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Original Article

FORMULATION OF SODIUM ASCORBYL PHOSPHATE (SAP) INTO O/W NANOEMULSION

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ABSTRACT

Objective: Wrinkles are a natural aging consequence that are most visible on sun-exposed skin, such as the face, neck, hands, and forearms. To meet the demands of the aesthetic market, anti-wrinkle active substances have been produced. Due to its capacity to shield skin from free radicals and promote collagen activity, water-soluble vitamin c has emerged as one of the essential anti-wrinkle options. Vitamin c as an antioxidant, can reduce damage caused by free radicals.

Methods: The aim of this study was to develop a water-soluble vitamin c nanoemulsion, identify the optimum formulation, and do stability tests. The method is to mix the ingredients with the same phase first, then mix the two and stabilize with surfactant. A standard procedure was used to produce the nanoemulsion, with the variation ratio of tween 80 to PEG 400 and glycerin. Then, the research went on to the evaluation involved a physical stability test utilizing centrifugation at 3375 rpm, six cycles of freeze-thaw and a thermal stability test at three distinct temperatures for four weeks.

Results: The results showed that the 10% glycerin increased SAP's solubility in the oil phase. A clear and transparent nanoemulsion with fine physical and thermal stability and globule sizes less than 200 nm was produced by the optimum formula, which contained Tween 80, PEG 40, glycerin, VCO, and phosphate buffer at pH 6 in a ratio of 17:9:10:3:61.

Conclusion: The formulation of Sodium Ascorbyl Phosphate has the good physical, chemical characteristic, and stability properties that makes it acceptable to use it as the primary or supplemental therapy for an anti-wrinkle agent with good efficacy and low side effects.

Keywords: Nanoemulsion, Sodium ascorbyl phosphate, Tween 80, PEG 400, Glycerin

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INTRODUCTION

Wrinkles are a natural aging consequence that are most evident on sun-exposed skin, such as the face, neck, hands, and forearms [1]. The occurrence of wrinkles on the skin can be accelerated when the skin is more frequently exposed to free radicals from the environment, and this condition leading to oxidative stress [2].

Nowadays, wrinkles are treated using topical retinoids (such as tretinoin, tazaroten, and adapalene) and over-the-counter wrinkle treatments containing glycolic acid or alpha hydroxy acid. However, there are some limitations or disadvantages, though, like dryness, burning, redness, or itching [3].

Given these restrictions, antioxidant compounds have emerged as a prime and viable option to treat or prevent wrinkles by reducing the production of free radicals, which has a higher potential and fewer adverse effects [3, 4].

Water soluble vitamin C or Sodium Ascorbyl Phosphate (SAP) is an antioxidant compound that has activity on inhibiting free radicals and inducing collagen activity on the skin [5, 6]. SAP is an active pharmaceutical ingredient that has been developed in various skin care to treat wrinkles [7]. However, there are some limitations related to permeability and penetration issues of this compound due to a fairly strong membrane barrier of the skin (stratum corneum) which is difficult for materials with low lipophilicity values, such as SAP to pass into the skin [8].

Nanoemulsions, also referred to as submicron emulsions, ultrafine emulsions, and mini emulsions, are isotropic dispersions of two immiscible liquids, such as water and oil, stabilized by an interfacial film made of a suitable surfactant and co-surfactant to form a single phase [9]. They are submicron-sized colloidal particulate systems that are considered to be thermodynamically and kinetically. In comparison to other dosage forms, nanoemulsions have a number of benefits, including [1] faster rate of absorption; [2] lower variability in absorption; [3] protection from oxidation and hydrolysis in O/W nanoemulsions; [4] delivery of lipophilic drugs after solubilization; [5] aqueous dosage form for water-insoluble drugs; [6] increased bioavailability for many drugs; [7] ability to combine lipophilic and hydrophilic drugs; [8] delivery systems to increase efficacy while reducing side effects, [9] as non-toxic and non-irritating vehicles for skin and mucous membrane delivery, and [10] release control [7, 8].

