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Research Article

Chlorogenic acid nanoemulsion for *Staphylococcus aureus* causing skin infection: Synthesis, characterization and evaluation of antibacterial efficacy

Bahar GOK[1], Yasemin BUDAMA-KILINC[2,3]*

[1]*Department of Bioengineering, Graduate School of Natural and Applied Science, Yildiz Technical University, Istanbul, 34349, Türkiye*
[2]*Department of Bioengineering, Faculty of Chemical and Metallurgical Engineering, Yildiz Technical University, Istanbul, 34349, Türkiye*
[3]*Health Biotechnology Joint Research and Application Center of Excellence, Istanbul, 34220, Türkiye*

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ABSTRACT

Staphylococcus aureus (S. aureus) causes many skin infections such as impetigo, infected abrasions, cellulitis, folliculitis, subcutaneous abscesses, infected ulcers and sores. In this study, it was aimed to develop a nanoformulation of chlorogenic acid that was efficient against *S. aureus*. In this context, ultrasonic emulsification method was used for production of the chlorogenic acid (CA) nanoemulsion formulation and was characterized in detail. In addition, the kinetic and thermodynamic stability of the CA nanoemulsion formulation was examined. Finally, the broth microdilution method was used to determine the antibacterial activity of the formulation. It was determined that the average droplet size of the CA nanoemulsion formulation was 120.4 ± 6.39 nm, the polydispersity index (PdI) was 0.180 ± 0.018, and the zeta potential value was -11.5± 1.15 mV. As a result of the thermodynamic stress tests of the CA nanoemulsion formulation, it was observed that there was no precipitation or phase separation. Moreover, *in vitro* release study showed a CA release of 75.49% after 48 hours. The antibacterial results revealed that the CA nanoemulsion formulation was efficient (95% inhibition) against *S. aureus* (ATCC 25923). As a result, it is thought that CA nanoemulsion is an effective, nano sized and controlled release system based formulation candidate that may be used in the topical treatment of *S. aureus* causing skin infection.

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***Corresponding author.**
*E-mail address: budama@yildiz.edu.tr

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INTRODUCTION

Bacterial infections of the skin and soft tissue are among the most common infections worldwide [1]. A well-known bacterium, *S. aureus*, a well-known bacterium, is the leading cause of infections in impetigo and infected abrasions. It is known that *S. aureus* causes superficial skin infections and soft tissue infections such as impetigo and infected abrasions. In addition, it causes more complex skin infections such as cellulitis, folliculitis/furunculosis, subcutaneous abscesses, infected ulcers, and lesions [2,3]. *S. aureus* usually starts with an inflammatory process in the epidermis, dermis, or subcutaneous tissues [4] with no pathogeny or symptoms of infection [5], and can spread to other parts of the body, cause severe infections such as endocarditis, osteomyelitis, necrotizing pneumonia, and sepsis [6,7]. It is known that topical antibiotics do not provide an adequate influence on the treatment of deep tissue infections due to their poor penetration ability [8]. In addition, most antibiotics may lose their effectiveness over time because of antimicrobial resistance [9]. The reason *S. aureus* infection is becoming increasingly difficult to treat is the breakout and rapid spread of methicillin-resistant *S. aureus* (MRSA)[10]. This bacterium is resistant to all known β-lactam antibiotics. Worryingly, Nannini et al. reported that there was a resistance to linezolid and daptomycin and reduction susceptibility to vancomycin in clinical strains of MRSA [11]. This issue is a major challenge in clinical practice globally [12-14]. The emergence of resistance to conventional antibiotic treatments has become a forefront the need for novel and safer antibacterial drugs and alternative controlled release systems [15-17]. The complex action mechanism of the plant polyphenols on bacteria compared to conventional antibiotics make them a strategic antibacterial candidate [18,19]. CA is a polyphenolic natural compound that has antibacterial, antifungal, antiviral, antiphlogistic, and antioxidant features [20,21]. It is commonly found in tea leaves, coffee beans, apples, and grapes [22]. It has been reported that antimicrobial activities on various Gram-positive and Gram-negative bacterial pathogens are through cell membrane permeability [20,23].

Intravenous injection (IV) and oral administration of antimicrobial agents for infections in the deep layers of the skin is more successful than the topical drug delivery system. However, these administration methods may become ineffective because of vascular insufficiency, diabetes and similar reasons [8]. For these reasons, the development of new nanocarriers, topical drug delivery systems that can carry the antimicrobial agent to the deep layers of the skin and exert its antimicrobial activity are of great importance [24-26].

