J Nanostruct 10(2): 268-278, Spring 2020

RESEARCH PAPER

Storage Stability of Wheat Germ Oil Encapsulated within Nanostructured Lipid Carriers

Mojgan Mirtalebi [1], Ahmad Rajaei [1], Manochehre Bahmaei [2], and Ahmad Yari Khosroushahi [3]**

[1] Department of Food Science and Technology, Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran

[2] Faculty of Chemistry, North Tehran Branch, Islamic Azad University, Tehran, Iran

[3] Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article History:
Received 17 December 2019
Accepted 06 February 2020
Published 01 April 2020

Keywords:
Lipid oxidation
Nanostructured lipid carriers
Poloxamer
Sodium dodecyl sulfate
Wheat germ oil

ABSTRACT

The present study aimed to evaluate the effect of surfactant composition on the physical properties of nanostructured lipid carriers (NLCs) containing wheat germ oil (WGO) and to investigate the influence of both surfactant composition and pH on the oxidative stability of WGO encapsulated within the NLCs. The results showed that the smallest particle size (52.7 nm) was related to the NLC with the poloxamer-to-lipid ratio of 1:1 (Polox-NLC-1). Polox-NLC-1 not only showed good stability during storage, but also indicated a suitable physical structure from differential scanning calorimetry (DSC) analysis. The oxidative stability results indicated that the NLCs were more successful than O/W emulsion in protecting the WGO from oxidation. Additionally, the oxidative stability of the NLC with the poloxamer-to-lipid ratio of 2:1 (Polox-NLC-2) looked promising. Furthermore, NLCs prepared with the surfactant of poloxamer as a non-ionic surfactant had greater oxidative stability at high pH, and NLC prepared with sodium dodecyl sulfate (SDS) as an ionic surfactant had greater oxidative stability at low pH. These findings indicated that NLC could be an effective delivery and protection system for the WGO as a source of bioactive compounds.

How to cite this article
Mirtalebi M, Rajaeia A, Bahmaei M , Yari Khosroushahi A. Storage Stability of Wheat Germ Oil Encapsulated within Nanostructured Lipid Carriers. J Nanostruct, 2020; 10(2):268-278. DOI: 10.22052/JNS.2020.02.007

INTRODUCTION

Nowadays there is a dramatic increase in the use of bioactive compounds in the food, pharmaceutical, and cosmetics industry. Products containing bioactive compounds such as ω-3 polyunsaturated oils (PUFAs), oil-soluble vitamins, phytosterols, and carotenoids possess certain health benefits and potential to decrease the risk of some diseases [1,2]. Wheat germ oil (WGO) obtained from wheat germ which is a by-product of wheat milling [3] is a well-known source of bioactive compounds such as tocopherols, phytosterols, policosanols, carotenoids , thiamin, and riboflavin [4]. This oil is also rich in polyunsaturated fatty acids (PUFAs). The use of WGO in the formulation of food, pharmaceutical, and cosmetic products as entire form possesses the limitation due to its lipophilic nature which cannot simply be dispersed directly into a polar phase, and its chemically unstable which can easily be oxidized [5]. Colloidal delivery systems have great potential for overcoming the challenges associated with using WGO [6].

Lipid-based carriers can be used for encapsulation and delivery of hydrophobic

* *Corresponding Author Email: ahmadrajaei@gmail.com*
Yarikhosroushahia@tbzmed.ac.ir

compounds in food, pharmaceutical, and cosmetic industries [7]. Lipid carriers include three groups of solid lipid nanoparticles (SLNs) with a solid lipid phase, emulsions with a fluid lipid phase and nanostructured lipid carriers (NLC) with a combination of solid and liquid lipids [8]. NLCs which are composed of a mixture of solid lipid and bioactive-containing liquid oil may be of great interest for encapsulation of unstable bioactive compounds [9]. The lipid carriers can enhance the chemical stability of bioactive compounds through reducing interactions with reactive agents at the interface or surrounding aqueous phase [10]. NLCs possess the physiological benefits of enhancing the bioavailability of the bioactive ingredient and enabling its sustained release as well the chemical stability of compounds sensitive to light, oxidation, and hydrolysis [6,11]. NLCs may be modified through the method of preparation or by altering components which can undergo the NLC qualities and application [12].

With the considering the enhanced effect of the water–oil interface in lipid oxidation, bulk oil oxidation is mechanistically different from the emulsified oil in oxidation process so various factors like free fatty acids content, pH, the polarity of the aqueous phase, antioxidant content and lipid droplet properties have a considerable effect on the vulnerable emulsified lipid [13]. The Previous studies verified the results which revealed the significant differences in the oxidative stability of emulsions, SLNs and NLCs as well the bulk lipid [6,7,10,12]. Recently, NLCs containing PUFAs have been prepared and findings showed significant protective effects for PUFAs against oxidization by using this encapsulation method [12].