Previous studies were conducted by Nursal *et al.*, 2020 [12], in which sodium ascorbyl phosphate was also prepared as a nanoemulsion for transcutaneous delivery; however, the studies were restricted to characterization and elementary evaluation. Keeping in mind that for highlight the novelty, we undertake research with various formulas, specifically on the variation of surfactant and co surfactan using phase diagrams, as well as physical and chemical stability evaluations, in order to determine the most optimum o/w nanoemulsion formulation, which has good permeability and penetration to pass through the skin barrier.

MATERIALS AND METHODS

Solubility of SAP in the oil phase

The solubility of SAP in the oil phase was determined using UV-vis spectrophotometry [13]. Multiple oil phases are used to dissolve the maximum amount of the SAP. The resulting mixture between the SAP and the oil phase is centrifuged to separate the supernatants from the filtrate solution. The aliquot of the supernatants was diluted with distilled water. The dissolved SAP was measured using UV-vis spectrophotometry.

Selection of mixture (surfactant and co-surfactant)

The nanoemulsion was formulated using VCO as the oil, Tween 80 as the surfactant, and an admixture of PEG 400 and glycerin as the co-surfactant. The surfactant and, co-surfactant (Smix) and VCO were mixed with a magnetic stirrer then titrated using aquadest. The emulsion selected is an emulsion that forms quickly and has a transparent color [14].

Construction of phase diagrams

Pseudoternary phase diagrams were used to obtain the nanoemulsion. Determined SAP solubility in the oil phase was done by dissolving SAP in various amounts in the oil phase [15]. Mixtures of SAP and oil phase were centrifuged to separate the supernatant from the filtrate. The aliquots supernatants were then diluted with aquadest and the amount of solubilized SAP was analyzed using UV-Vis spectrophotometry. The weight ratio of the Smix varies between 3:1 and 2:1. Aquadest was titrated to the Smix and oil mixture and stirred using a magnetic stirrer until a transparent nanoemulsion formed. The ratio of the Smix, oil, and aquadest were recorded to complete the pseudoternary phase diagrams [16].

Preparation of SAP-loaded nanoemulsion system

According to the nanoemulsion area in the phase diagrams, the SAP-loaded nanoemulsion formulation was chosen. The nanoemulsion was obtained by mixing the SAP, Smix, and the oil, and stirred using a magnetic stirrer until the SAP dissolved completely. The oil phase was then sonicated for 15 min [17].

Visual characterization

The optimized nanoemulsion containing SAP was stored at 4 °C, 25 °C, and 40 °C for 4 w, and then the clarity and phase separation were investigated [18].

Globule size measurement

The globule size and polydispersity index (PDI) were measured by the Delsa™ Nano C Particle Analyzer [19].

Freeze thawing

Freeze thawing was conducted to determine the stability of the nanoemulsion. The formulations were subjected to 6 freeze-thaw cycles, including freezing at 4 °C for 24 h and thawing at 40 °C for 24 h. The phase separation of the formulations were then observed [20].

Centrifugation test

The samples were centrifuged at 3375 rpm for 5 h at room temperature then phase separation was observed for each hour [21].

Accelerated stability test

The samples were stored at room temperature at 40 °C for 4 w. Every week the samples were taken to evaluate their pH, phase separation, SAP concentration, droplet size, and PDI [22].

RESULTS

Solubility of SAP in the oil phase

Table 1: SAP solubility at different glycerin concentrations.

Component %(w/w)			
Component	**K 1**	**K 2**	**K 3**
SAP	1.5	1.5	1.5
Tween 80	17	17	17
PEG 400	9	9	9
Glycerin	0	5	10
VCO	3	3	3
SAP Solubility (% w/w)	5.74±0.32	38.08±2.01	94.55±0.26

Conducted on 3 formulas of experiment with different concentrations of glycerin. K1: 0%, K2: 5% and K3: 10%. (n = 3; with standard deviation of the mean)

Selection of mixture (surfactant and co-surfactant)

Table 2: Observation results of formulations after went through 6 cycles of freeze-thaw

Component	(% w/w)						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Tween 80	17	15	20	22	17	20	30
PEG 400					9	10	5
Glycerin	10	10	10	10	10	10	10
VCO	3	3	3	3	3	3	3
Buffer	70	72	67	65	61	57	52
	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Globule size (nm)	33.3	22.4	37.4	43	50.8	132.4	55.8
PDI	0.429	0.342	0.42	0.401	0.344	0.3	0.42
After 6 cycles of freeze-thaw	Milky-white	Milky-white	Milky-white	Milky-white	Clear	Milky-white	Milky–white

Conducted on 7 formulas of experiment with different concentrations of Tween 80, PEG 400 and buffer. The experiment measured globule size, PDI and condition of SAP after 6 cycles of freeze-thaw.