Nanoemulsions (NEs) are nano-sized droplets used as carriers against enveloped viruses, fungi and bacteria [27-30]. Meral *et al*., (2019) synthesized thyme oil NEs and investigated their antibacterial effects on *Pseudomonas aeruginosa*, *Escherichia coli and Salmonella typhimurium*. Zeta potential and PdI of thyme oil NEs (TONa and TONb) with diameters of 219 nm and 163 nm, respectively, were defined as 19.77, 0.24 and -24.80, 0.054, and they reported that these NEs have effective antibacterial effects [31]. Ceylan *et al*., (2020) synthesized curcumin and rosemary oil NEs (CUR and RON) with average diameters of 184.3 nm and 158.3 nm by sonication technique [32]. They evaluated the effectiveness of NEs against *P. aeruginosa, E. coli* and *S. typhimurium*. It has been reported that CUR shows varying levels of antibacterial activity against three bacterial species, while RON shows antibacterial activity only against *S. typhimurium.* In another study, a nanoemulsion was developed with glyceryl monocaprylate (GMCY), which is known to have bactericidal activity for the treatment of infected wounds. Three common wound bacteria (*S. aureus*, *P. aeruginosa* and *E. coli*) were used for this [33]. As a result of the study, it was shown that GMCY nanoemulsion exhibited antibacterial properties against *S. aureus* and *E. coli.* NEs are thermodynamically stable systems with a translucent appearance [34,35]. It can be prepared using high-energy methods such as ultrasonication or homogenization [36,37]. NEs are formulations with small droplet size (<200 nm) that provide a large surface area in contact with the skin [38-40]. These systems are widely used for the controlled release of nutraceuticals and active pharmaceutical ingredients into the skin layers of the skin [41,42].

In our study, CA-NE formulation was produced by using the ultrasonic emulsification method, and then characterized. The parameters such as average droplet size, zeta potential PdI were analyzed by Dynamic laser light scattering (DLS). Drug content assay and *in vitro* release profile were determined by UV-Vis spectrophotometer. The kinetic and thermodynamic stability of the CA-NE formulation was examined by accelerated stability tests (centrifuge and thermal stress tests). Finally, the antibacterial effect of CA-NE formulation against *S. aureus* was determined by Minimum Inhibitory Concentration (MIC) test.

MATERIALS AND METHODS

Materials

Pluronic® F-68, ethanol, DL-alpha tocopherol acetate, Muller-Hinton broth and Muller Hinton agar from Sigma-Aldrich; Chlorogenic Acid from Alfa Aesar; Isodecyl neopentanoate (DUB VCI 10) from Stearinerie Dubois; Undecyl alcohol (Sensiva® PA 30) was purchased from Schülke and Caprylic/capric triglyceride (Labrafac Lipophile WL 1349) was purchased from Gattefosse.

Methods

Preparation of CA-NE

CA-NE was synthesized using ultrasonic homogenization method in NE type, in which the aqueous phase is a continuous phase (O/W) [43]. The water phase of NE was

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prepared with distilled water to be 15% Pluronic® F-68. In the oil phase, 1% Isodecyl neopentanoate, 2% undecyl alcohol, 11.675% Caprylic capric triglyceride, and 0.2% DL-alpha tocopherol acetate, 2.5% Transcutol, 5% Labrafil and 17.8 mg CA were used. The water phase and oil phase were prepared in separate beakers and then the water phase was mixed into the oil phase. It was then mixed for 5 minutes at 8100 rpm using a homogenizer (Witeg, Germany). After premixing, the emulsion was ultrasonically emulsified using a 20 kHz and 750 W Sonicator (Ultrasonics, USA). Ultrasonication was performed at 50% amplitude for 20 minutes.

Dynamic Light Scattering (DLS) Analysis of CA-NE

The critical parameters such as average droplet size, PdI and zeta potential values of the NE were analyzed at 25 °C by using Zeta Sizer Nano ZS (Malvern Instruments, UK). Samples were prepared in distilled water (1:100 v/v), and then analyzed within 24 hours in triplicate [44].

Electrical Conductivity and pH Measurements of CA-NE

The probes of a pH meter (Ohaus® STARTER 3100M) were used for the determination of the electrical conductivity and the pH values of formulation. The analyzes were determined at 25 °C in triplicate.

