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Development of cottonseed-based emulsions for encapsulation of vitamin A

Madiha IFTIKHAR[1,2], Maria MUNIR[3], WAJEEHA[4], Muhammad Rizwan TARIQ[5]*, Shinawar Waseem ALI[5], Muhammad SHAFIQ[6], Sajid ALI[7], Zulfiqar AHMAD[8], Amna HAMEED[2], Waseem SAFDAR[9], Anwar AHMAD[1], Zujaja UMER[5], Maham KHALID[10], Munawar IQBAL[11]

Abstract

Nanoemulsions are nanoscale self-assembled entities which are manufactured for improving the delivery of active pharmaceutical ingredients. Nanoemulsion technology is one of the best ways to encapsulate the functional components as it enhances the stability and bioavailability of the compound. In the present study, nanoemulsions were prepared by ultrasonic homogenization of dispersed phase (10-22%) and surfactants (3-9%) with vitamin A (0.25-2.5%). Nanoemulsions were characterized through various optical techniques and stability was assessed using thermal shock and freeze-thaw cycle. The results revealed that nanoemulsions with high percentage of surfactants were more stable and retained smaller particle size as compared to nanoemulsions with low surfactants percentage and high dispersed phase, contributed to phase separation and colour change. These emulsions can be further tested for any toxic/allergic effect on the biological model and may be incorporated into food products.

Keywords: nanoemulsions; encapsulation; emulsion stability; vitamin A; surfactant.

Practical Application: Food industries frequently demand supplements that are manufactured from the natural sources. Food safety authorities are concerned regarding the use of synthetic ingredients due to their several toxicological effects. Therefore, production of supplements from natural sources using novel technologies is a commendable way to meet industrial requirements. Cotton seed oil possesses various functional properties that makes it a best fit for development of trans-free products. On the other hand, Vitamin A is highly sensitive to environmental conditions such as high temperature and its exposure time. The preliminary cause of fat-soluble vitamin loss especially the vitamin A is the presence of moisture and pro-oxidants i.e. iron, copper, and manganese hence, encapsulation is the best way to prevent Vitamin A.

1 Introduction

The Food industries tend toward supplementing the food products while nanoemulsions deliver the oil soluble micronutrients gradually along with prolonged physical stability, enhanced mouth feel, and faster flavor release (McClements, 2011). The nanoemulsions used for food additives delivery such as colors, flavors, and vitamins, have gained substantial interest in the food sciences (McClements et al., 2007). Some of these additives have the potential for industrial application due to their hydrophobic nature and ability to protect against environmental damage (Ozturk et al., 2015; Rao & McClements, 2012). Nanoemulsions are kinetically unstable but thermodynamically stable, self-assembled isotropic colloidal dispersions with applications in the food, cosmetic, pharmaceutical, and petrochemical industry (Kumar et al., 2011).

Food grade and non-food grade nanoemulsions differ due to their composition i.e. surfactant and oil. The food industry prefers nanoemulsion due to its unmistakable appearance and ease of preparation, which promotes product acceptance, enhanced bioavailability, and longer shelf life (McClements et al., 2007). Nanoemulsions retain opacity and turbidity of water and other transparent drinks because of less light scattering ability (Mason et al., 2007; Velikov & Pelan, 2008; Wooster et al., 2008).

Humans require vitamin A (retinol) for the normal functioning of the visual system. Retinol is transported to ocular tissue and the eye's retina by intracellular binding and transport protein, which plays an essential part in the formation of rhodopsin, an essential visual pigment, particularly for dim-light vision. Moreover, vitamin A helps to maintain growth, epithelial cellular integrity, and immune function in the body (Gonnet et al., 2010). Thus, in vitamin A loss, the number of goblet cells is reduced in epithelial tissues, reducing mucous secretions (Rosenberg,