To the best of our knowledge, although the microencapsulation method was used to increase the storage stability of WGO [2,14], there is no study on encapsulating WGO in NLCs. Moreover, there is limited understanding of the effect of both type and concentration of surfactant as well as pH on the oxidative stability of NLCs incorporating PUFAs. Therefore, the present study was set (i) to encapsulate the WGO within NLC, (ii) to investigate effect of type (ionic and non-ionic) and concentration of surfactant on the thermal behavior of NLCs and (iii) to study the effect of both type and concentration of surfactant as well as pH on the oxidative stability of NLCs incorporating WGO.

MATERIAL AND METHODS

Materials

Wheat germs were provided from the wheat milling company of Ettehad-e-Karaj Flour (Iran). Crude sunflower oil was obtained from Ghoo Oil Company (Iran). Tween 80, lecithin, span 40, sodium dodecyl sulfate (SDS), poloxamer 407 and percirol ATO5 (glyceryl palmitostearate) were provided from Sigma-Aldrich (Sigma–Aldrich, Steinheim, Germany). Chloroform, methanol, ammonium thiocyanate, Iron (II) and cumene hydroperoxide were purchased from Merck (Merck, Darmstadt, Germany). All materials were used without further purification. Also, ultra-pure water was used throughout the study.

Physicochemical properties of WGO

The WGO was extracted from the wheat germs according to the AOCS Official methods using n-hexane as a solvent [15]. The WGO was then stored at -18 °C until analysis. The fatty acid composition of WGO was determined by gas chromatography (GC) (Agilent Technologies, Palo Alto, CA, U.S.A.) equipped with a flame ionization detector and an SP-2380 column (60 m ×0.25 mm i.d., 0.20 µm film thickness, Supelco, Bellefonte, PA, U.S.A.) using helium as a carrier gas. Tocopherol composition of WGO was measured by high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) using AOCS Ce 8-89 method [15]. AOCS Cd1-25 method was used to determine iodine value [15]. The oxidative stability of WGO and crude sunflower oil as a reference was evaluated using a 743 Rancimat™ analyzer (Metrohm AG, Herisau, Switzerland). In brief, three grams of WGO and crude sunflower oil were weighed in a reaction vessel, and the reaction vessel was placed in a preheated heating block at 120 °C with airflow set at 20 l/h [16]. A differential scanning calorimeter (Metter Toledo, Switzerland) was used to determine the melting and crystallization behavior of the WGO. In this test, 16 mg of WGO was placed in an aluminum pan and hermetically sealed, and an empty pan was used as a reference. The WGO was heated from -75 to 50◦C at 5◦C/min and then cooled down to -75◦C at 5◦C/min.

Preparation of lipid particles

The ultrasound method was used to prepare the different lipid particle systems. First, solid lipid (percirol ATO5) was fully melted in a water bath at

85 ºC and the heated WGO (85 ºC) was dispersed in the solid lipid by stirring. Next, the different hot surfactant solutions (85 ºC) were added to the hot lipid mixture. The lipid and aqueous phases were sonicated at 65 ºC for 10 minutes using a Sonics VCX-400 sonicator (Sonics & Materials Inc., Newtown, Conn., U.S.A.). Finally, the samples were sealed and cooled at ambient temperature for lipid solidification and recrystallization to form NLC dispersions. In addition, an O/W emulsion was prepared to be compared with the NLCs in terms of thermal behavior and oxidative stability. For emulsion formula, lipid phase was only composed of the WGO. Moreover, the surfactant (poloxamer 407) to lipid phase ratio of 1:1 was used in the O/W emulsion.

Determination of particle size, zeta potential and NLC stability

The particle size parameters of lipid particles such as hydrodynamic diameters (z-average), and polydispersity index (PDI) of each dispersion were determined by the dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments Ltd.,Worcestershire, U.K.), at a scattering angle of 90^{o} and at a temperature of 25 ºC. To prevent multiple scattering effects and to achieve an adequate scattering intensity prior to the measurement, the dispersion samples were diluted in deionized water then were subjected to analysis. The particle size analysis using intensity distribution is reported as the mean hydrodynamic diameter (z-average) based on Stokes–Einstein equation, and the polydispersity index (PDI) ranging from 0 (monodisperse) to 1.0 (very broad distribution), was calculated based on three individual measurements.

The electrophoretic mobility (zeta potential) of the NLCs incorporating WGO was measured using the same device by the electrophoretic light scattering procedure. Before zeta potential measurements, the samples were diluted 1:100 with sodium phosphate buffer (10 mM, pH 7) and placed in a capillary cell (DTS 1070, Malvern Instruments, Malvern, U.K.). The zeta potential was calculated based on the Helmholtz–Smoluchowski equation.

To evaluate the physical stability during storage, the NLC particle size (only sample with the smallest particle size) stored at 25 ºC in darkness was determined after 15 days from the production time.

Thermal analysis of lipid particles

Differential scanning calorimetry (DSC) analysis of lipid particles was performed using a differential scanning calorimeter (Metter Toledo, Switzerland). For DSC measurement, 4-6 mg of the dried lipid particles were placed in an aluminum pan and hermetically sealed as well an empty pan was used as a reference. In following, the samples were heated from -70 to 70◦C at 5◦C/min and then cooled down to -70◦C at 5◦C/min.