Accelerated Stability Studies of CA-NE

The accelerated thermodynamic stability of NE was performed within 24 hours by centrifugation and thermal stress tests. 15 g of CA-NE formulation was centrifuged at 25±1°C at 4,500 rpm for 20 minutes. Macroscopically, any phase separation or turbidity was evaluated [45]. Thermal stability test was accomplished to determine the organoleptic analysis of CA-NE formulations, such as color, odour, consistency, presence of precipitate and phase separation. 10 g of CA-NE formulation was exposed to a temperature increase of 5 °C from 40 °C to 80 °C in a water bath. Data on the appearance (such as no phase separation, agglomeration or creaming) of the formulation were obtained as a result of centrifugal and thermal stress tests.

Analysis of Active Substance Content

For the determination of active substance (CA) content, 0.5 mL of CA-NE was taken into a flask, its volume was completed to 10 mL with methanol. After it was extracted in an ultrasonic bath for 30 minutes, it was analyzed by UV-Vis spectrometer at 324 nm. Finally, the concentration of CA was calculated with the calibration curve (R^2 = 0.999).

***In Vitro* Release Study of CA-NE**

Since the CA-NE formulation was developed to use for topical application, the *in vitro* release study was conducted at skin pH (5.0-6.0) and temperature (32°C) [46]. 1 mL of CA-NE was put in a dialysis capsule, and the samples were taken from 5 mL of release medium at specified time intervals in a shaking water bath at 120 rpm, and replaced with an equal amount of fresh release medium. The absorbance values of each sample were analyzed by the UV-Vis spectrometer at 324 nm. The release (%) of CA was calculated using Equation (1).

$$Release\ \% = \frac{Released\ CA}{Total\ CA} \times 100 \quad (1)$$

Antibacterial Activity

The antibacterial activity of the free CA and CA-NE formulation on *S. aureus* ATCC 25923 was determined by the broth microdilution method. Bacterial culture was activated on Mueller-Hinton Agar (MHA) at 37°C for 24 hours. After incubation, serial dilutions were prepared by adding compounds to 96-well plates with 100 µL of Mueller Hinton Broth (MHB). Serial dilutions were prepared to start from 0.178 mg/mL for the free CA, and 1 mg/mL for the CA-NE formulation. Bacteria were incubated at 35-37 °C for 18-24 hours. The plate was analyzed with an ELISA reader. The percentage (%) of bacterial growth inhibition was determined as Equation (2); where A_c is the absorption value of the negative control and A_t is the absorption value of samples.

$$Inhibition\ (\%) = \frac{A_c - A_t}{A_c} \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Characterization of CA-NE

It has been stated in the literature that the ultrasonic homogenization method is quick and effective technique in order to prepare stable NE with the desired droplet size and low PdI [47,48]. In our study, NE formulation was synthesized by ultrasonic homogenization method, and then characterization and stability studies were conducted. DLS, which is one of the most preferred method, was used to determine the hydrodynamic size, PdI and zeta potential values of the synthesized NE [49-53]. The results were given in Figure 1 and Figure 2. The blank NE had average droplet size of 110.8 ± 0.14 nm. On the other hand, CA-NE had average droplet size of 120.4 ± 6.39 nm. It is known that the nanoemulsions are nanosystems with smaller droplet size between 20 and 200 nm compared to emulsions [54,55]. NEs with the small droplet sizes provides advantages in order to overcome the challenges in nano-size study such as gravity separation, flocculation, and coalescence [31,54]. In many studies in the literature, it has been stated that the droplet sizes ranging from 100 to 200 nm can provide advantages for NEs in topical applications because of their superior penetration and controlled release properties [37,55,56]. Based on this information and our average droplet size result, CA-NE formulation is considered suitable for topical applications.