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[1] *Department of Food Technology, Institute of Food and Nutritional Sciences, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi, Pakistan*
[2] *Department of Diet & Nutritional Sciences, Ibadat International University, Islamabad, Pakistan*
[3] *Faisalabad Medical University, Faisalabad, Pakistan*
[4] *University Medical and Dental College, Faisalabad, Pakistan*
[5] *Department of Food Sciences, University of the Punjab, Lahore, Pakistan*
[6] *Department of Horticulture, University of the Punjab, Lahore, Pakistan*
[7] *Department of Agronomy, University of the Punjab, Lahore, Pakistan*
[8] *Department of Food Science and Technology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*
[9] *Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan*
[10] *Punjab University College of Pharmacy, Lahore, Pakistan*
[11] *College of Statistical Sciences, University of the Punjab, Lahore, Pakistan*
*Corresponding author: rizwan.foodsciences@pu.edu.pk

1961). Vitamin A is of great significance because of its potential utilization during processing however prolonged exposure to high temperature causes degradation of this essential vitamin (Chen, 2016). Nanoemulsions with high diffusion rate and shelf stability are designed to cope with such challenges (Mason et al., 2007).

In the present study, cottonseed-based nano emulsions are developed with the incorporation of Vitamin A acetate as a functional ingredient for improving health. Cottonseed oil depicts health-promoting nutritional qualities. Cotton seed oil is an excellent source of oleic acid- 22%, linoleic acid- 52%, and linolenic acid-1% (O'Brien & Wakelyn, 2005). The current study is designed to develop food grade nanoemulsions and to evaluate their thermal stability and vitamin retention properties.

2 Materials and methods

2.1 Material procurement

Cotton seed oil was procured from Punjab Oil Mills, Pakistan whereas Vitamin A (B.A.S.F, German) was acquired from S.B pharma. The deionized water required for all experiments was provided by Laboratory of National University of Medical sciences, Rawalpindi, Pakistan.

2.2 Preparation of nanoemulsion

Vitamin A (0.25-2.5%) was effectively mixed with cotton seed oil in the the dispersed phase. The aqueous phase and the surfactant "tween 80" was taken in a concentration less than 4% and blended in continues phase. The samples were prepared by using test tubes possessing screw caps carrying 30 s vortex. These were allowed to equilibrate in a water bath for further analysis. The nanoemulsions were prepared using Ultrasonic Homogenizer (E.W.- 04711-70 Cole-Parmer ultrasonic homogenizer, U.S.A.). Coarse particles were formed initially and afterward, leading to formation of stable nanoemulsions.

2.3 Selection of suitable continuous phase ratio and homogenization time

The different proportions of the continuous and dispersed phases were mixed for 15-20 minutes to attain a suitable proportion for forming nanoemulsions as shown in Table 1. The proper proportions for continuous and dispersed phases were selected based on particle size and the stability against phase separation. The preferred treatments (T1 to T4) were subjected to stirring for three minutes. Later, these treatments were homogenized by an ultrasonic homogenizer (EW-04711-70 Cole-Parmer ultrasonic homogenizer, U.S.A.) for varying lengths of time (1-6 minutes).

2.4 Effect of homogenization time on temperature

Four peculiar treatments were formulated, possessing 90% continuous phase and 10% dispersed phase, for evaluating the effect of homogenization time on the temperature. These treatments were subjected to ultrasonic homogenization for variant time lengths. It was followed by observing and evaluating the temperature for each time interval.

2.5 Selection of suitable surfactant ratio

The dispersed Phase and continuous Phase were mixed to assist the desired surfactant ratio as shown in the Table 2. In the beginning, vitamin A (0.25-2.5%) was effectively mixed in the cotton seed oil during the dispersed phase. The aqueous phase and the surfactant "Tween-80" that was taken in different concentrations were blended in a continuous phase. Nanoemulsions were prepared by combining 90% continuous and 10% dispersed phases.

2.6 Homogenization effect on temperature and vitamin retention

Continuous Phase of 90% and the dispersed Phase of 10% were used to determine the effect of homogenization on temperature and vitamin retention. The treatments were further subjected to the ultrasonic homogenization for different intervals as shown in the Table 3.

2.7 Particle size measurement

The Particle size was determined prior to using Perkin Elmer Lambda 25 UV-VIS Spectrometer. Deionized water was used to dilute the samples to achieve maximum clarity at the ratio of 0.5 mL of the purified sample and 16 mL of deionized water. Only the samples with a particle size ≤ 100 nm were chosen for the formation of phase diagram. Characterization for thermal stability, palpable firmness under freeze and thaw cycles, viscosity, and storage endurance was performed for its stability test.