Oxidative stability

The formation of lipid hydroperoxides in the different NLCs and the O/W emulsion incorporating the WGO at three pH of 3, 5 and 8 at 45 °C during 35 days of storage was determined [6]. In brief, first, the samples were added to 9.8 ml chloroform-methanol (7+3, v/v) and mixed for 5s on a vortex mixer. Then, ammonium thiocyanate solution (50µL) was added to the mixture. In the following, 50µL iron (II) solution was added. After 5 min incubation at room temperature, the absorbance of samples was measured at 500 nm against a blank by a spectrophotometer (Milton Roy Company, Rochester, NY, USA). The amount of lipid hydroperoxides was calculated using an external standard curve made of cumene hydroperoxide.

Statistical analysis

Means and standard deviations (SD) were calculated using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). The lipid oxidation results were analyzed with a one-way ANOVA and Duncan posthoc test using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Differences at $p \leq 0.05$ were considered as significance.

RESULTS AND DISCUSSION

Physicochemical properties of WGO

As Table S1 shows, the total unsaturated and saturated fatty acids of WGO were approximately 81.97 and 17.89%, respectively. The most abundant saturated fatty acid was palmitic acid, which was 95% of the saturated fatty acids. In addition, linoleic acid (18:2 n6) as an essential fatty acid formed 56% of the fatty acid composition. These results are in good agreement with the previous study [17]. It is worth mentioning that the WGO is prone to oxidation and degradation due to high amount of linoleic acid [18].

As Table S1 presents, the WGO had a high

content of tocopherols up to approximately 3319 (mg/kg oil), consisting of α and β-tocopherol. These results are in good agreement with the previous studies [19]. Earlier studies have shown that tocopherols are potent antioxidants [22]. The oxidative stability of WGO was measured with the rancimat test to determine whether this level of tocopherols could prevent enough oxidation. In addition, the oxidative stability of crude sunflower oil (possessing an iodine value quite close to the iodine value of WGO) was measured with the rancimat method to better understand the oxidative stability of WGO. The rancimat test results showed that the oxidative stability of crude sunflower oil (4.5 h) was higher than the oxidative stability of WGO (3.8 h) (Table S1). These results correspond with the previous studies [5,22].

In addition, the thermal behavior of WGO investigated by DSC exhibited two exothermic peaks at -43.3 °C and -19.5 °C (Fig. 1a) and two superimposed endothermic peaks at -29.5 °C and -12.3 °C (Fig.1b), being in good agreement with the previous study [23].

These results show that the WGO is a good source of bioactive compounds, but its use would be limited due to its low oxidative stability. Therefore, increasing the WGO application in different products requires protection by a suitable encapsulation method such as the NLC method.

Preparation of NLC

Effect of type of surfactant

In preliminary tests, different surfactants with various HLB values were used to obtain the best formula of NLC incorporating the WGO in terms of particle size and stability (Table S2). Preliminary tests showed that the surfactants of poloxamer 407 and SDS were the best surfactants to produce dispersions with the particle size below1µm. Poloxamer 407 is a non-ionic surfactant with HLB number 22 composed of the block copolymer of polyethylene oxide and polypropylene oxide [24]. Furthermore, the surfactant of SDS is a highly hydrophilic anionic surfactant (HLB ~ 40) [25]. The common feature of both surfactants is their high HLB value compared to other surfactants (Table S2). Therefore, it can be stated that in production of NLC, surfactants with higher HLB value have higher efficiency. It is necessary to mention that in previous studies, better results were achieved with combination of different surfactants such as ionic and non-ionic surfactants [26].

Effect of concentration of surfactant

In the following, the effect of concentration of poloxamer and DSD, selected as the best surfactants in the previous step, was investigated on the particle size and appearance of NLC systems. According to Table 1, the SDS-to-lipid ratio of 1:1 (SDS-NLC) was able to produce lipid particles with the size of 255.1 nm. With increasing SDS-to-lipid ratio from 1:1 to 2:1, we could not obtain the suitable formula due to formation of high foam in the production process. The high formation of foam by SDS at high concentrations is due to its ionic nature and its high HLB value [25]. Concerning