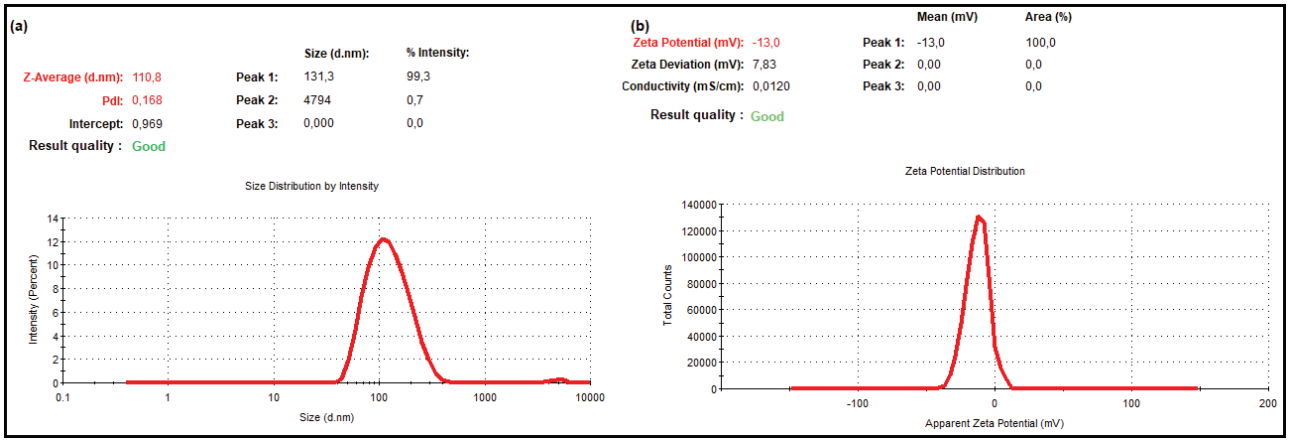

Figure 1. DLS analysis of blank NE; (a) average droplet size, and (b) Zeta potential graphs.

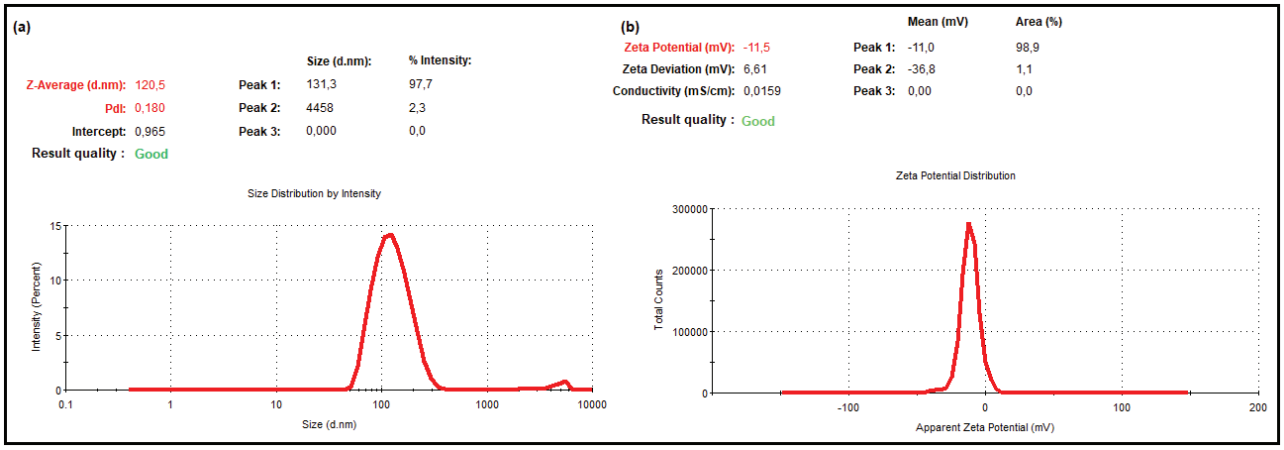

Figure 2. DLS analysis of CA-NE; (a) average droplet size, and (b) Zeta potential graphs.

The PdI value is a measure of the homogeneity and stability of the droplet size in the NE. A smaller value of less than 0.1 for PdI means that monodisperse size distribution for droplets, while large value greater than 0.2 for PdI means heterogeneity within the size distribution of droplets [31]. It was found that the blank NE had PdI value of 0.168 ± 0.006, and CA-NE had PdI value of 0.180 ± 0.018. From the obtained results, it was determined that the formed droplets had a narrow size distribution.

The zeta potential is an indirect measurement method of the electric charge of particles in the colloidal system, and it is used to determine approximately the surface charge of NE droplets [57]. The oils used in the oil phase of the formulation consist of fatty acids. The negative charge of the droplets' zeta potential values is due to the carboxylic acid groups of fatty acids [58]. It was found that the blank NE had zeta potential of -13 ± 4.03 mV, and CA-NE had zeta potential of -11.5 ± 1.15 mV. These results were similar to the reported in previous studies in the literature [56,59-62].

pH and Conductivity

The pH value is crucial to evaluate the stability of nanoemulsions. pH changes that may occur in the formulation may indicate the possibility of chemical reactions that may cause problems in the quality and stability of the final product [63]. The pH value of human skin is approximately between 4.6 and 5.8 [46]. It was found that the pH values obtained from our study were suitable for topical application (Table 1).