Construction of diagram phase

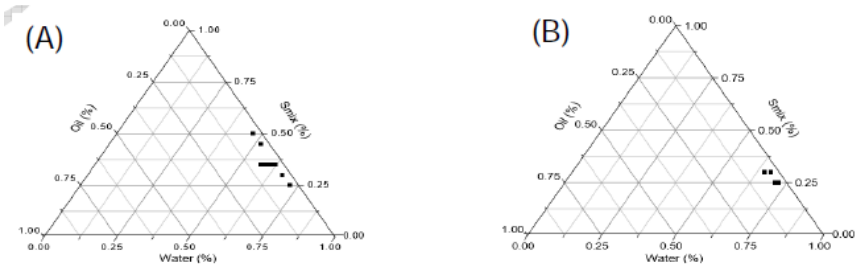


Fig. 1: Pseudo-ternary phase diagrams of o/w nanoemulsion composed of VCO as oil, Tween 80 as surfactant, and an admixture of PEG 400 and glycerin as co-surfactant (Smix 3:1) (A); VCO as oil, Tween 80 as surfactant, and glycerin as co-surfactant (Smix 2:1) (B)

Freeze thawing

Table 3: F5 characteristics during each cycle of freezing and thawing

Cycle	pH	Globule size (nm)	PDI	Phase separation
1	6.26±0.09	46.70±7.80	0.322±0.007	-
2	6.30±0.09	41.63±7.54	0.352±0.023	-
3	6.36±0.11	43.23±3.85	0.381±0.019	-
4	6.44±0.08	44.93±6.49	0.398±0.018	-
5	6.52±0.11	44.73±2.71	0.430±0.044	-
6	6.54±0.12	52.96±10.04	0.450±0.041	-

Conducted on 3 formulas of experiment measured pH, globule size, PDI and condition of SAP after 6 cycles of freeze-thaw. (n = 3; with standard deviation of the mean)

Centrifugation test

Table 4: Results of a centrifugation test

Hour	Globule size (nm)	PDI	Phase separation
0	101.46±6.271	0.299±0.063	-
1	121.43±4.177	0.373±0.021	-
2	127.36±3.92	0.385±0.025	-
3	152.56±17.20	0.422±0.033	-
4	152.93±14.13	0.455±0.035	-
5	183.13±14.48	0.489±0.075	-

Conducted on 3 formulas of experiment measured globule size, PDI and phase separation of centrifugation test. (n = 3; with standard deviation of the mean)

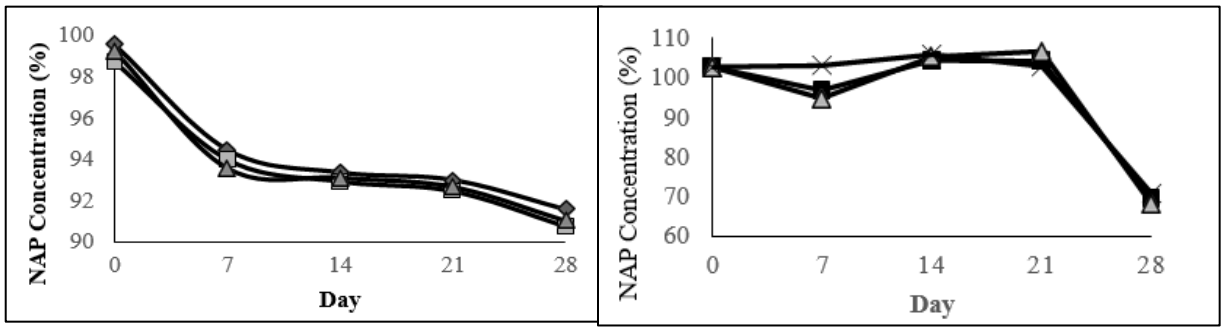
Physical stability

Table 5: The SAP nanoemulsion's physical stability at various thermal conditions the data are shown as mean±SD (n = 3)