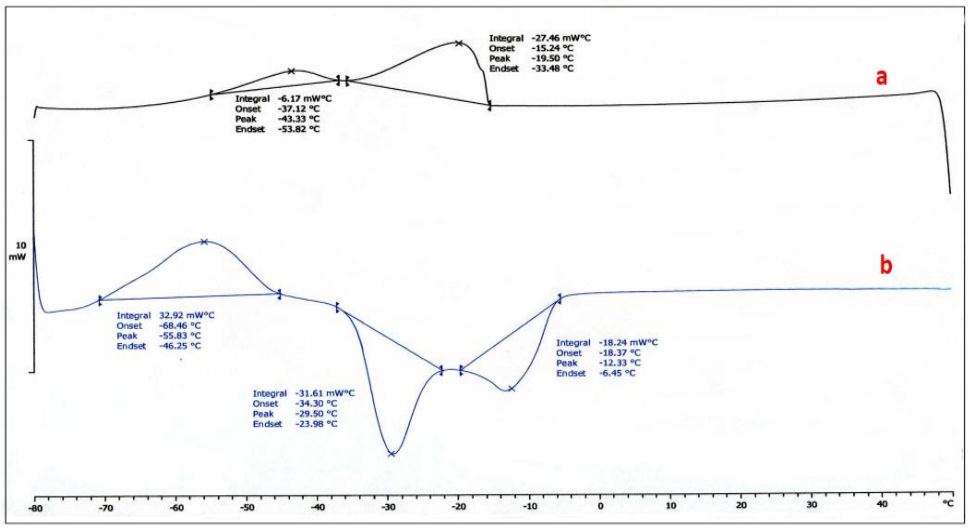

Fig. 1. DSC heating (a) and cooling (b) curves of wheat germ oil.

poloxamer, with increasing poloxamer-to-lipid ratio from 1:1 (polox-NLC-1) to 2:1 (polox-NLC-2), the particle size increased from 52.7 to 99.09 nm, respectively (Table 1). In addition, the formula with the ratio of 1:1 (polox-NLC-1) was clearer than the formula with the ratio of 2:1 (polox-NLC-2) (Fig. 2a). This finding is in agreement with others studies [24,27,28] reporting that increasing surfactant concentration to a certain limit reduces the size of the particles, but increasing further increases the particle size. The increase of particle size above a certain surfactant concentration can be due to formation of a highly viscous liquid phase [27,29]. Furthermore, a slight increase in the size may be due to an increase in the surfactant layer formed around the lipid particles.

Zeta potential of NLCs

The zeta potential of lipid particles was measured to determine the action of electrostatic repulsion forces of NLCs. Table 1 shows the results of zeta potential. Based on the nonionic nature of poloxamer 407 and other constituents of the system, the zeta potential of poloxamer NLCs was very small (close to zero), and the increase of surfactant concentration did not have any effect on the increasing or decreasing potential zeta of particles. Considering the polymeric and bulky structure of poloxamer 407, the stability of poloxamer NLCs could be due to steric repulsion of the surfactant molecules in these systems, and the electrostatic repulsion of the particles did not play a significant role in their sustainability. Polyhydroxy surfactants such as poloxamer 407, due to their non-ionic nature as well as low and zero zeta potential, stabilize systems by creating spatial exclusion [30]. According to Table 1, the zeta potential of SDS-NLC was -28.8. Therefore, the stability of the particles in the SDS-NLC during storage was related to the electrostatic repulsions [31]. It has been shown in previous works that better stability can be obtained by utilizing mixtures of non-ionic and ionic surfactants due to their synergic effect [26,32].

Stability of NLC

According to Table 1, the polox-NLC-1 (the smallest particle size) was chosen to study particle size stability during storage (15 days) at ambient temperature. The particle size and size distribution

