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MICROEMULSION „WATER IN OIL“ AS A POTENTIAL SYSTEM FOR ASCORBIC ACID ENCAPSULATION

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Abstract

Microemulsions are optically isotropic and thermodynamically stable systems of water, oil and surfactant, often in combination with a cosurfactant which is required for optimal formation of microemulsion with a smaller aggregate radius and greater curvature of the interfacial layer. The full potential of microemulsion systems has yet to be realized and a lot of innovation in the field of microemulsion technology is expected. They have numerous advantages due to spontaneous formation, ease of manufacturing and scale-up, thermodynamic stability, ability to improve drug solubilization, and bioavailability. This work describes microemulsion systems which contain nonionic surfactants Tween 80 and Span 80, isopropyl myristate as the oil phase and purified water. From the most suitable microemulsion region, samples were selected and analyzed, blank and with encapsulated ascorbic acid, by measuring the size distribution of microemulsion aggregates, refractive index, electrical conductivity, and surface tension.

Keywords: microemulsion; micellization; phase diagram; surfactant; cosurfactant, dynamic light scattering, refractive indeks, electrical conductivity, surface tension.

МІКРОЕМУЛЬСІЯ «ВОДА В ОЛІЇ» ЯК ПОТЕНЦІЙНА СИСТЕМА ДЛЯ ІНКАПСУЛЯЦІЇ АСКОРБІНОВОЇ КИСЛОТИ

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Анотація

Мікроемульсії є оптично ізотропними і термодинамічно стабільними системами, що складаються з води, олії і поверхнево-активної речовини, часто у поєднанні з ко-сурфактантом, який потрібний для оптимального формування мікроемульсії з меншим агрегатним радіусом і більшою кривизною міжфазного шару. Потенціал мікроемульсій ще не повністю реалізований, і тому очікується багато інновацій в області технології мікроемульсій. Вони мають численні переваги завдяки спонтанному утворенню, простоті виробництва і масштабування, термодинамічній стабільності, здатності покращувати солюбілізацію ліків і біодоступність. У цій роботі описані мікроемульсивні системи, що містять неіонні поверхнево-активні речовини Tween 80 і Span 80, ізопропілмірістат в якості масляної фази і очищену воду. За допомогою вимірювань розподілу розмірів мікроемульсивних агрегатів, показника заломлення, електропровідності і поверхневого натягу з найбільш відповідної області мікроемульсії були відібрані і проаналізовані зразки, як порожні, так і з інкапсульованою аскорбіновою кислотою.

Ключові слова: мікроемульсія; мицелізація; фазова діаграма; ПАР; ко-сурфактант, динамічне розсіяння світла, коефіцієнт заломлення, електропровідність, поверхневий натяг.

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Introduction

In recent years, researchers' interest in microemulsion has increased significantly as a system that could improve the drug delivery system due to its significant properties [1–10]. Microemulsion is a unique system that is a transparent and optically isotropic phase consisting of oil, water and surfactants. It is considered an ideal system for delivering active ingredients due to its thermodynamically stable, low viscosity, easy to produce and has a small range of droplet sizes [7; 8]. Due to the small size of the droplet (20-200nm), the microemulsion is able to improve the penetration and absorption of the drug or active ingredient into the skin [11; 12]. In addition, the microemulsion system could provide a greater degree of solubility of lipophilic and hydrophilic active ingredients and prevent their decomposition [4; 6].

Ascorbic acid, unique for its high reactivity with all aggressive oxygen radicals, is the main and only essential antioxidant in the aqueous cell compartment [13]. It is believed that oral supplementation of ascorbate does not increase its concentration in the skin enough. Despite its very low lipophilicity, mouse skin is relatively high permeable to ascorbic acid, indicating its possible local application [14]. Topical use of ascorbic acid is also recommended due to its depigmentation activity and its ability to stimulate collagen synthesis [15]. Since ascorbic acid in the solution is subject to rapid oxidation, its use in pharmaceutical products is limited above all by its poor stability [10; 11].