2.8 Characterization techniques

The characterizations of cotton seed oil-based nanoemulsion were carried out by visual observations and evaluation of thermal stability, palpable firmness under freeze-thaw cycles, viscosity, and storage endurance.

2.9 Visual observation

The O/W nanoemulsions were examined on a regular basis to estimate phase separation and pellucidity for a period of one

Table 1. Continuous phase ratio selection treatments.

Treatments	Dispersed Phase	Continuous Phase	Time (Min.)
T1	10	90	15
T2	14	86	15
T3	18	82	15
T4	22	78	15

Table 2. Surfactant ratio selection.

Treatment code	Dispersed phase (%)	Cont. Phase (%)	
		Water %	Surfactant %
W1	10	81	9
W2	10	83	7
W3	10	85	5
W4	10	87	3

Table 3. Treatments for determining the homogenization time effect on temperature.

Treatments	Dispersed phase (10%)		Continuous phase (90%)		Homogenization (Min)
	Cottonseed oil %	Vitamin A %	Deionized Water %	Tween 80%	
V_1	9	1	86	4	4
V_2	9	1	86	4	7
V_3	9	1	86	4	10
V_4	9	1	86	4	13

Table 4. Particle size of treatments for the selection of continuous phase ratio.

Treatments	Dispersed Phase	Continuous Phase	Time (Min.)	Particle Size in nm
T1	10	90	15	195
T2	14	86	15	200
T3	18	82	15	230
T4	22	78	15	250

month. The samples were selected on the basis of stability to phase separation (Edris & Malone, 2012).

2.10 Turbidity measurement

The turbidity of Nano-emulsified samples was estimated through UV-Vis spectrophotometer at 600 nm for 30 days (S-200D) (McClements, 2004).

2.11 Thermal stability

The thermal stability of nanoemulsion samples was determined by executing thermal stress (40-70 °C) in the oven. The temperature was elevated by 10 °C per day. The meticulousness of the thermostat was ± 2 °C. The experiment procedure was initiated by maintaining the temperature of the void oven at 40 °C for a day. Ultimately, the vapor pressure was cumulated in storage vials (Edris & Malone, 2012).

2.12 Palpable firmness under freeze and thaw cycles

All the treatments were inspected against alternate freeze-thaw cycles (12 h) for an interval of one week. The samples were subjected to freezing temperature (4 °C) for about 12 hours followed by storage at room temperature (25 °C) for additional 12 hours to achieve thawing and retained at room temperature for one week in order to calibrate these samples. The methods of Edris & Malone (2012) were considered for performing visual observation to determine stability.

2.13 Viscosity measurements

Viscosity of Nano-emulsions was investigated as described by Berry (2011), Vibrio viscometer (S.V. Series Viscometer 640SV10) was used at operating conditions of 30 Hz, < 1 mm amplitude, and temperature of 25 ± 0.5 °C.

2.14 Statistical analysis

The experimental data was statistically analysed using ANOVA, regression, and correlation at 95%. The analysis was carried out using statistical software Minitab 16 (Minitab Inc, U.S.A.), according to the methods of Steel et al. (1997). All the deduced experimental results were recorded as arithmetic means ± SD, and each one of the measurements was carried out and counter-checked nearly three times.

3 Results and discussion

3.1 Continuous phase ratio and time selection for homogenization of oil in water nanoemulsion

Particle size as a parameter for continuous phase ratio selection

The statistical analysis by the equation Y = 628.3 – 4.875X (Y represents the particle size and X denotes the continuous phase) proved that with every change in the ratio of the continuous phase, there was a decrease in particle size at the rate of 4.875.

The values of the dispersed phase (10%) and the continuous Phase (90%) of the treatment T1 reduced the particle size to 195 nm. In comparison, the treatment T4 with the dispersed Phase (22%) and continuous Phase (78%) resulted in the increased particle size (250 nm) as shown in Table 4. The particle size decreased with the increase in the continuous phase ratio. Interfacial tension is lowered by using a high proportion of continuous phases, ultimately lowering particle size (Jafari et al., 2008).