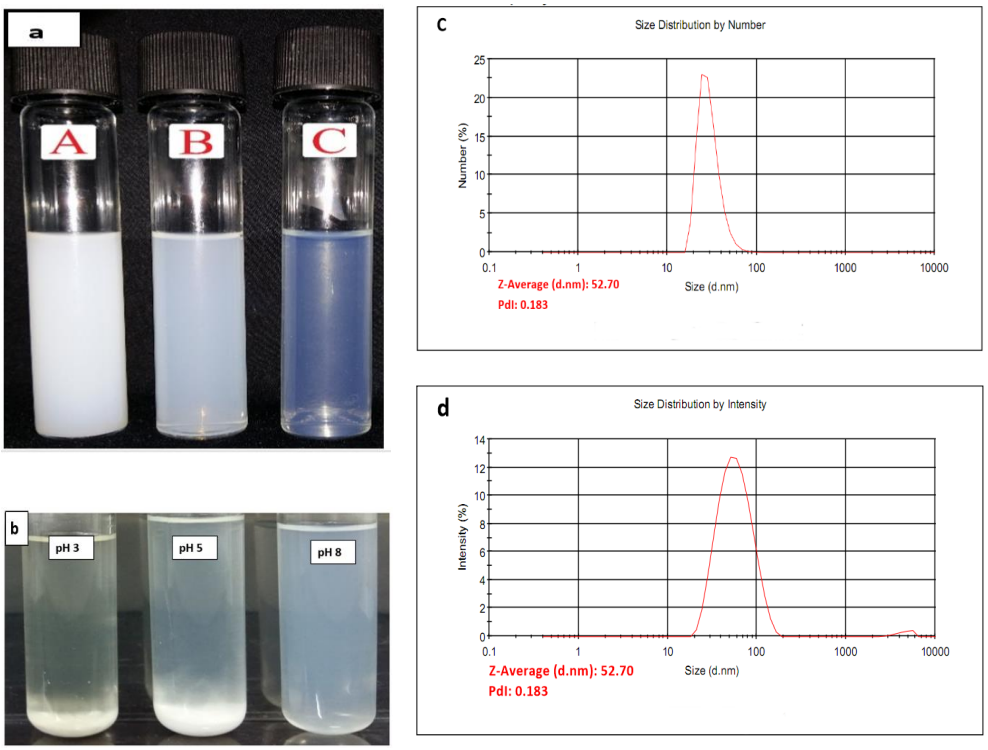

Fig. 2. (a) the appearance of formulations of polox-NLC-2 (A), SDS-NLC (B) and polox-NLC-1 (C) produced one day after production; (b) the appearance of SDS-NLC at pH 3, 5 and 8 after 3 months' storage at room temperature; (c) size distribution of polox-NLC-1 after 1 day of storage and (d) size distribution of Polox-NLC-1 after 15 days of storage.

Table 1. Mean particle diameter d43, PDI and zeta potential of lipid particles measured by dynamic light scattering at day 1.

Particle system	Lipid composition	Surfactant concentration	Mean particle diameter (nm)	PDI	Zeta potential
Polox-NLC-1	0.26% (w/w) percirol, 0.14% (w/w) WGO	0.4% (w/w) poloxamer	52.7	0.183	-1.46
Polox-NLC-2	0.26% (w/w) percirol, 0.14% (w/w) WGO	0.8% (w/w) poloxamer	99.09	0.209	-1.46
SDS-NLC	0.26% (w/w) percirol, 0.14% (w/w) WGO	0.4% (w/w) SDS	255.1	0.41	28.8

were measured after 1 and 15 days of storage that their related results are shown in Fig. 2c and d, respectively. The mean particle size (52.7 nm) and PDI (0.183) within 15 days showed no significant change. However, a second peak (4535 nm) was seen after 15 days that was very low at approximately 1.5 percent. In general, the results showed that polox-NLC-1 possessed relatively good stability.

Thermal behavior of lipid particles

The aim of these series of experiments was to evaluate the thermal properties of different lipid carriers. For this purpose, four formulas, including (i) polox-NLC-1, (ii) polox-NLC-2, (iii) SDS-NLC and (iv) O/W emulsion were used.

Fig. 3A shows the endothermic curves of different formulas. Upon heating from 20°C, one endothermic peak was observed at 52°C for the polox-NLC-1. The melting points of precirol and poloxamer 407 are 56 and 53-57°C, respectively [33,34]. The absence of the melting peaks of solid and liquid lipids in the structure of polox-NLC-1 could be due to crystallization of two compounds and good compatibility with each other, creating a new composition with new thermal properties. For the polox-NLC-2 similar to the polox-NLC-1, one large endothermic peak was observed at 52°C. Additionally, in polox-NLC-2 curve, a small endothermic peak at 58°C was detected, which could be attributed to more poloxamer used in the polox-NLC-2 in comparison with that used in the polox-NLC-1. This result suggests that the thermal behavior of NLC can be improved up to a certain surfactant concentration; however, using more surfactant does not have a positive impact on the thermal behavior of the NLC. For the O/W emulsion, only one endothermic peak at 56°C was observed. This peak was due to applying poloxamer as a surfactant. The intensity of endothermic curves obtained in polox-NLC-2, polox-NLC-1, and O/W emulsion was as a result of applying poloxamer. In the SDS-NLC, one wide endothermic peak at 52°C and one small peak at 18°C were observed. In the particle system of SDS-NLC, the SDS surfactant was used, having a melting point of 206°C [7] and consequently, SDS had no role in forming the endothermic curve in the SDS-NLC.

Fig. 3B shows the exothermic curves of different formulas. Two exothermic peaks in poloxamer NLCs are a consequence of applying percirol and poloxamer. The heat difference between the two peaks in the polox-NLC-2 could be due to more surfactant compared to the polox-NLC-1. This difference was less in polox-NLC-1, and the peaks appeared closer. In the O/W emulsion, only one exothermic peak was observed due to presence of poloxamer. Two small peaks observed in the SDS-NLC were similar to small peaks observed in the endothermic curve of O/W emulsion.

The effect of particle system and pH on the oxidative stability of lipid particles

In the following, the effect of different lipid particle systems (polox-NLC-1, polox-NLC-2, SDS-NLC and O/W emulsion) and pH (3, 5 and 8) on formation of hydroperoxides during 35-day storage at 45 °C was investigated, and Table 2 shows the related results.

In the particle system of polox-NLC-1 in the early days, the change in peroxide values was not significant ($P>0.05$) at different pHs. However, most hydroperoxides were observed at pH 3 on the 35th day, showing a significant difference ($p \leq 0.05$) with that at pH 5 and 8 (Table 2).