The aim of this study is to produce a microemulsion of water in oil for topical application. This is achieved by constructing a phase diagram using isopropyl myristate, mixed surfactant Span 80 and Tween 80 in different mass ratio and water. From the phase diagram for physical characterization, a combination with a minimum amount of surfactant and the maximum amount of water is selected. Microemulsion with the most desirable physical properties is filled with ascorbic acid and tested its physical stability.

Experimental Section

Chemicals. Polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate (Span 80), ascorbic acid and isopropyl myristate were purchased from Merck, France. Suprapure water (declared conductivity 0.04 μS cm^{-1}) was prepared by a Millipore Simplicity (USA) unit.

Procedures and Apparatus

Particle Size and Polydispersity Index (PDI). The mean particle size (Z-average) and PDI were measured using the Anton Paar Litesizer 500 (Anton Paar, Austria) at 25.0 °C. The microemulsion was loaded in a clear quartz cell with a 1 cm path length. The particle size was measured in triplicate where each measurement consisted of 12 runs on the sample, and the average particle size was obtained.

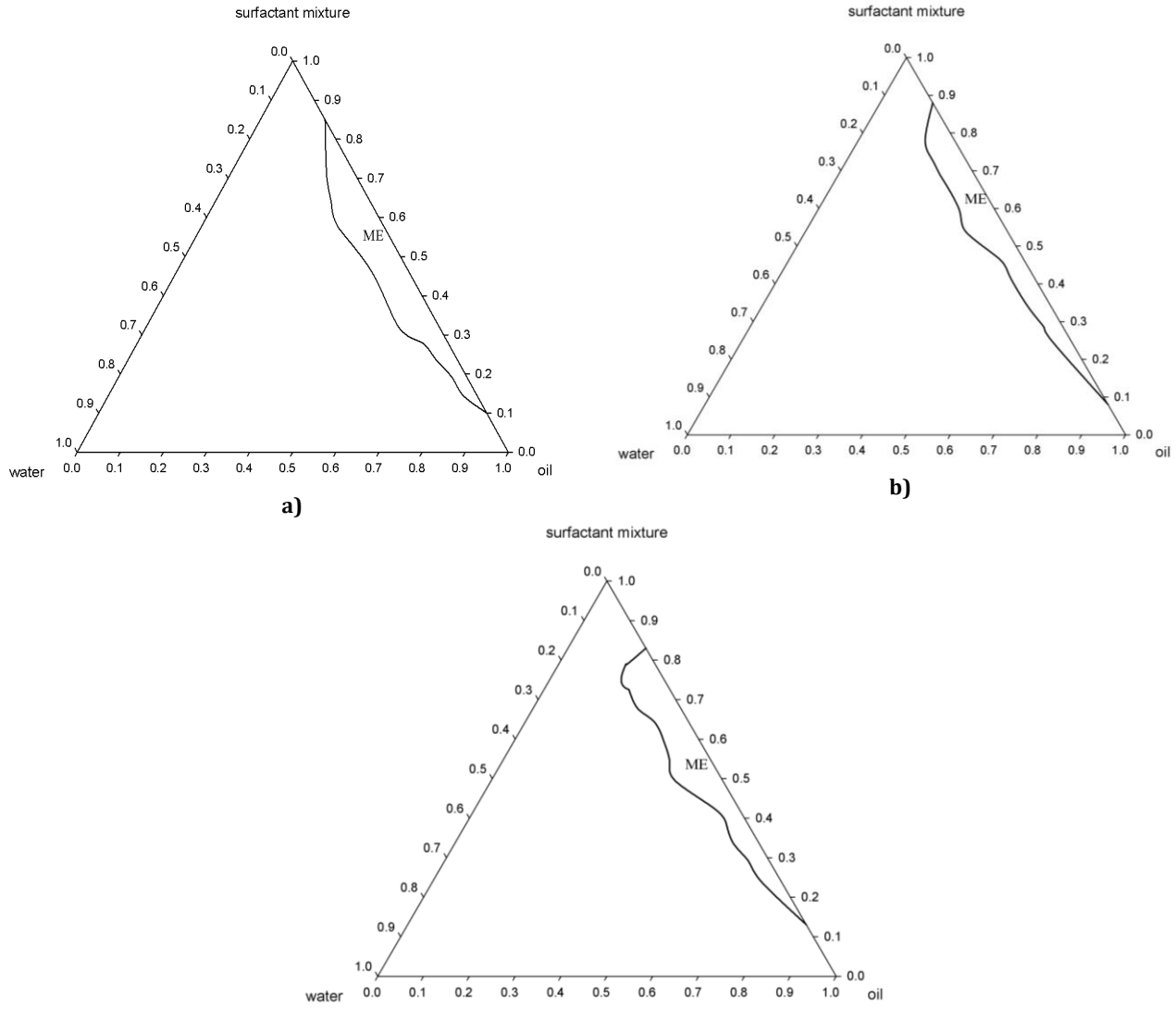
Electrical Conductivity. The electrical conductivity of the microemulsion samples was measured using a Mettler Toledo Five easy conductivity meter (Metter Toledo, Switzerland). All measurements were run in triplicate.