Homogenization time selection

The effect of independent variable X (homogenization time) and dependent variable Y (particle size) of the treatment T1 (Table 5) is elaborated by the equation Y = 287.0 – 6.500 X. With every unit increase in time, there was a decrease in the size by 6.500 times. The possible reason for the decrease in the particle size was the

Table 5. Effect of homogenization time on particle size.

Treatments	Dispersed Phase	Continuous Phase	Homogenization	Particle Size
	Percentage	Percentage	Time (Min)	(nm)
T_1	10	90	15	195
T_2	10	90	12	200
T_3	10	90	9	230
T_4	10	90	6	250

Table 6. Effect of surfactant ratio on particle size.

Treatments	Dispersed Phase (10%)		Continuous Phase (90%)		Particle Size
	oil %	Vitamin A %	DI water	Tween 80%	(nm)
W1	9	1	81	9	195
W2	9	1	83	7	200
W3	9	1	85	5	230
W4	9	1	87	3	250

DI water: Deionized Water.

homogenizer-generated cavitation and the shear force (Cheong et al., 2008). The regression model showed that the analysis of variance was significant, and the negative correlation (r = - 0.970) proved that the increase in time decreased the particle size.

3.2 Surfactant ratio selection

Using the variance analysis (ANOVA), the variable Y decreased by 8.350 times, as shown in the equation; Y = 275.8 – 8.250 X. Here, Y is the particle size, and X is the surfactant ratio. The particle size starts decreasing by increasing the amount of surfactant. Hence the W1 (Table 6) was selected as the resulting particle size of the emulsions was the smallest among all samples. The available surfactant covered small particles (newly formed) and ultimately showed a decrease in size (Jafari et al., 2008). The particle size of the emulsion is negatively correlated i.e. r = -0.997, with the surfactant concentration.

3.3 Effect of homogenization time on temperature and vitamin retention

Effect of homogenization on temperature

The fitted equation for the linear model describes the relationship between Y (temperature) and X (homogenization time): Y = 19.43 + 2.567 X. With every unit increase in the homogenization time, there is an increase in the temperature by 2.567 times. The result indicated that by increasing homogenization time, the temperature increases. The rise of temperature is the transfer of more mechanical energy with the increase in time. Table 7 shows that the homogenization time and temperature are correlated (r = 0.999). When homogenization is done for a long time, the transfer of mechanical energy causes an increase in temperature (McClements, 2004). This experiment showed the maximum variation in the temperature up to 99.9%.

Effect on the vitamin retention

Vitamin A is very sensitive to high temperature and causes its deterioration. The total homogenization time affected vitamin retention, which is given in Table 7. With an increase in the homogenization time, vitamin retention was decreased. The regression equation Y = 103.2 – 1.700 X justifies the statement as the increase in homogenization time vitamin retention decreased with the rate of 1.700. Hence, the mechanical energy is increased over time, resulting in high temperatures. Ultimately, Vitamin A destruction occurs (Lee et al., 2004). The homogenization time increases the system's temperature, which may destroy vitamin content structure. The decrease in the overall contents of vitamin A. r = - 0.997 show that the homogenization time and vitamin retention are negatively correlated. By lengthening the homogenization process, the temperature of the system rises, which causes the destruction of vitamin A.

Table 7. Vitamin retention and temperature affected by homogenization.

Treatments	Homogenization Time (Min)	Temperature	Vitamin Retention
V1	4	30	96
V2	7	37	92
V3	10	45	86
V4	13	53	81

3.4 Nanoemulsion characterization

Palpable firmness under freeze and thaw cycle

Table 8 shows the stability status of the samples. All treatments showed stability for two weeks, but S_1 and S_2 were unstable in the third and fourth weeks, while S_3 and S_4 were stable in four weeks. The instability of these samples was the result of a lower surfactant amount. Less amount of surfactant makes some of the particles of the sample less or not bound to the surfactant, hence becoming unstable (Charlton & Doherty, 2001)

Thermal stability

The thermal stability of the prepared nanoemulsions is already shown in Table 9. All samples were stable till 60 °C. T4 exhibited

Table 8. Palpable firmness under freeze and thaw cycle.