In the particle system of polox-NLC-2, the effect of pH on the hydroperoxide changes in the early days was not significant ($p> 0.05$), but the formation of hydroperoxides at pH 3 indicated a

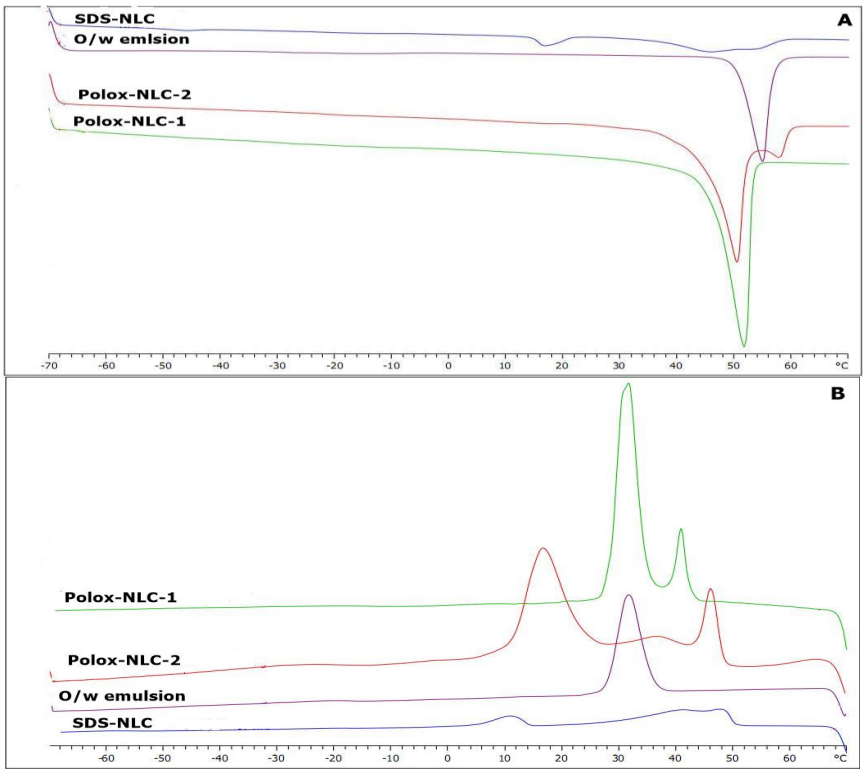

Fig. 3. DSC heating (A) and cooling (B) curves of formulations of polox-NlC-2, SDS-NLC, polox-NLC-1 and O/W emulsion.

significant ($p \leq 0.05$) increase on the 7th and 14th days. At the end of storage, most hydroperoxides were observed at pH 3, indicating a significant difference ($p \leq 0.05$) with that at pH 5, but did not show a significant difference with that at pH 8 (Table 2).

In the particle system of SDS-NLC, the hydroperoxide changes on different days increased at all pHs in the beginning and then decreased. The formation of hydroperoxides at pH 3 was less than that at pH 5 and 8 in mid times that the observed difference was significant ($P \leq 0.05$). On the 35th day, the hydroperoxide changes in the SDS-NLC at different pHs had not a significant difference ($p > 0.05$) with each other. In general, the formation of hydroperoxides at pH 5 and 8, was almost the same, and no significant difference was observed ($P > 0.05$) (Table 2).

In the particle system of O/W emulsion, the formation of hydroperoxides at all pHs first increased and then decreased. This result can be due to breaking the primary oxidation compounds into secondary oxidation compounds [35]. On the 7th, 14th and 35th days, most hydroperoxides were observed at pH 3, showing a significant difference ($p \leq 0.05$) with that at pH 5 and 8 (Table 2).

As Table 2 shows, at pH 3, most hydroperoxides were observed in the O/W emulsion, polox-NLC-1, SDS-NLC and polox-NLC-2, respectively. The O/W emulsion at pH 3 on the 14th day showed most hydroperoxides (504.3±26.7 µmol/g oil), with a significant difference with other treatments ($P \leq 0.05$). At pH 5 and 8, similar to pH 3, the lowest and highest hydroperoxides were observed for the polox-NLC-2 and the O/W emulsion, respectively.

Schematic illustration: Fig. 4 shows a schematic diagram for the proposed reasons related to the formation of hydroperoxides in various formulas at different pHs. Generally, by comparing the formation of hydroperoxides in the O/W emulsion with different NLCs (Polox-NLC-1, Polox-NLC-2 and SDS-NLC), it can be concluded that the NLCs have been able to provide greater protection against WGO oxidation than O/W emulsion (Table 2). This result is in good agreement with the previous studies [6,12]. Salminen et al. (2014) showed that by encapsulating fish oil in the NLC, the formation of lipid hydroperoxide, propanal and hexanal compared to fish oil in water emulsion decreased by 72%, 53% and 57%, respectively. Furthermore,

Table 2. Effect of type of particle systems and pH on the formation of hydroperoxides (μ mol/g oil) during 35 d storage at 45 °C. Each value is an average of three independent measurements ±standard deviation.