Viscosity, surface tension and refractive index. The viscosity of microemulsion samples was measured at 25 °C with a Brookfield viscometer (LVDV-E, Brookfield Engineering Laboratories, Middleboro, MA, USA) using spindle no. 61. with a shear rate of 30 rpm. The surface tension and refractive index of microemulsion samples were also measured at 25 °C with a tensiometer (SIGMA 703D, Biolin Scientific Oy, Finland) and Abbe refractometer ORT 1RS (Roth, Germany).

Results and Discussion

Ternary Phase Diagrams. The ternary phase diagrams (Fig. 1) show that the water-in-oil systems form arc-shaped (binodal curve) regions at low water content in the presence of surfactant ranging between 20 and 90 % (w/w). Microemulsions form within a narrower region between 30 and 50 % using Span 80 with Tween 80 mixed surfactant (ratio span 80/tween 80 = 60/40; span 80/tween 80 = 55/45) and within a broader range using Span 80 with Tween 80 mixed surfactant (ratio span 80 / tween 80 = 70/30). In order to accommodate the higher number of water-solubilized active ingredients, a higher water phase is required in the selected surfactant range. Therefore, microemulsions samples formed using Span 80 with Tween 80 mixed surfactant (ratio span 80/tween 80 = 70/30) were selected for further characterization and discussion (Fig. 2).

Size distribution, electrical conductivity, surface tension and refractive index. Microemulsion particle size results are given in Table. One of the factors that contributes to changes in microemulsion particle size is the packing parameter of the surfactants present in the formulation [17]. All three microemulsions have similar compositions used in the formulation. Hydrophobic fatty acid tail facing outwards to the oil phase.



Fig/ 1. Ternary phase diagram for the system of isopropyl myristate-water and surfactant mixtures a) span 80 / tween 80 = 70/30; b) span 80 / tween 80 = 60/40; c) span 80 / tween 80 = 55/45. ME is a single-phase region, while the labeled solid curve represents the binodal curve

