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ORIGINAL ARTICLE / ÖZGÜN MAKALE

PREPARATION AND CHARACTERIZATION OF COMBINED SALICYLIC ACID AND POVIDONE-IODINE CONTAINING NANOEMULGELS: A PRELIMINARY STUDY

KOMBİNE SALİSİLİK ASİT VE POVİDON-İYOT İÇEREN NANOEMÜLJELLERİN HAZIRLANMASI VE KARAKTERİZASYONU: ÖN ÇALIŞMA

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ABSTRACT

Objective: *The aim of this preliminary study is to prepare and characterize combined salicylic acid and povidone-iodine-containing nanoemulgels for use in disease models such as wounds and burns in the future.*

Material and Method: *Within the scope of the study, first of all, analytical method validation of salicylic acid was performed. Then, oil solubility studies were carried out and nanoemulsions and nanoemulgels were prepared. Morphology, zetasizer analysis, type and pH determination, FTIR analysis, spreadability, and in vitro release studies were performed to determine the characterization of the formulations.*

Result and Discussion: *Nanoemulsions and nanoemulgels have been prepared successfully. Nanoemulsions with spherical droplet structure and outer phase water were obtained, and their morphology and zeta sizer results were compatible. In the 1-month stability study, only the F1 formulation did not decompose. There was not much change in pH after holding. At the end of the FTIR analysis, it was seen that there was no interaction between the items. In the release study performed with pH 5.5 phosphate buffer, approximately 40% of the release occurred after 8 hours. This study is a preliminary study, and formulations with long-term stability and release rate can be developed by conducting more detailed studies in the future. Salicylic acid and povidone-iodine were used in combination for the first time. This combination can be translated into formulations that may be beneficial for skin diseases in the future.*

Keywords: *Characterization, nanoemulgel, povidone-iodine, salicylic acid, skin diseases*

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ÖZ

Amaç: *Bu ön çalışmanın amacı, gelecekte yapılması planlanan yara ve yanık gibi hastalık modellerinde kullanılmak üzere kombine salisilik asit ve povidone-iyot içeren nanoemüljeller hazırlamak ve karakterize etmektir.*

Gereç ve Yöntem: *Çalışma kapsamında öncelikle salisilik asitin analitik yöntem validasyonu yapılmıştır. Daha sonra yağda çözünürlük çalışması yapılıp, nanoemülsiyonlar ve nanoemüljeller hazırlanmıştır. Hazırlanan formülasyonların karakterizasyonunu belirlemek için morfoloji, zetasizer analizi, tip ve pH tayini, FTIR analizi, yayılabilirlik ve in vitro salım çalışmaları yapılmıştır.*

Sonuç ve Tartışma: *Nanoemülsiyonlar ve nanoemüljeller başarıyla hazırlanmıştır. Küresel damlacık yapısına sahip dış fazı su olan nanoemülsiyonlar elde edilmiştir ve morfolojileri ile zetasizer sonuçları uyumlu çıkmıştır. Yapılan 1 aylık stabilite çalışmasında sadece F1 formülasyonunda ayrışma gerçekleşmemiştir. Bekletme sonrasında pH'larda fazla değişim olmamıştır. FTIR analizi sonunda maddeler arasında etkileşimin olmadığı görülmüştür. pH 5.5 fosfat tamponuyla yapılan salım çalışmasında 8 saat sonunda yaklaşık % 40 oranında salım gerçekleşmiştir. Bu çalışma bir ön çalışma olup ilerde daha detaylı çalışmalar yapılarak uzun süreli stabiliteye ve salım oranına sahip formülasyonlar geliştirilebilir. Salisilik asit ve povidon-iyot ilk defa kombine halde kullanılmıştır. Bu kombinasyon gelecekte cilt hastalıkları için faydalı olabilecek formülasyonlara dönüştürülebilir.*

Anahtar Kelimeler: *Cilt hastalıkları, karakterizasyon, nanoemüljel, povidon-iyot, salisilik asit*

INTRODUCTION

Human skin diseases are one of the most important public health problems, and there has been a severe increase in skin diseases recently. Between 30 and 70% of people worldwide are affected by these problems, which are the most common reason for consultation in general practice. More than 3000 skin diseases, both acute and chronic, have been described, affecting people of all age groups [1, 2]. Since the history of ancient medicine, the skin has been the oldest organ widely used in administering many drugs. Similarly, in modern medical practices in recent years, dermal drug delivery provides an alternative to oral drug delivery and makes essential contributions to health services [3]. The dermal delivery of drugs has always been both attractive and challenging to research. Advances in modern technologies allow dermal delivery of both hydrophobic and hydrophilic small molecule and large molecule drugs. Dermal drug administration, which is a comfortable and painless way for patients, has many advantages compared to other administration ways, making it one of the most preferred. Among the important advantages are the avoidance of hepatic first-pass metabolism and the gastrointestinal tract for drugs with low bioavailability [4].

