 2. The parity plot of the predicted value versus the experimental values for $D_{(3,2)}$ of ACM stabilized O/W-type nanoemulsion gave a good indication of a good agreement between predicted and experimental responses (Figure 2). It has been reported that a p -value lower than 0.05 is significant [26,57]. The p -value of the ANOVA results for the effect of the three independent variables on $D_{(3,2)}$ response was < 0.0001 which is lower than 0.05 and is significant (Table 2). The adequate signal-to-noise ratio for $D_{(3,2)}$ response was given as 104.158 (Table 2). Studies have reported that adequate prediction compares the range of the predicted values at the design points to the average prediction error and a ratio greater than 4 is desirable [58,59]. It can be observed that the signal-to-noise ratio for $D_{(3,2)}$ is much higher than 4 as it is 104.158. Hence, it can be inferred that the stability design space as a function of $D_{(3,2)}$ for ACM stabilized O/W-type nanoemulsion contains minor errors i.e., the experimental data set transmits more useful information about the developed stability model for $D_{(3,2)}$.

Figure 2. Parity plot of predicted and experimental values of Sauter mean diameter of ACM O/W-type stabilized nanoemulsion.

3.5. Optimization of Response

Final polynomial equations were obtained for the effect of BS% flux and particle size (Equation (5)) and the constituent was constrained with lower and upper limits. The 3 components in real scale amounted to 100% (1% to 5% for ACM, 3% to 10% for oil, and 85% to 96% for water), respectively listed in the design. The numerical optimization of an O/W-type nanoemulsion formulation was carried out according to the desirability function using the Design-Expert (Stat-Ease Inc., Minneapolis, MN, USA, version 10.0.7) statistical software. The research objective was to evaluate how the physicochemical properties of ACM affect its efficacy as an emulsifier in sunflower O/W-type nanoemulsion using ultrasonication to optimize the emulsion formation to provide the food-grade, cosmetics, drug delivery, and personal care industry with a low cost, eco-friendly alternative emulsifier. Desirability functions were chosen to either maximize BS% flux (γ_1) and minimize Sauter mean diameter (γ_2) or maximize BS% flux (γ_1) and maximize Sauter mean diameter (γ_2). This desirability function was set to answer sub-research objective questions on whether maximizing BS% flux (γ_1) and minimizing Sauter mean diameter (γ_2) or maximizing BS% flux (γ_1) and maximizing Sauter mean diameter (γ_2) would affect the desirability options. The sub-research objective questions were also set to enable choices to be made for suitable mixture constituents' composition to fulfil emulsifier roles in the cosmetics, drug delivery, and personal care industry.

Diverse nanoemulsion solutions representing different combinations of mixture constituents and independent variable levels were found by using the Design-Expert statistical software. These solutions were dependent on the degree to which the dependent variables BS flux (γ_1) and Sauter mean diameter (γ_2) were minimized and maximized. The overall desirability of the chosen formulation for option A (EOR 4.2:3) which involved maximizing BS% flux (γ_1) and minimizing Sauter mean diameter (γ_2) was 100%, which means that this formulation substantially satisfied the targeted constraints. Similarly, the overall desirability of the chosen formulation for option B (EOR 5:10) which involved maximizing BS flux (γ_1) and maximizing Sauter mean diameter (γ_2) was 95%, which means that this formulation substantially satisfied the targeted constraints. Authors have reported that O/W emulsions stabilized with octenyl succinic anhydride (OSA) starch having OSA starch concentration above 10% had drastically reduced creaming index values and stated that this

was due to network structure formation through inter-molecular bonding of OSA starch molecules [49,50]. Hence, the structure formed increased the viscosity of the continuous phase, slowed down oil droplet movement, and reduced the rate of creaming, which means that increase in OSA starch concentration led to an increase in the physical stability of the investigated emulsions [49,50]. The authors concluded that an increase in the concentration of oil in emulsions stabilized by OSA starch caused a significant delay of creaming [49,50]. Furthermore, the authors also reported that as oil concentration increased from 5% to 60% the apparent viscosity of the stabilized emulsions increased [49,50]. Similarly, the authors of [49,50] investigated the effect of oil concentration (10–50%) on the rheological, structural, and microstructural properties of ethanol-induced whey protein stabilized soy-bean oil emulsions: the authors observed that stabilized emulsions with higher oil concentration exhibited a higher degree of shear-thinning as the apparent shear viscosity decreased with increasing shear rate. The authors concluded that the shear-thinning displayed was consistent with the rheological properties of colloidal dispersions containing aggregated particles [49,50]. The result obtained in our investigation i.e., maximizing ACM and oil concentration (EOR) could enhance microstructural stability of the emulsion by reducing creaming and increasing viscosity, consistent with the literature [49,50].