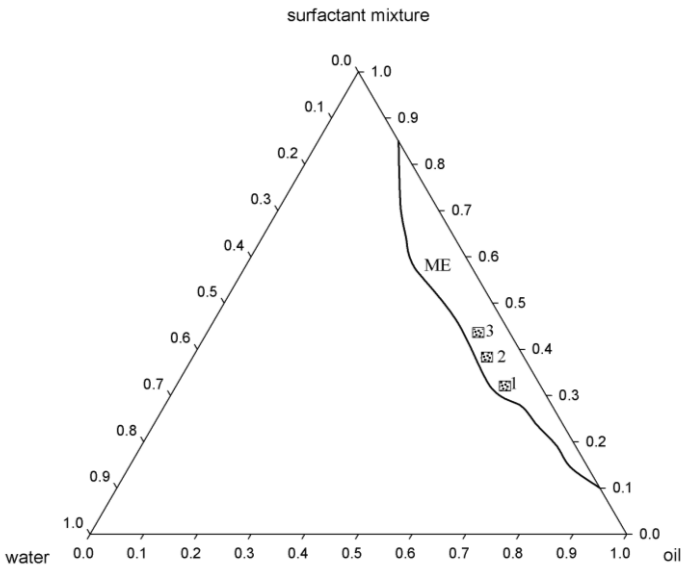


Fig. 2. Selected samples (marked with squares) of a ternary system of surfactant mixtures (span 80 / tween 80 = 70/30) – isopropyl myristate-water for measuring the size distribution of microemulsion aggregates by dynamic light scattering, refractive index, electrical conductivity and surface tension

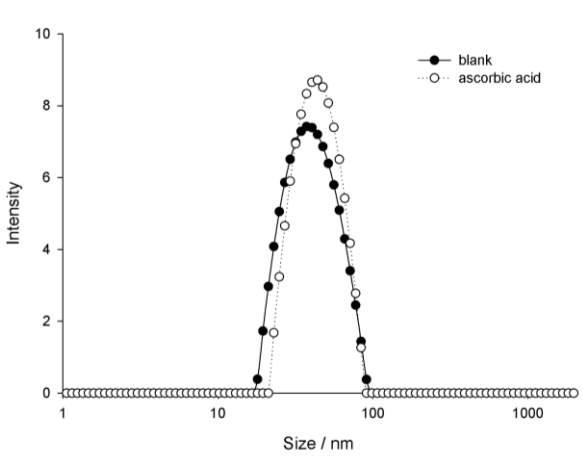
The particle size is also affected by the surfactant's HLB value. Surfactants with lower HLB values tend to have a less hydrated headgroup compared to surfactants with a higher HLB value. Surfactant headgroups that do not swell greatly can produce a more compact structure and give a smaller particle size [18]. Microemulsions with narrow distribution values around 0.15 suggest that the microemulsion droplets are uniform in size, which is the best case in microemulsion sample 1.

It is well known that there is a strong correlation between the specific structure of the microemulsion systems and their electrical conductive behavior, where phase systems ("oil in water" or "water in oil") of the microemulsions were determined by measuring electrical conductivity [19]. In this study, microemulsion formulations had an electrical conductivity less than 0.05 mS/cm for all samples, which indicates the "water in oil" structure of microemulsions. Also, "water in oil" structure of microemulsions is confirmed with color tests. The mean viscosity of formulations was 160 cps for sample 1, 178.2 cP for sample 2 and 273.4 for sample 3 which indicates formation of water-in-oil microemulsions. The particle size results correlate with viscosity, whereby microemulsions with small particle sizes have low viscosity [15; 16].

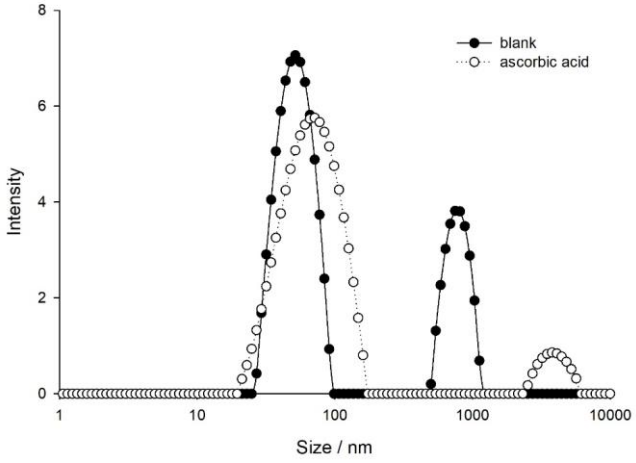
Table

Average size, PDI, refractive index, electrical conductivity, surface tension and viscosity for blank and encapsulated microemulsion samples