Dermal drug delivery systems can come in different physical forms, from liquid to powder, and the most popular of these are preparations in semi-solid forms, such as creams and ointments. Among semi-solid products, the use of gels has increased considerably in both cosmetic and pharmaceutical preparations. Gels are dosage forms formed by entrapping large amounts of aqueous or hydroalcoholic liquid in a colloidal network that can be created using natural or synthetic polymers. In particular, they have a high water content that allows the active ingredients to dissolve more. Compared to ointment or cream bases, gels enable the active ingredient to dissolve easily through the liquid carrier because of

having a higher aqueous component. In addition, gels are superior in terms of ease of application and patient compliance. However, gels show a significant limitation in delivering hydrophobic active substances. Therefore, studies on different gel types have increased recently to overcome these limitations. Especially with the inclusion of oil-containing systems into gels, different dosage forms have started to be developed. Among the various nanolipoidal delivery systems such as solid lipid nanoparticles, liposomes, microemulsions, and nanoemulsions, which have a very important place among the new generation oil-containing systems, especially nanoemulsions are among the most successful delivery systems for lipophilic and hydrophilic active substances applied in various ways, including the topical route [3, 5].

Nanoemulsions are oil-in-water, or water-in-oil biphasic dispersions of two immiscible liquids stabilized using a suitable surfactant and they can typically be formed with less surfactant than other colloidal dispersions and have more excellent kinetic stability properties than coarse emulsions [6]. Loading poorly water-soluble drugs into nanoemulsions increases their wettability and/or solubility, improving their pharmacokinetics and pharmacodynamics by different routes of administration. The advantages of nanoemulsions such as optimum drug release, long-term efficacy, drug intake control, low side effects, and drug protection from enzymatic or oxidative processes have been reported in recent years [7, 8].

Nanoemulgels are emulsion-based topical gel formulations in which nano-sized emulsion droplets are gelled by adding a suitable gelling agent. Because nanoemulgels contain both nanoemulsion and gel base, they are among the suitable options as drug delivery systems. The nanoemulsion component of the nanoemulgel protects the active substance from enzymatic degradation and reactions such as hydrolysis, and the gel base provides thermodynamic stability to the emulsion by increasing the viscosity of the aqueous phase by reducing the interface and surface tension. In the presence of suitable penetration enhancers, the droplet size in nano form can increase the formulation's effectiveness by improving the permeability and spreadability of the drug [3, 9].

In this study, salicylic acid (SA) and povidone-iodine (PI) were used as active ingredients. SA is a natural ingredient derived from the bark of the willow tree (*Salix alba*). It has been used worldwide for centuries for its analgesic, antipyretic and anti-inflammatory properties. SA is highly irritating to the gastric mucosa when taken orally, so topical use is preferred. The absorption of SA in the topical application is variable. The systemic effects of SA in topical applications are minimal when applied in low to moderate doses to intact skin. However, if there is deterioration in the structure of the stratum corneum, measurable levels of SA may be present in the body. SA can be used topically as a keratolytic, bacteriostatic, fungicide, and photoprotective. Today, it is frequently used to treat warts, calluses, localized hyperkeratosis, plaque psoriasis, actinic keratosis, ichthyosis, and comedonal acne [10]. PI is

a complex formed with iodine with antiseptic properties and povidone, a synthetic carrier polymer that does not have microbicidal activity. In an aqueous medium, free iodine is released from the PI complex into the solution. The antiseptic activity increases and iodine release continues until an equilibrium is established [11]. PI is also a broad-spectrum antiviral agent against enveloped and non-enveloped viruses such as adenovirus, rotavirus, rhinovirus, human immunodeficiency virus, herpes virus, and measles, polio, rubella, measles, and influenza viruses [12]. This preliminary study aims to prepare and characterize combined SA and PI-containing nanoemulgels for use in disease models such as wounds and burns in the future. SA and PI were combined for the first time.

MATERIAL AND METHOD

Materials

SA was purchased from Riedel-de-Haën (Germany). Olive oil and mineral oil were purchased from Doğa İlaç (Turkey). Sesame oil, linseed oil (LSO), and ethanol were purchased from Sigma (Germany and USA). Sunflower oil and hexane were purchased from Hasyalçın Dış Tic. (Turkey) and J. T. Baker (Holland), respectively. Tween 20 (T20), Tween 60 (T60), Span 80 (S80), and polyethylene glycol (PEG) 400 were purchased from Merck (Germany). PI and hydroxypropyl methylcellulose (HPMC) E15 were kindly received as a gift from BASF (Turkey) and Santa Farma İlaç A.Ş (Turkey).

Development of Quantification Method for Salicylic Acid

A stock solution of SA in ethanol was prepared at a concentration of 100 µg/mL in a multipoint magnetic stirrer (2mag, MIX 15 eco, Germany). After finding the wavelength at which SA gave maximum absorbance, the absorbance of the series was measured in a UV-VIS spectrophotometer (Beckman Coulter DU 730, USA) by dilution from this stock solution. The calibration equation with the calibration curve was found, and then the method was validated. The validation parameters like accuracy, precision, LOD, LOQ, and selectivity were studied (n=6) [13].