3.6. Structural Characteristics of O/W-Type Nanoemulsion Formulation

The Cryo-TEM micrographs of the ACM stabilized O/W-type nanoemulsion showed that ACM encapsulated the O/W-type nanoemulsion and formed a consistent aggregate structure (Figure 3). Cryo-Tem images revealed that ACM encapsulated the micelles at oil/water interfaces of the nanoemulsion to ensure stability (Figure 3). This was evidenced by the smaller particle sizes of nanoemulsions with higher ACM-EOR (Figure 3). Authors have reported that emulsifiers adsorb at the oil and water interface to enable dispersion of oil droplets in water [60,61]. Additionally, emulsifiers adsorb more to the surface of smaller droplets to ensure that the emulsion is stable [61]. Authors have reported that an emulsion can attain stability when its particle size is reduced [8,17,62]. The literature established that the lower the particle size the more stable an emulsion becomes [8,18]. Hence, it can be inferred that ACM encapsulated the oil/water interfaces of the nanoemulsion to ensure stability as depicted by lower particle size.

As a result of the mucoadhesive nature of ACM and the microstructural stability of the nanoemulsion formulated, it could be used in the controlled release of drugs by reducing the speed of drug absorption by the body. Therefore, the structural stability achieved in O/W nanoemulsions can be employed in biomedical and personal care applications.

Figure 3. Structural characteristics of ACM stabilized O/W-type nanoemulsions with emulsifier–ACM oil ratios of 1% (a–d), midpoints (e–g), and 5% (h–k).

4. Conclusions

African catfish mucilage (ACM) was extracted from African catfish and used to encapsulate O/W nanoemulsions. D-optimal mixture design was used to describe the synergy between mixture components ACM, oil, and water on particle size and optimize the stability of ACM stabilized O/W-type nanoemulsion formulations for cosmetic, personal care, and drug delivery products. Piepel trace plots showed that stability (low particle size) increased as ACM-EOR increased but started to reduce at certain ACM-EOR concentrations as evidenced by curvature. The quadratic mixture model for particle size was significant ($F [235.71, 0.13] = 1805.99$; p -value < 0.0001). The model's lack of fit ($F [0.17, 0.11] = 0.3104$; $p = 1.49$) was not significant, and the predicted R-squared value was 0.9977 and adequate precision was 104.158 indicating a model with adequate goodness-of-fit. The study determined that the higher the concentration of the ACM the more stable the emulsion. Hence, the emulsifier oil ratio influences the stability of the emulsion. This study becomes significant as ACM encapsulated nanoemulsion as a result of the mucoadhesive nature of the ACM. Hence, the microstructural stability attained in the formulated stable nanoemulsions could be applied to incorporate functional components into cosmetics, personal care, and drug delivery products for health benefits as the African catfish mucilage can be applied as a natural emulsifier.

5. Patents

This work is being considered for a patent.

Author Contributions: A.O.O., D.I.I.-O., V.A.J. and S.K.N. designed the experiment; A.O.O. performed the characterization and stability experiments and drafted the manuscript; D.I.I.-O., V.A.J. and, S.K.N. contributed research materials and provided necessary inputs for the revision of the manuscript as research advisors. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the Guidelines for Ethical Conduct in the Care and Use of Nonhuman Animals in Research (protocol code Record number: 6e360 (legacy id: 15205) and the Institutional Ethics Committee of the Faculty of Engineering ethical committee of Cape peninsula University of Technology and was approved on 15 January 2018.

Informed Consent Statement: The study did not involve humans.

Data Availability Statement: The data is available on the Cape Peninsula University of Technology institutional MediaTum and Figshare platforms but the data is being held because the project is being considered for a patent.

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