	Sample 1	**Sample 2**	**Sample 3**
Average size (nm) - blank	39.03±1.08	67.61±2.11	1327.6±38.14
PDI - blank	0.186	0.260	0.354
Average size (nm) – a. acid	44.78±4.28	122.3±5.02	851±18.22
PDI – a. acid	0.188	0,264	0.358
Refractive index - blank	1.4633	1.4625	1.4630
Refractive index – a. acid	1.4630	1.4629	1.4631
Electrical conductivity (mS cm^{-1}) - blank	0.0240	0.0220	0.0211
Electrical conductivity (mS cm^{-1}) – a. acid	0.0243	0.0218	0.0210
Surface tension (mNm^{-1}) – blank	30.05	30.16	30.09
Surface tension (mNm^{-1}) – a. acid	30.10	3.15	30.15
Viscosity (cP) – blank	160.2	178.2	273.4
Viscosity (cP) – a. acid	160.6	177.6	273.9



a)



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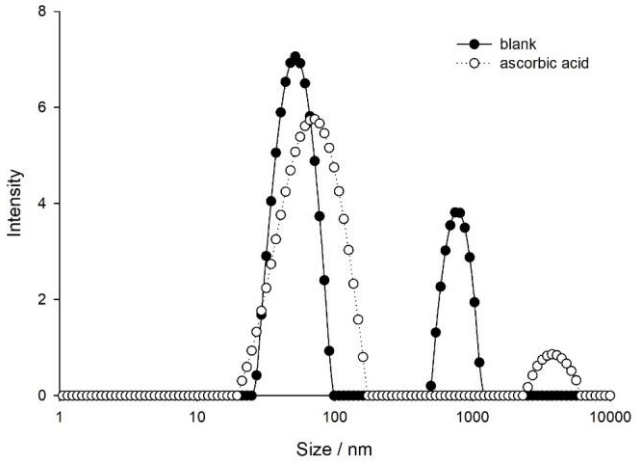

c)

Figure 3. The droplet size distribution by intensity for: a) sample 1, b) sample 2 and c) sample 3, blank and with ascorbic acid (0.5 % w/w concentration)

The surface tension of the formulations was around 30 mN/m for all microemulsion samples. It can be concluded that all samples had a "water-in-oil" structure because the surface tension and refractive index values were very close to the values of an oil continuous phase. The visual examination experiment was carried out over a period of 6 months in weekly intervals for the first 3 months and monthly intervals for the subsequent months. The visual observation showed for first two samples no evidence of phase separation or any precipitation or flocculation, but for sample 3 flocculation occurred. These first two samples also revealed no sign of phase separation under centrifugation stress at 8000 rpm for 20 min. The centrifugation tests showed that microemulsions remained homogenous without any phase separation, which points to their good physical stability. These two blank samples were further used for encapsulation ascorbic acid. Furthermore, physical characterisation visual examination experiment was conducted with encapsulated samples over a period of six months. No phase separation, turbidity, precipitation or coagulation of the samples was observed, and no significant change in physical parameters confirming that these microemulsions are stable. It was also proved that the addition of ascorbic acid did not affect the stability of the basic microemulsion composition [20–24].

Conclusion

The ternary phase diagrams show that the water-in-oil systems form arc-shaped (binodal curve) regions at low water content in the presence of surfactant ranging between 20 and 90 % (w/w). Microemulsions form within a narrower region between 30 and 50 % using Span 80 with Tween 80 mixed surfactant (ratio span 80/tween 80 = 60/40; span 80/tween 80 = 55/45) and within a broader range using Span 80 with Tween 80 mixed surfactant (ratio span 80/tween 80 = 70/30). All three microemulsions have similar compositions used in the formulation. Hydrophobic fatty acid tail facing outwards to the oil phase. The particle size is also affected by the surfactant's HLB value. Surfactants with lower HLB values tend to have a less hydrated headgroup compared to surfactants with a higher HLB value. Surfactant headgroups that do not swell greatly can produce a more compact structure and give a smaller particle size. It can be concluded that all samples had a "water-in-oil" structure because the surface tension and refractive index values were very close to the values of an oil continuous phase. The visual observation showed for first two samples no evidence of phase separation or any precipitation or flocculation, but for sample 3 flocculation occurred. These two blank samples were further used for encapsulation ascorbic acid. Physical characterisation visual examination experiment was conducted with encapsulated samples over a period of six months. No phase separation, turbidity, precipitation or coagulation of the samples was observed and no significant change in physical parameters confirming that these microemulsions are stable. It was also proved that the addition of ascorbic acid did not affect the stability of the basic microemulsion composition.