Solubility of Salicylic Acid in Different Oils and Phosphate Buffer

The saturation solubility study of SA in different oils and pH 5.5 phosphate buffer was found by stirring in a magnetic stirrer for a long time at room temperature. For this purpose, sesame oil, olive oil, LSO, sunflower oil, and mineral oil were used. Concentrated suspensions of SA in oils/phosphate buffer (4 g) were prepared and stirred for 72 hours on a multipoint magnetic stirrer (1200 rpm) at room temperature. Afterward, the samples were centrifuged (Hettich Micro 200, Germany) at 12500 rpm for 30 min, and the supernatants were diluted at certain ratios with the validation medium and/or hexane. The amounts of dissolved SA were determined by the validated UV-VIS spectrophotometric method (n=3) [14].

Preparation of Salicylic Acid Nanoemulsions

SA-containing nanoemulsions were prepared by the ultrasonication method at room temperature. First, SA (50 mg) was dissolved at a certain ratio in the oil medium (1000 mg), where it was maximum soluble via the ultrasonicator (Bandelin Sonopuls HD 2070, Germany). Then, an appropriate surfactant/co-surfactant (T20 and S80 and/or PEG 400) was added and homogenized with the ultrasonicator. Finally, ultrapure water (1650 mg) was added to the homogeneous oil solution, and nanoemulsion formation was carried out with the ultrasonicator at cycle 3 and power of 100% for 4 minutes (minimum n=6) [15]. Nanoemulsions are coded with the letter E.

Morphology of Nanoemulsions

Morphology of nanoemulsions was determined by optical microscope (Zeiss Primo Star, Germany) and transmission electron microscopy (TEM, Hitachi HighTech HT7700, Japan). For TEM determination, nanoemulsions were dispersed in ultrapure water, and one drop of diluted nanoemulsions was placed on a 400-mesh carbon-coated copper grid. The grid was then dried at room temperature overnight. The TEM imaging was conducted at 120 kV. This analysis was carried out at the East Anatolian High Technology Research and Application Center (DAYTAM) of Atatürk University [14].

Zetasizer Analysis of Nanoemulsions

Droplet size distribution, zeta potential, polydispersity index, and conductivity of nanoemulsions were determined with the zetasizer device (Malvern Zetasizer Nano ZSP, United Kingdom). Nanoemulsions were diluted with ultrapure water at a ratio of 1:9. Zeta sizer measurements were taken in the DTS1070 cell at 25°C, and 3x12 measurements were taken with a measurement angle of 173° utilizing a laser of 633 nm and 10 mW. This analysis was carried out at the East Anatolian High Technology Research and Application Center (DAYTAM) of Atatürk University [16].

Type Determination of Nanoemulsions

Type determination of nanoemulsions was made according to the dilution method. Nanoemulsions were diluted with ultrapure water at a ratio of 1:9 on the watch glass [17]. The outer phase of the nanoemulsions, which form a homogeneous mixture with water and no phase separation is observed, was accepted as the aqueous phase.

Preparation of Nanoemulgels with Povidone-Iodine

Preformulation studies have been made with natural and synthetic polymers such as sodium alginate, pectin, and HPMC E15. As a result of the preformulation studies, it was decided to use HPMC E15. HPMC E15 (500 mg) was swollen in ultrapure water at room temperature. Then, a specific concentration of PI (100 mg) and T60 (400 mg) were added and mixed manually until homogeneous.

Then, nanoemulsions containing SA were added to these gel bases and mixed manually until homogeneous again (minimum n=6) [18]. Nanoemulgels are coded with the letter F. In order to determine the phase separation stability, the nanoemulgels were kept at room temperature and in the refrigerator (4±2 °C) for 1 month. The caps of the vials are tightly closed with parafilm in an airtight manner.

pH Determination of Nanoemulgels

The pH of the freshly prepared nanoemulsions and nanoemulgels was measured with a pH meter (WTW inoLab, Germany). When the nanoemulgels' pH was lower than 5.5, the pH was adjusted to 5.5 with NaOH solution. After the pH was adjusted to 5.5, the nanoemulgels were kept at room temperature and in the refrigerator for 1 month to determine the pH stability [14].

Spreadability of Nanoemulgels

The spreadability of the nanoemulgels was determined using two transparent plastic plates. For this purpose, a circle with a diameter of 1 cm was drawn inside one of the plates, and 0.5 g of each nanoemulgel was weighed on this circle. The second plate is placed on top of the first plate. Finally, a weight of 1 kg was placed on the upper plate and left for 5 minutes. The spreadability of the nanoemulgel was evaluated by measuring the increasing diameter at the end of time [19]. In order to determine the spreadability stability, the nanoemulgels were kept at room temperature for 1 month.

***In Vitro* Drug Release of Nanoemulgels**

The dialysis membrane method of Topal et al. was used for *in vitro* drug release studies. For this purpose, a dialysis membrane (MWCO: 20 kD, Spectra/Por®Biotech, USA), pH 5.5 phosphate buffer, and a shaking water bath (Memmert WNB 14, Germany) were used. pH 5.5 phosphate buffer was prepared by adding HCl into pH 5.8 phosphate buffer (USP 30/NF 25). The dialysis membrane was incubated in the release medium for 15 minutes. 0.5 g of accurately weighed nanoemulgels and 0.5 g of pure SA's were placed in the middle of the dialysis bags. Then, dialysis bags and 50 mL pH 5.5 phosphate buffer at 37±0.5 ºC were added to each amber vial. At the specified time intervals, 2 mL samples were taken, and the same amount of release medium was placed in the bottles to maintain the sink conditions. The amounts of SA in the samples were determined by the validated UV-VIS spectrophotometric method (n=3) [20].