	pH			Globul Size (nm)			PDI		
Days	Temperature								
	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C	4 °C	25 °C	40 °C
0	6.43±0.05	6.43±0.05	6.43±0.05	37.83±4.74	40.90±3.81	40.90±3.83	0.28±0.07	0.282±0.07	0.282±0.07
7	6.52±0.07	6.53±0.09	6.52±0.13	41.36±5.97	36.96±7.62	40.46±1.05	0.30±0.04	0.381±0.05	0.352±0.01
14	6.49±0.04	6.51±0.08	6.53±0.02	67.30±4.29	54.90±5.54	57.83±9.05	0.37±0.01	0.391±0.02	0.370±0.08
21	6.50±0.06	6.48±0.08	6.50±0.07	73.53±8.97	79.80±7.72	65.70±2.85	0.40±0.01	0.421±0.01	0.406±0.06
28	6.44±0.02	6.43±0.02	6.42±0.02	75.10±4.76	89.40±2.54	114.80±2.91	0.39±0.01	0.426±0.03	0.407±0.02

Conducted on 3 formulas of experiment measured pH, globule size and PDI after physical stability various thermal conditions test. (n = 3; with standard deviation of the mean)

Accelerated stability



A **B**

Fig. 2: Stability of the SAP in nanoemulsion (A) and water (B)

DISCUSSION

Sodium Ascorbyl Phosphate (SAP) is an active ingredient that functions as an antioxidant and is able to overcome the problem of wrinkles on the skin. However, SAP has a disadvantage because of its low lipophilicity (Log P,-0.005), making it difficult to penetrate the skin and to reach its action site [6, 12]. To overcome this problem, in this study, SAP was formulated into a nanoemulsion where this emulsion is a heterogeneous system consisting of two immiscible liquid phases with submicron droplet sizes between 20-200 nm [10]. These small sizes of droplets have the advantage of increasing the performance of the active ingredient in the delivery [11]. The SAP nanoemulsion formulas consisted of 3 variations which were distinguished from the ratio of the amount of co-