References

[1] Wang, X., Zan, M., Amuti, A., Shu, Q., Wang, Z. (2021). Evaluation of the oxidation stability and anti-cancer cell activity of Paeonia ostii seed oil and its linolenic acid fractions delivered as microemulsions, *J. Mol. Liq.*, 342, 117579. https://doi.org/10.1016/j.molliq.2021.117579

[2] S. Sargazia, Hajinezhadb M. R., Baranici, M., Rahdard, A., Shahrakia, Sh., Karimie, P., Cucchiarinif, M., Khatamig, M., Pandey, S. (2021). Synthesis, characterization, toxicity and morphology assessments of newly prepared microemulsion systems for delivery of valproic acid, *J. Mol. Liq.*, 338, 116625 https://doi.org/10.1016/j.molliq.2021.116625

[3] Amuti, A., Wang, X., Zan, M., Lv, S., Wang, Z. (2021). Formulation and characterization of curcumin-loaded microemulsions: Evaluation of antioxidant stability and in vitro release, *J. Mol. Liq.*, 336, 116881. 10.1016/j.molliq.2021.116881.

[4] Špaglová M., Čuchorová M., Šimunková V., Matúšová D., Čierna M., Starýchová L., Bauerová K. (2020). Possibilities of the microemulsion use as indomethacin solubilizer and its effect on in vitro and ex vivo drug permeation from dermal gels in comparison with transcutol®, *Drug Dev. Ind. Pharm.*, 46(9), 1468–1476. 10.1080/03639045.2020.1802483.

[5] Mitsou, E., Pletsa, V., Sotiroudis, G. T., Panine, P., Zoumpanioti, M., Xenakis, A. (2020). Development of a microemulsion for encapsulation and delivery of gallic acid. The role of chitosan," *Colloids Surfaces B Biointerfaces*, *190*(March), 110974. 10.1016/j.colsurfb.2020.110974.

[6] Benigni, M. Pescina, S. Grimaudo, M. A. Padula, C. Santi, P. Nicoli, S. (2018). Development of microemulsions of suitable viscosity for cyclosporine skin delivery, *Int. J. Pharm.*, *545*(1–2), 197–205. 10.1016/j.ijpharm.2018.04.049.

[7] Tabosa, M. A. M., de Andrade, A. R. B., Lira, A. A. M., Sarmento, V. H. V., de Santana, D. P. L., Leal, (2017). Microemulsion Formulations for the Transdermal Delivery of Lapachol, *AAPS Pharm Sci Tech*, *19*(4), 1837–1846, 2018. 10.1208/s12249-018-0995-2.

[8] Froelich, A., Osmałek, T., Snela, A., Kunstman, P., Jadach, B., Olejniczak, M., Roszak, G., Białas, W. (2017). Novel microemulsion-based gels for topical delivery of indomethacin: Formulation, physicochemical properties and in vitro drug release studies, *J. Colloid Interface Sci.*, 507, 323–336. 10.1016/j.jcis.2017.08.011.

[9] Kaur G., Mehta, S. K. (2017). Developments of Polysorbate (Tween) based microemulsions: Preclinical drug delivery, toxicity and antimicrobial applications," *Int. J. Pharm.*, 529(1–2), 134–160. 10.1016/j.ijpharm.2017.06.059.

[10] J. Fuchs, "Potentials and limitations of the natural antioxidants RRR-alpha- tocopherol, L-ascorbic acid and β-carotene in cutaneous photoprotection," *Free Radic. Biol. Med.*, vol. 25, no. 7, pp. 848–873, 1998, doi: 10.1016/S0891-5849(98)00161-0.