FT-IR Analysis of Nanoemulgels

FT-IR spectra were taken to evaluate whether there is an interaction between the active ingredients (SA, PI), the excipients (LSO, S80, T20, T60, HPMC E15), and the nanoemulgels (F1-F6). This analysis was carried out at the East Anatolian High Technology Research and Application Center (DAYTAM) of Atatürk University [21].

Statistical Analysis

The statistical analysis between the samples were evaluated with the "One-Way Analyses of Variance (ANOVA)" test (according to the homogeneity of the variances and the size of the population). Results at the $p<0.05$ level were considered significant.

RESULT AND DISCUSSION

Development of Quantification Method for Salicylic Acid

The quantification method and validation studies for SA were successfully completed quickly and easily with the UV-VIS spectrophotometric method. The calibration curve, equation, and validation study results are given in Figure 1 and Table 1. The maximum absorbance was seen at 302 nm. The desired linearity was achieved, and the validation study was carried out within the desired limits. LOD and LOQ values were found to be 0.294 µg/mL and 0.892 µg/mL, respectively. In the study of Ahmad et al., in the presence of combined benzoic acid and SA in ethanol in a UV-VIS spectrophotometer, SA gave an absorbance at 303 nm [14]. In the validation study of Sinha et al. with methanol in a UV-VIS spectrophotometer, SA gave an absorbance at 301 nm, and linearity was obtained in the range of 1-10 µg/mL [22]. Our results were similar to the results of these studies.

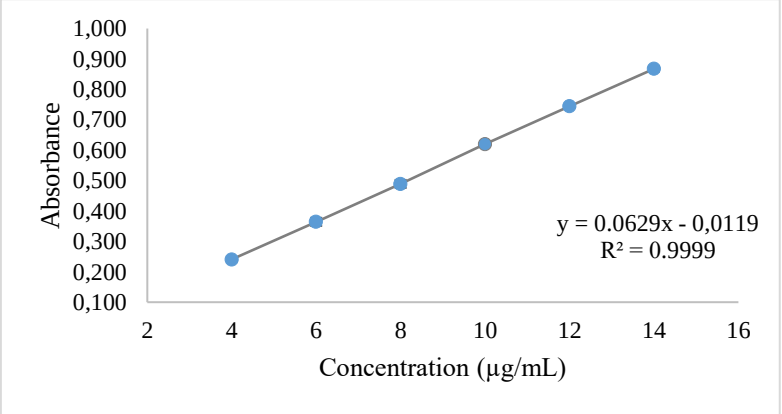

Figure 1. Calibration curve and equation of salicylic acid

Table 1. Validation study results of salicylic acid (mean±standard deviation)

	Theoretical Concentration (µg/mL)	Accuracy (% Relative Error)	Precision (% Variation Coefficient)
Inter-day	5	0.33±1.41	1.49±0.73
	9	0.79±0.77	0.99±0.38
	13	0.29±0.85	1.11±0.36
Intra-day	5	0.67±2.07	1.09±0.64
	9	1.71±0.23	1.02±0.75
	13	1.57±0.36	1.19±0.29

Solubility of Salicylic Acid in Different Oils and Phosphate Buffer

When preparing oil-containing formulations of water-insoluble active substances, it is essential that they are soluble in oil. For this reason, the solubility study was carried out to find the oil with the highest capacity to dissolve the SA which is insoluble in water. The solubility study results are given in Table 2 below. There are not many studies on the solubility of SA in oils in the literature. Many oils have been tested in the solubility study of SA in oils by Ashara et al. [23]. The study was carried out in a shaking incubator at 30 °C for 1 day. The results were evaluated by UV-VIS spectrophotometric method. Olive oil and mineral oil were used together in both our study and theirs. In Ashara et al.'s study, solubility results were obtained at 9.66±0.03 mg/g in olive oil, 2.5±0.07 mg/g in mineral oil, and 10.65±1.30 mg/mL and 0.56±0.03 mg/mL in our study, respectively. Although data showed similar results in olive oil, lower results were obtained in our study in mineral oil. However, when all the results were examined, the highest solubility value was found in LSO. For this reason, LSO was used as the oil phase in the preparation of nanoemulsions. Low solubility was also obtained in the solubility study of SA with pH 5.5 phosphate buffer. Similar low solubility data were obtained in the study of Teng et al., and the results were found to be 2.205±0.020 mg/mL in water and 5.208±0.010 mg/mL in pH 5.0 phosphate buffer at 25 °C [24].

Table 2. Solubility results of salicylic acid (mean±standard deviation).

Oil	**Sesame Oil**	**Olive Oil**	**Linseed Oil**	**Sunflower Oil**	**Mineral Oil**	**pH 5.5 Phosphate Buffer**
Solubility (mg/mL)	10.62±0.36	10.65±1.30	13.66±0.66	11.52±0.85	0.56±0.03	1.65±0.06

Preparation of Salicylic Acid Nanoemulsions

Many modifications have been made while preparing nanoemulsions. The ratios of the formulation components are given in Table 3 below. When SA studies in the literature are examined, it is seen that SA is used at ratios of 0.5% to 40% [10, 25, 26]. For this reason, the dose of SA (50 mg) in our study was chosen to be 1% in nanoemulgels. In our study, the use of dual surfactants was preferred, and Tween 20 and either Span 80 or PEG 400 were used. These surfactants are frequently used to form nanoemulsions [27, 28].