Particle systems	pH	Storage time (days)					
		1	7	14	21	28	35
Polox-NLC-2	3	57.4±2.4^{aB}	110.4±8.5^{aC}	143.1±11.9^{bB}	177.3±18.8^{aA}	225.2±21.7^{aA}	399.2±4.0^{aA}
	5	62.2±5.1^{aB}	96.2±10.2^{aC}	182.0±14.2^{aC}	155.4±23.1^{aB}	128.2±10.4^{bB}	310.5±11.3^{bA}
	8	61.2±1.9^{aB}	111.8±12.8^{aC}	134.4±17.6^{bC}	169.6±10.9^{aA}	189.2±23.3^{aA}	215.8±4.7^{cB}
Polox-NLC-1	3	56.3±7.0^{aB}	206.0±29.1^{aB}	147.0±13.4^{aB}	98.9±8.3^{bC}	136.1±15.1^{aB}	227.8±9.0^{aC}
	5	59.0±3.0^{aB}	88.5±14.5^{bC}	107.0±4.6^{bD}	115.5±9.0^{aC}	127.6±10.7^{aB}	172.0±13.9^{bB}
	8	60.2±1.3^{aB}	75.4±15.0^{bD}	74.3±16.2^{bD}	86.3±7.2^{bB}	119.4±9.1^{aBC}	197.9±20.8^{abBC}
SDS-NLC	3	74.3±5.0^{aA}	98.0±1.1^{bC}	106.6±4.1^{bC}	85.0±6.5^{bC}	89.6±2.3^{bC}	150.0±21.8^{aD}
	5	71.5±3.1^{aA}	148.1±6.9^{aB}	305.5±22.9^{aB}	180.0±11.8^{aB}	125.5±16.1^{aB}	168.0±12.0^{aB}
	8	71.3±2.8^{aA}	149.4±13.9^{aB}	284.3±19.9^{aB}	186.6±20.5^{aA}	138.2±17.0^{aB}	178.1±9.1^{aC}
o/w emulsion	3	79.1±4.3^{aA}	327.5±23.1^{aA}	504.3±26.7^{aA}	136.1±3.1^{cB}	120.2±14.4^{bB}	320.5±22.9^{aB}
	5	75.8±6.0^{aA}	226.0±13.5^{bA}	405.1±27.6^{bA}	289.6±17.1^{aA}	178.0±18.6^{aA}	159.0±8.1^{cB}
	8	76.1±3.9^{aA}	195.9±10.3^{bA}	347.1±22.1^{cA}	173.3±12.2^{bA}	94.9±6.1^{bC}	284.6±14.9^{bA}

Within a column, different letters (a-c) represent statistically significant difference (P ≤0.05) among different pH of each particle system.
Within a column, different letters (A-D) represent statistically significant difference (P ≤0.05) among different particle systems with the same pH

Krasodomska et al. [12] used PUFA-rich oils of blackcurrant, blackberry, raspberry, strawberry, and plum as components of NLCs. Their results showed that the NLC was an effective method to increase oxidative stability of the PUFAs. In our work, by encapsulating WGO in NLCs, a percirol shell is most likely formed around the WGO through heterogeneous crystallization in the solidified interfacial layer (Fig. 4). This shell may have acted as a physical barrier reducing oxidation by limiting the access of oxygen, prooxidants, and light. In the O/W emulsion (containing 100% WGO as a lipid phase), this protective shell is missing (Fig. 4) so that the oxygen, prooxidants, and light could interact to a greater degree [6].

Overall, from Table 2, we can conclude that the polox-NLC-2 had the highest oxidative stability, which can be due to a higher ratio of surfactant to lipid phase. More ratio of surfactant to lipid phase in the polox-NLC-2 compared to other particle systems, probably not only caused to increase the thickness of the interfacial layer around lipid particles (Fig. 4), but also the amount of non-adsorbed poloxamer present in the continuous phase caused to increase the viscosity of the continuous phase [27]. Poloxamer in the continuous phase can retard lipid oxidation by slowing down the mobility of peroxidants in the continuous phase. These results are in agreement with the previous studies [36,37]. Results also indicated that the oxidative stability of lipid particles prepared with the nonionic surfactant of poloxamer (polox-NLC-1, polox-NLC-2 and O/W emulsion) was lower at low pH than at high pH (Table 2). This phenomenon is presumably due to the higher solubility of metals such as iron at low pH, which can act as peroxidant agents on the surface of oil droplets (Fig. 4)[38]. Previous studies have shown that the lipid oxidation increases when transition metals (positive charge) are electrostatically attracted to the surface of the lipid droplets (negative charge) due to close interaction between the lipid substrate and transition metals [27,38]. In the lipid particle system of SDS-NLC, higher oxidative stability was observed at lower pH (Table 4). This result can be explained by the