[11] Su, R. Fan, W., Yu, Q., Dong, X., Qi, J., Zhu, Q., Zhao, W., Wu, W., Chen, Zh., Li Y., Lu, Y. (2017). Size-dependent penetration of nanoemulsions into epidermis and hair follicles: Implications for transdermal delivery and immunization, *Oncotarget*, 8(24), 38214–38226. 10.18632/oncotarget.17130.

[12] Nastiti, C. M. R. R., Ponto, T., Abd, E., Grice, J. E., Benson, H. A. E., Roberts, M. S. (2017). Topical nano and microemulsions for skin delivery, *Pharmaceutics*, 9(4), 1–25. 10.3390/pharmaceutics9040037.

[13] Padayatty, S. J., Katz, A., Wang, Ya., Eck, P., Kwon, O., Lee, Je-H., Chen, Sh., Corpe, Ch., Dutta, A., Dutta S. K., Levine M. (2003). Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention, *J. Am. Coll. Nutr.*, *22*(1), 18–35. 10.1080/07315724.2003.10719272.

[14] Ae-Ri Cho Lee, K. T. (1998). Characterization of Skin Permeation of Vitamin C : Theoretical Analysis of Penetration Profiles and Differential Scanning Calorimetry Study, *Chem. Pharm. Bull.*, *46*, 174–177. 10.1248/cpb.46.174.

[15] Yi, N., Chiang, Z. (2017). Topical Vitamin C and the Skin, *Jcad J. Clin. Aesthetic Dermatology*, *14*(7), 14–17.

[16] Gallarate, M., Carlotti, M. E., Trotta, M., Bovo, S. (1999). On the stability of ascorbic acid in emulsified systems for topical and cosmetic use, *Int. J. Pharm.*, *188*(2), 233–241, 10.1016/S0378-5173(99)00228-8.

[17] Liu, J., Zhang, X., Zhang, H. (2014). Water /AOT/IPM/alcohol reverse microemulsions: Influence of salts and nonionic surfactants on structure and percolation behavior, *J. Chem. Thermodyn.*, 72, 1–8, 10.1016/j.jct.2013.12.026.

[18] Mishra, B.K., Kuanar, M., Chauhan, G. (2000). Behaviour of non-ionic microemulsion containing Tween-80, iso-amyl alcohol, hexane and water, 7(Novemb.

[19] Eicke, H. F. Borkovec, M. Das-Gupta, B. (1989). Conductivity of water-in-oil microemulsions: A quantitative charge fluctuation model, *J. Phys. Chem.*, 93(1), 314–317. 10.1021/j100338a062.

[20] Liović, N., Bošković, P., Drvenica, I., Jambrak, A. R., Dropulić, A. M., Krešić, G., Nedović, V., Bugarski, B., Zorić, Z., Pedisić, S., Bilušić T. (2019). Phenolic Extracts from Vaccinium corymbosum L. Loaded in microemulsions and liposomes as enhancers of olive oil oxidative stability, *Polish J. Food Nutr. Sci.*, 69(1), 23–33. 10.31883/pjfns-2019-0003.

[21] Rozman B., Gašperlin, M. (2007). Stability of vitamins C and E in topical microemulsions for combined antioxidant therapy, *Drug Deliv.*, *14*(4), 235–245. 10.1080/10717540601067786.

[22] Liu, W., Sun, D., Li, C., Liu, Q., Xu, J. (2006).Formation and stability of paraffin oil-in-water nano-emulsions prepared by the emulsion inversion point method, *J. Colloid Interface Sci.*, *303*(2), 557–563. 10.1016/j.jcis.2006.07.055.

[23] Hsu, J. P., Nacu, A. (2003). Behavior of soybean oil-in-water emulsion stabilized by nonionic surfactant, *J. Colloid Interface Sci.*, 259(2), 374–381. 10.1016/S0021-9797(02)00207-2.

[24] Yew, H. C., Bin Misran, M. (2016). Nonionic Mixed Surfactant Stabilized Water-in-Oil Microemulsions for Active Ingredient in Vitro Sustained Release. *J. Surfactants Deterg.*, *19*(1), 49–56. 10.1007/s11743-015-1753-z.