Table 3. Formulation components of nanoemulsions (mg)

Formulation Code	**Salicylic Acid**	**Linseed Oil**	**Tween 20**	**Span 80**	**PEG 400**
E1	50	1000	100	200	-
E2	50	1000	150	150	-
E3	50	1000	200	100	-
E4	50	1000	200	-	100
E5	50	1000	150	-	150
E6	50	1000	100	-	200

Morphology of Nanoemulsions

In addition, the optical microscope images of the nanoemulsions are given in Figure 3 below. As can be seen from the images, nanoemulsions with very homogeneous size distribution have been successfully prepared by the ultrasonication method.

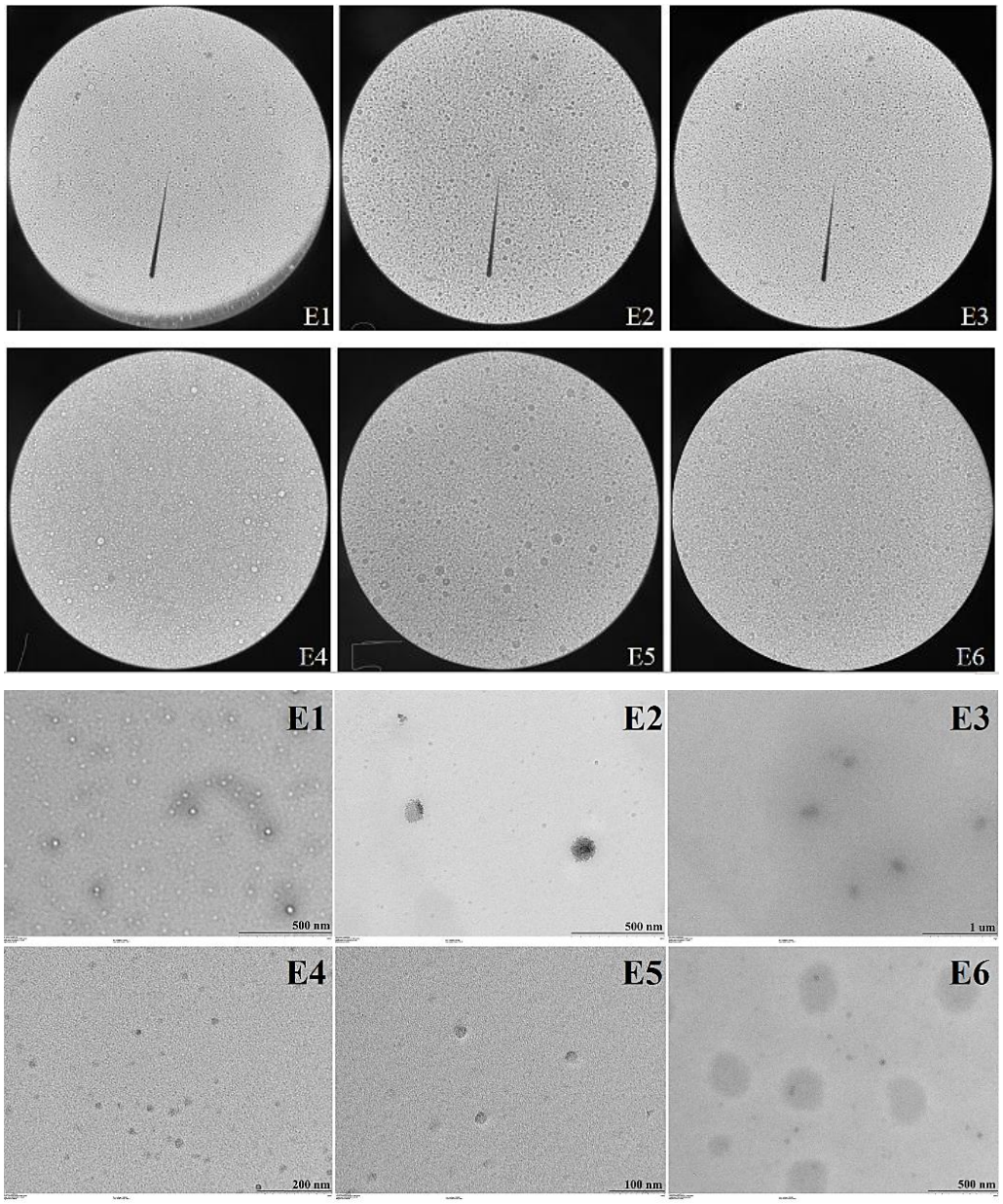

Figure 3. The optical microscope (scale: 100x) and TEM images of the nanoemulsions