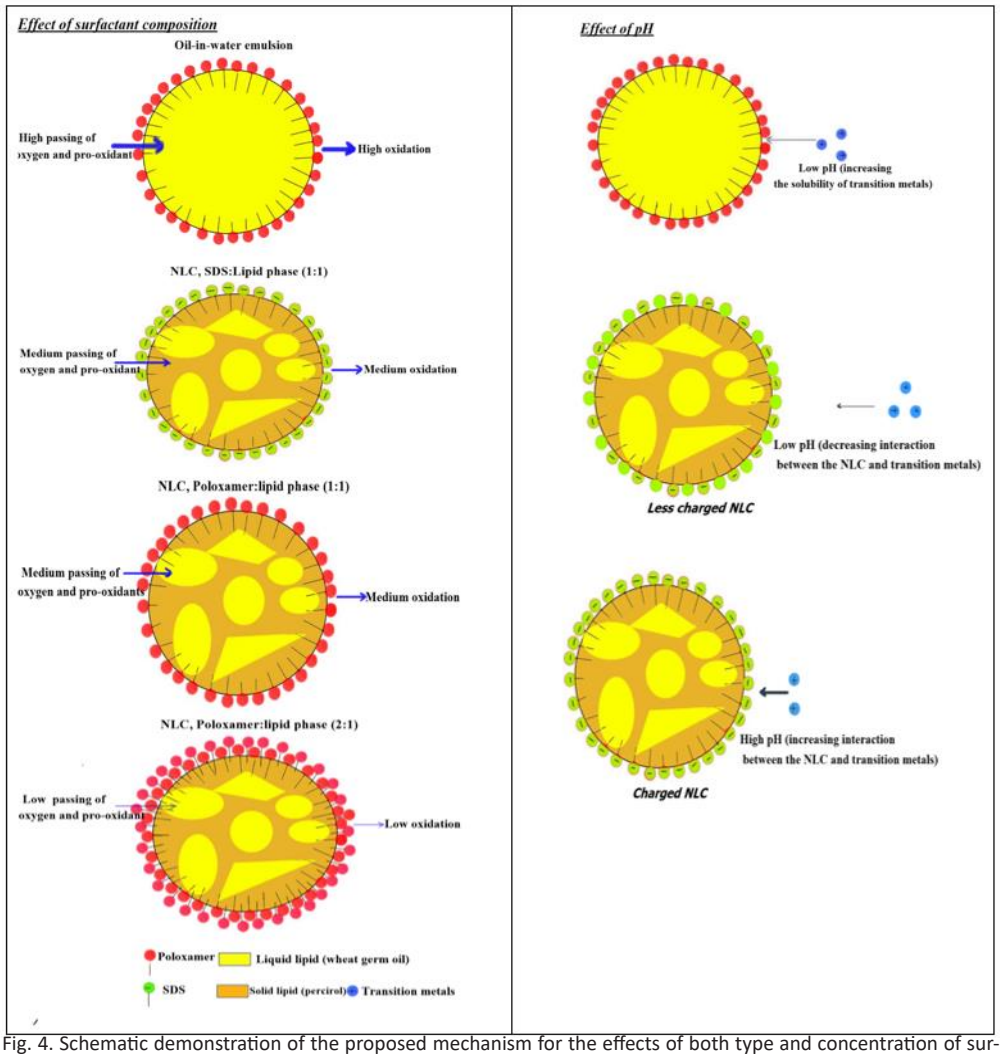

Fig. 4. Schematic demonstration of the proposed mechanism for the effects of both type and concentration of surfactant as well as pH on the formation of hydroperoxides in the different lipid particle systems (polox-NlC-2, SDS-NLC, polox-NLC-1 and O/W emulsion).

fact that the lipid particles prepared by anionic surfactants such as SDS are less charged at low pH [36]. Therefore, the interaction between the lipid substrate and transition metals at low pH can occur less than at high pH (Fig. 4). The ionic characteristic of SDS at low pH can slightly prevent oxidation, but it can unstable the system since uncharged particles do not repel each other, leading to form the sedimentation. As Fig. 2 b shows, after 3-month storage, the sedimentation was observed in the acidic samples of SDS-NLC (pH 3, and 5); however, the alkaline sample (pH 8) was clear.

It is necessary to mention that the delivery of bioactive compounds to different sites within the body is directly affected by the particle size. Therefore, nanoencapsulation has the potential to improve controlled release, enhance bioavailability, and enable precision targeting of the bioactive compounds in a greater extent than microencapsulation [39]. In previous studies, microencapsulation method was used to encapsulate WGO [2,14] . However, in the present work, for the first time, the NLC method was used as one of the nanocapsulation methods to encapsulate and increase the oxidative stability of WGO, the results of which indicated that NLC was successful in increasing the oxidative stability of WGO.

CONCLUSION

In this study, NLCs containing WGO less

than 100 nm were successfully prepared using poloxamer as an emulsifier. The results showed that the emulsifier type was effective on particle size so that the non-ionic emulsifier performed better than the ionic one. In addition, pH was an effective factor in the oxidative stability so that the NLCs prepared with poloxamer had greater oxidative stability at higher pH. The findings also showed that the oxidative stability of NLCs was higher than the oxidative stability of O/W emulsion. These results demonstrated that NLC could be used as a promising approach to protect unstable bioactive compounds such as WGO. Furthermore, a suitable NLC incorporating WGO with the high chemical stability can be achieved by choosing the appropriate both type and concentration of surfactants as well as pH of the matrix.

ACKNOWLEDGEMENTS

The authors would like to thank the Shahrood University of Technology through the financial support of this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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