surfactant used in the formulation. The details of the formula can be seen in table 1. The concentration of SAP used was 1.5% (w/w) as the active substance; excipients were also used as additives in the nanoemulsion formula. Additional materials that are often used in nanoemulsions are surfactants, co-surfactants, water phase, and oil phase [24]. Surfactants are surface active materials used to stabilize the interfacial tension of the globules to avoid mixing that can form aggregates and phase inversion. In this study, the surfactant used was Tween 80 with a concentration of 17%. The surfactant is assisted by other materials called co-surfactants in stabilizing the surface tension. The co-surfactants used were PEG 400 and glycerin with a ratio of 3 variations in concentration were 9:0, 9:5, and 9:10. The oil phase used was VCO with a concentration of 3%. Before carrying out the nanoemulsion formulation, the solubility of the SAP in the oil phase was determined first. The steps taken were dissolving a maximum amount of SAP in the oil phase. The mixtures of SAP and oil phase were then centrifuged to separate the supernatant and filtrate. The supernatants were then diluted with aquadest and the amount of solubilized SAP was analyzed using UV-vis spectrophotometry [13]. Based on the measurements carried out, it can be concluded that the highest solubility was in formula 3 with a co-surfactant PEG 400 concentration of 9% and glycerin concentration of 10%. This result shows that the co-surfactants that have good yields are the ones obtained from the combination of two surfactants which are almost the same in concentration. The use of a combination of glycerin and PEG 400 as an additive in nanoemulsion has also been demonstrated in several studies [9, 14]. The next step was to determine the amount of surfactant and co-surfactant. The Tween 80 was used as the surfactant and a mixture of PEG 400 and glycerin used as the co-surfactant. The mixture of surfactant and co-surfactant (Smix) and VCO was then mixed using a magnetic stirrer and then titrated using aquadest. The emulsion selected was the one that formed emulsion quickly with clear/transparent color. From 25 formulation variations, only 7 formulas were fast in forming emulsions and with clear colors (Tabel 2). In table 2 it can be seen that all formulations produced clear nanoemulsions with globule size less than 200 nm and PDI<0.5. The next step was the construction of a pseudoternary phase diagram to obtain the nanoemulsion area. [26]. The weight ratio of the Smix varies between 3:1 and 2:1. The pseudoternary phase diagram of the o/w nanoemulsion consisting of VCO as oil, Tween 80 as the surfactant, and a mixture of PEG 400 and glycerin as co-surfactant (3:1 mixture) as shown in fig. 1A. The pseudoternary phase diagram of the o/w nanoemulsion consisting of VCO as oil, Tween 80 as surfactant, and glycerin as co-surfactant (2:1 mixture) as shown in fig. 1B. After the phase diagram was obtained, the SAP nanoemulsion formulation was then being carried out. According to the nanoemulsion area in the phase diagram, the nanoemulsion containing the formulation SAP was selected. The nanoemulsion system was obtained by mixing SAP, Smix, and oil stirred with a magnetic stirrer until the SAP was completely dissolved. The oil phase was then sonicated for 15 min. The preparation that was carried out was dissolving the material that was soluble in the oil phase into the oil phase and the material that was soluble in the water phase was dissolved into the water phase. The SAP was a material that is soluble in the water phase, thus it was dissolved first in the water phase then the oil and surfactant phases were mixed and stirred. The mixture was added with a co-surfactant and then homogenized using a homogenizer. After the mixture was obtained, characterization and evaluation were carried out on the SAP nanoemulsion preparation. Physical stability testing was carried out through 6 freeze-thaw cycles, where samples were frozen at 4°C for 24 h followed by thawing at 40 °C for 24 h. Observations on pH, globule size, PDI, and phase separation were observed in each cycle. Up to the 6th freeze-thaw cycle, only formula F5 produced a clear nanoemulsion with a globule size of less than 200 nm and PDI<0.5, where no phase separation occurred (table 3). These results indicated that the nanoemulsion was well formed and met the requirements of the 20-200 nm test. In addition to the freeze-thaw test, a centrifugation test was also carried out to see the separation of the preparations. In formula F5, 10 grams were taken and centrifuged at 3375 rpm for 5 h at room temperature and then observed every hour for phase separation. The results showed that there was no phase separation, the globule size was less than 200 nm, and PDI<0.5 (table 4). In formula F5, 10 grams were taken and centrifuged at 3375 rpm for 5 h at room temperature and then observed every hour for phase separation. The results showed that there was no phase separation, the globule size was less than 200 nm, and the PDI<0.5. These results suggest that F5 will remain stable for a year. Physical stability tests had also been carried out, which included tests for pH, globule size, PDI, and phase separation during storage for 4 w at three different temperatures, namely 4 °C, 25 °C, and 40 °C. Based on the evaluation results, it was found that the pH of the SAP nanoemulsion remained in the range of 5.5-6.8 which is the pH of human skin, no phase separation occurred for up to 4 w, the globule size remained less than 200 nm, and the PDI remained<0.5 (table 5). The accelerated stability test was carried out for 4 w at three different temperatures, namely 4 °C, 25 °C, and 40 °C. The stability of the SAP in the nanoemulsion and in water was analyzed every 7 d using a UV-VIS spectrophotometer. The initial concentration of SAP was 1%. After 4 w, more than 91% of the non-degradable compounds remained in the nanoemulsion. According to the literature, SAP is the most stable derivative of ascorbic acid. The high stability is obtained from the results of its chemical structure. The phosphate group at the second position of the cyclic ring protects the molecular enediol system against oxidation so that it cannot act as an antioxidant agent to stabilize the formulation [17, 18].

CONCLUSION

This study reported that the addition of 10% glycerin in the nanoemulsion formulation increased the solubility of SAP in the oil phase. The optimum formula containing Tween 80, PEG 40, glycerin, VCO, and phosphate buffer at pH 6 with a ratio of 17:9:10:3:61 produces a clear/transparent nanoemulsion with fine physical and thermal stability, and the globule size is smaller than 200 nm.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declare none

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