Zetasizer Analysis of Nanoemulsions

The droplet size, zeta potential, polydispersity index, and conductivity results of the nanoemulsions are given in Table 4. Relatively small droplet sizes were obtained. When the droplet size

was evaluated statistically, E4, E5, and E6 were found to be significant (p<0.05). This significant difference was thought to be caused by PEG 400 found in E4- E5 and E6. Zeta potential determines the surface charge of nanoemulsions and it is very important for their physical stability [14]. When our zeta potential results were examined, our values were found in the range of (-)11.7±4.04-(-)23.5±3.08 mV. All our zeta potential values were negative. This is because non-ionic surfactants were used when developing formulations [29]. In a study by Acharya et al., nanoemulsions were developed using tweens. In the stability study conducted at room temperature and 2-8 °C for 3 months, it was stated that the stability of the formulations was quite good even at approximately -12.8 mV [14]. The highest zeta potentials were seen in the E3 and E4 formulations. When these formulations are evaluated in terms of content, it can be said that the amount of Tween 20 is the highest (200 mg) in both of them, and the increase in the amount of Tween 20 also increases the zeta potential. When the zeta potential values were evaluated statistically, E1, E3, and E4 were found to be significant (p<0.05). This significant difference was thought to be caused by Tween 20, which is high in E3 and E4, and Span 80, which is high in E1. PDI gives information about the width of the particle size distribution, and the large size distribution can cause stability problems. A PDI value of 0.1-0.25 indicates a narrow distribution, while a PDI value greater than 0.5 indicates an extensive size distribution [30, 22]. When our PDI results were examined, our values in the range of 0.181±0.019-0.228±0.006 were below 0.25, which means that the distribution is narrow. This result was also found to be compatible with the optical microscope and TEM images. Very high conductivity values were obtained in the range of 245±2-333±0 µS/cm. The high electrical conductivity values indicate that the outer phase of the nanoemulsions is water. Similar results were found in the nanoemulsion study of Arbain et al. [31].

Table 4. The droplet sizes, zeta potentials, polydispersity indexes, and conductivity results of nanoemulsions (mean±standard deviation)

Formulation Code	Droplet Size (nm)	Zeta Potential (mV)	Polydispersity Index	Conductivity (µS/cm)
E1	269.9±2.07	-11.7±4.04	0.199±0.016	333±0.00
E2	308.2±1.04	-17.5±5.47	0.207±0.005	283±2.08
E3	295.2±1.65	-23.5±3.08	0.228±0.006	245±2.08
E4	248.2±1.27	-23.0±1.71	0.182±0.006	292±2.52
E5	249.6±1.39	-15.2±3.90	0.181±0.019	318±3.61
E6	315.0±4.46	-17.8±3.59	0.195±0.012	291±3.00

Type Determination of Nanoemulsions

The images obtained by diluting the nanoemulsions with water are given in Figure 4. The fact that they are immediately miscible with water and a homogeneous mixture was obtained. This means that their outer phase is water. This result was also compatible with the electrical conductivity results.

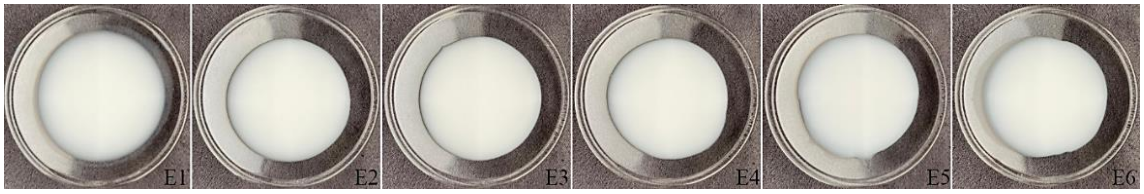

Figure 4. The images obtained by diluting the nanoemulsions with water

Preparation of Nanoemulgels with Povidone-Iodine

Nanoemulgels containing both SA and PI (100 mg) combined have been successfully prepared. The images of the nanoemulgels are given in Figure 2 below. When the PI studies in the literature are examined, it is seen that PI is generally used at the rate of 0.5% to 10% [12, 32, 33]. Therefore, in our study, the dose of PI was chosen as 2% in nanoemulgels. Since the color of PI was yellowish-brown/reddish-brown, the color of the nanoemulsion was as seen in the figure. Separation was observed in all nanoemulgels, except F1, which were subjected to 1-month stability at both room and refrigerator temperatures. The most separation was observed in F6 and decreased towards F2.