Treatments	Stability			
	Week 1	Week 2	Week 3	Week 4
S_1	Stable	Stable	Stable	Stable
S_2	Stable	Stable	Stable	Stable
S_3	Stable	Stable	Cloudy	Yellow
S_4	Stable	Stable	Yellow	Phase separation

Table 9. The stability of nanoemulsions under thermal stress.

Storage	Storage	Total	Storage	Effect of Thermal Stress			
(Days)	(hrs)	Storage (hrs)	Temp.	T1	T2	T3	T4
1	24	24	40	No Effect	No Effect	No Effect	No Effect
2	24	48	50	No Effect	No Effect	No Effect	No Effect
3	24	72	60	No Effect	No Effect	No Effect	No Effect
4	24	96	70	No Effect	No Effect	No Effect	Cloudy

instability by the rise of temperature, and the destabilized samples can be reversed at room temperature (Streletzky & Phillies, 1995; Charlton & Doherty, 2001).

The higher proportion of Tween 80 in other samples showed stability up to 70 °C because the non-ionic surfactant showed its effect against the phase inversion. Hence, the nanoemulsions containing a higher proportion of surfactant showed thermal stability (Martino & Kaler, 1990).

Measurement of viscosity

The rise in temperature causes changes in the continuous phase and dispersed phase viscosity of samples 3 and 4, as depicted in Table 10. The viscosity of sample 3 was 1.28 at 20 °C; the viscosity was changed to 1.23 when 10 °C was raised. As the system's temperature rise by 10 °C; viscosity decreases to 1.20. Similarly, the decreasing trend was seen by further increasing temperature from 50 °C to 60 °C; viscosity was decreased from 1.20 to 1.15.

When sample 4 was tested for viscosity, a different trend was seen as the viscosity at 20 °C was 1.05 but increased temperature to 30 °C increased the viscosity to 1.2. Further increasing temperature by 10 °C, the viscosity of nanoemulsion was increased up to 1.8. A different trend was seen when a further rise in temperature decreased the viscosity up to 1.08.

The droplet size depended on the viscosity of the dispersed phase to the continuous phase during the functioning of an ultrasonic homogenizer. Tiny droplets were produced during processing when the viscosity value was nearer to the unity (Lee et al., 2004).

A significant change was observed in the viscosity of the continuous and dispersed phase with the increase in temperature. It indicates the dependence of relative viscosity on the temperature.

Table 10. Effect of temperature on viscosity.

Temperature °C	Viscosity (B3) mPa	Viscosity (B4) mPa
20	1.28 ± 0.01	1.05 ± 0.04
30	1.23 ± 0.04	1.2 ± 0.04
40	1.20 ± 0.06	1.3 ± 0.02
50	1.15 ± 0.04	1.08 ± 0.02

Storage endurance and retention of vitamin A

The storage stability and vitamin A retention was assessed for one month. All samples showed stability, but T4 became unstable after 21 days and lost its transparency. Instability was reduced by increasing the surfactant amount; therefore, the increase in cottonseed oil amount caused further instability. Sample (T1) was the best as it showed highest stability than other samples as the particle size increased when the storage time was increased. Significant effect of storage time and the treatments was noted on the retention of Vitamin A. Emulsions were stored at room temperature for 30 days to check the vitamin stability. The results indicated that treatments and storage time significantly affected the vitamin retention of oil in water nanoemulsions. Similarly, an interaction between treatments and storage time was found to be significant during one-month storage study. After 30 days of storage, 90% retention was observed in emulsion samples stored at the temperature of 4 °C while the retention of vitamin A was 87% in emulsion samples stored at 25 °C.

4 Conclusion

The O/W nanoemulsions using cottonseed oil were developed through ultrasonic homogenization as an effective delivery system for nutraceuticals and food additives. The nano emulsion prepared using 90% continues phase, 10% dispersed phase

and 4% surfactant was most stable through the study period. The optimum condition for emulsion preparation was 3 minutes followed by 15 minutes of processing. Ultrasonic homogenizer increases the temperature gradually, that can be maintained using ice bath. Food additives patterns for the nanoemulsion release require further studies by the use of different surfactants and other cooking oils.

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