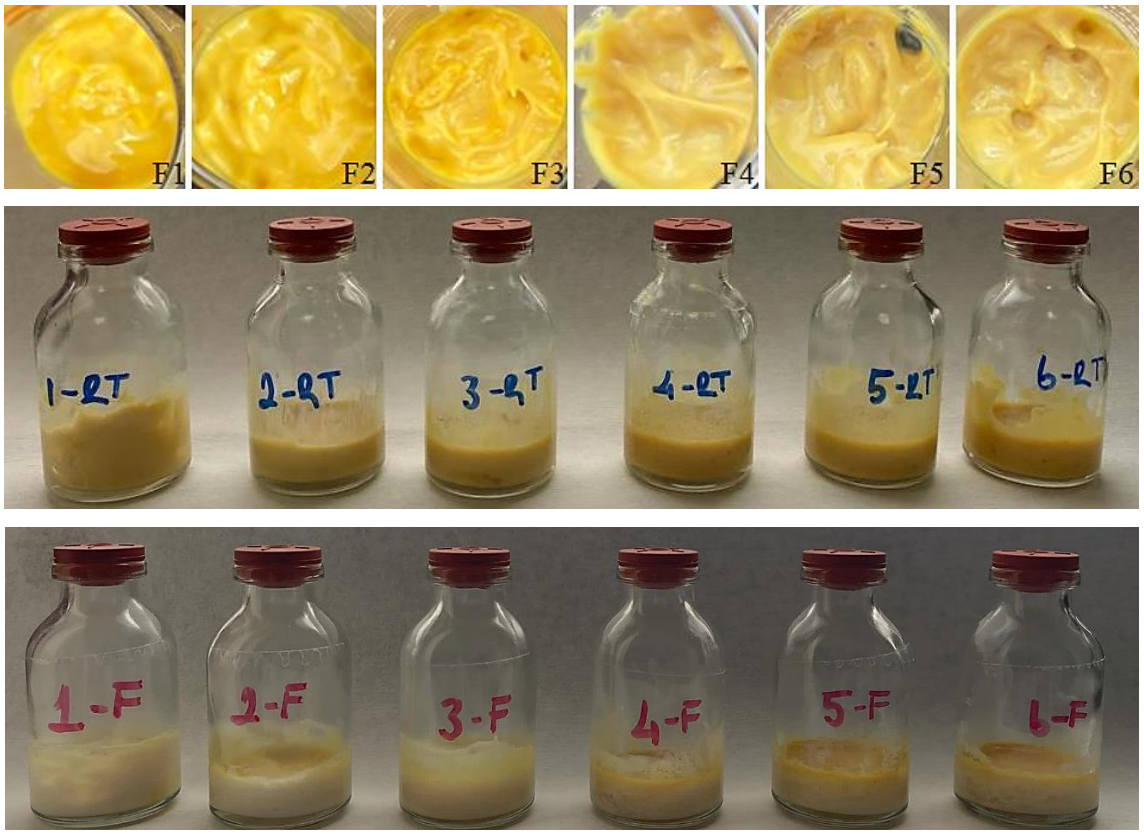

Figure 2. The images of freshly prepared (top) and kept for 1 month-nanoemulgels (RT: room temperature (middle) and F: refrigerator temperature (down))

pH Determination of Nanoemulgels

The pH measurement results of the nanoemulsions and nanoemulgels are given in Table 5. When the results are examined, it is seen that the pH's of both nanoemulsions and nanoemulgels are acidic. In addition, it was observed that the pH decreased more by gelling the nanoemulsions. The pH of the nanoemulgels was adjusted to 5.5 by the addition of NaOH and subjected to 1-month stability at room and refrigerator temperature. Normal skin pH is between 4.5 and 6.0. In the 1-month stability study, the pH values of the nanoemulgels were found in this range. Although separations were observed in most of the nanoemulgels after 1 month, the pH changes were not too much. There were no significant differences in pH between freshly prepared nanoemulsions and nanoemulgels, and nanoemulgels that were kept for 1 month at room and refrigerator temperatures ($p>0.05$).

Table 5. The pH measurement results of the nanoemulsions and nanoemulgels.

Formulation Code	Freshly Prepared-Room Temperature	Formulation Code	Freshly Prepared-Room Temperature	After 1 Month-Room Temperature	After 1 Month-Refrigerator Temperature
E1	2.80	**F1**	2.14	5.50	5.62
E2	2.78	**F2**	2.01	5.39	5.42
E3	2.76	**F3**	2.12	5.23	5.28
E4	2.68	**F4**	2.08	5.01	5.10
E5	2.64	**F5**	2.01	4.96	5.08
E6	2.62	**F6**	2.05	4.82	4.90

Spreadability of Nanoemulgels

Spreadability results of nanoemulgels which are freshly prepared and kept for 1 month are given in Table 6. Nanoemulgels are easily spreadable by applying a small force. When all the results were examined, it was observed that the spread was more in the F2 and F5 formulations where surfactants were used equally. On the contrary, these formulations showed minimal spread after 1 month of storage. It is thought that the reason for this is the higher water loss in these formulations. Similar results were seen in the study by Sharma and Tailang [34].

Table 6. Spreadability results of nanoemulgels which are freshly prepared and kept for 1 month.

Formulation Code	Spreadability (cm)	
	Freshly Prepared	After 1 Month-Room Temperature
F1	2.9	3.1
F2	4.1	2.5
F3	3.4	3.7
F4	1.9	4.0
F5	3.0	2.9
F6	2.1	4.2

In Vitro Drug Release of Nanoemulgels

As a result of the 1-month stability study performed, the release study was carried out with the F1 formulation since decompositions were observed in other formulations except for the F1 formulation. Pure SA was also evaluated in the release study. *In vitro* drug release profile of F1 and pure SA is given in Figure 6. The release medium had no further effect on the solubility of SA. It was found to be compatible with the results obtained from the solubility study. When the release profile is examined, it is seen that the pure SA is released slightly more than the nanoemulsion. However, this is a normal situation seen in water-insoluble active substances. When SA in the formulation passes from the oil phase in which it is soluble to the high viscosity aqueous phase in which it is insoluble, its release is slowed due to decreased solubility [35]. The polymer concentration that forms the hydrogel outside the droplets causes the release to be delayed due to the network-like structures formed in the gel [19]. In our study, after 8 hours, F1 released approximately 40%, while pure SA released approximately 45%. Sinha et al. prepared nanoemulsions of SA and conducted a release study using a dialysis membrane in a pH 7.4 phosphate buffer. At the end of 8 hours of the release study, approximately 50% of the SA nanoemulsion was released. However, pure SA solution in methanol showed about 99% release after 2 hours [23]. When compared with our study, it is quite clear that entrapping the SA nanoemulsion in a hydrogel slows the release of SA a little more. There are other studies in the literature that found similar results to ours [36].

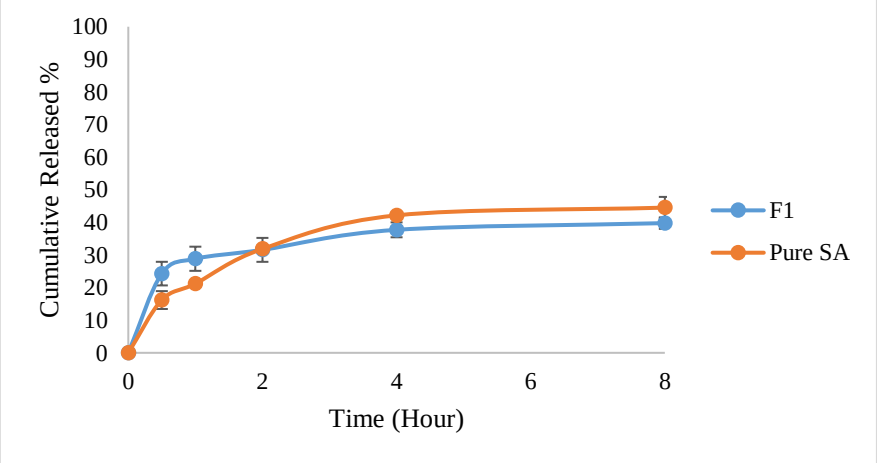

Figure 6. The release profile of F1 and pure salicylic acid

FT-IR Analysis of Nanoemulgels

The FT-IR spectra of the active substances, the nanoemulgels, and all the excipients used in the nanoemulgels are given in Figure 5 below. FTIR analysis of SA and PI is not very common in the literature. Since PI also contains groups similar to SA, it gave a similar FTIR spectrum [37]. SA has two specific functional groups, a carboxylic acid, and a phenol group. According to the chemical structure

of SA, the unique O-H phenol group gives specific peaks in the range of 3250-2750 cm^{-1} (O-H and C-H stretching 3233 cm-1 and 2999-2831 cm^{-1}), the O-H carboxylic acid group in the ranges of 2750-2250 cm^{-1} and 750-500 cm^{-1} (=C-H bending 760-669 cm^{-1}), and the C=O carboxylic acid group in the range of 1700-1500 cm^{-1} (C=O (COO-) asymmetric stretching 1652-1670 cm^{-1}) [38, 39]. When the results are examined, it is seen that the active substances and excipients in the formulations do not interact, and there is no change in the spectrum of the active substances.

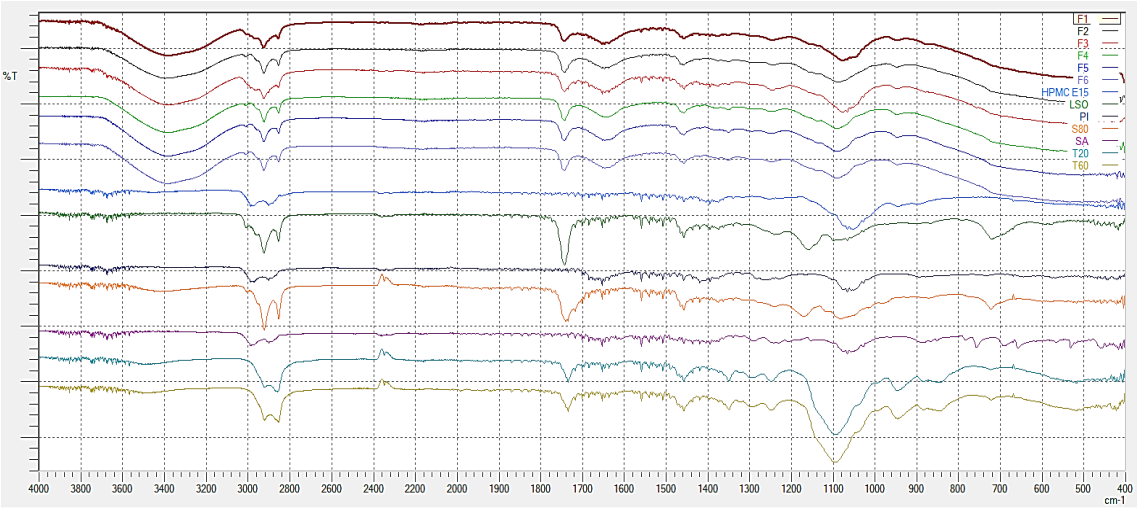

Figure 5. The FT-IR spectra of the active substances, the nanoemulgels, and all the excipients

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there are no actual, potential, or perceived conflicts of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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