Similar to pH change, electrical conductivity changes can also provide information about the quality and stability of NEs [45,64]. Electrical conductivity measurement for NE

Table 1: pH and conductivity results of NE formulation. (Data are presented as mean ± SD)

Formulation	pH	Conductivity (µS/cm)
Blank-NE	5.70 ± 0.06	87 ± 0.30
CA-NE	5.31 ± 0.33	96.2 ± 1.58

is a simple, inexpensive, and essential method. Electrical conductivity measurement means that the aqueous phase is the continuous phase (O/W), and high electrical conductivity values are observed in such nanoemulsions [64]. In the present study, high conductivity values were obtained for the blank NE and CA-NE formulations. It indicated that the external phase of the synthesized NE formulation was water (Table 1).

Accelerated Stability Tests

Accelerated stability tests provide useful information in predicting the stability of colloidal systems [65]. Centrifugal and thermal stress test were performed to evaluate the thermodynamic stability of the formulation. Thermodynamic stability problems in colloidal systems are observed as coalescence, creaming, and phase separation. The results showed that none of the stability problems were observed in the developed formulation (Table 2).

Table 2: Accelerated stability test results of NE formulation

Formulation	Organoleptic characteristics
Blank-NE	milky and bluish aspect
CA-NE	milky and bluish aspect

Analysis of Active Substance Content

In this study, CA-NE formulation was synthesized as oil-in-water type nanoemulsion, and its continuous phase was the aqueous phase. Extraction was performed by using methanol to determine the active ingredient content (CA) of the formulation. Then, the sample was analyzed with a UV-Vis spectrometer [66] at 324 nm. The active substance content of CA-NE formulation was calculated via the calibration curve of CA (R^2 = 0.999), and it was found to be 99.7 ± 0.96 %.

***In Vitro* Release Profile of CA-NE Formulation**

NEs are unique systems for the delivery of active ingredients to the target area without degradation, and their controlled release ability. In our study, the *in vitro* release study was conducted by simulating skin pH and temperature to determine the controlled release profile of the CA-NE formulation when applied to the skin. In Figure 3, it was shown that *in vitro* release plot of CA-NE was plotted depending on time and cumulative release of CA (%). As seen in the graph, the release profile shows that 65.04 ± 2.00% of CA is released in the first 6 hours. This may be due to its initial burst release [67]. It was determined that 70.85 ± 4.00% and 75.49 ± 1.09% of CA was released in the first 24 hours and 48 hours from the nanoemulsion formulation, respectively. In conclusion, our results showed that the CA-NE formulation released CA in a controlled and exhibited a slow release profile after 24 hours.

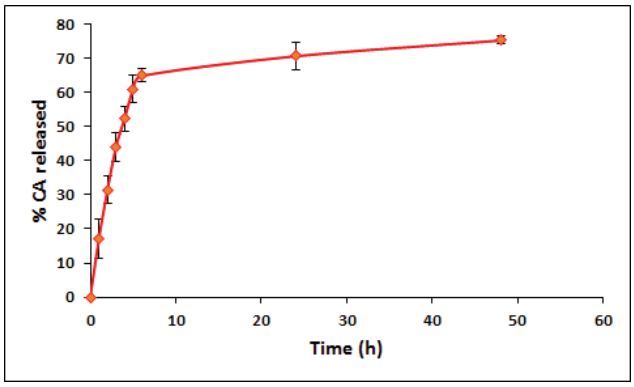

Figure 3. *In vitro* release profile of CA (%).

Bacterial Activity Assay

The antimicrobial activity of CA, CA-NEs was evaluated by using the broth microdilution method. The initial concentration of CA and CA-NE was determined according to the *in vitro* release profile. Bacterial growth inhibition values of free CA and CA-NEs on *S. aureus* were found to be 85% and 95%, respectively (Table 3).

Tablo 3: Determination of % inhibition values of CA and CA-NE

Treatment	% inhibition
CA	85%
CA-NE	95%

Up to now, various studies have been carried out to develop a nanoemulsion formulation with desired physicochemical properties and biological activity such as antibacterial efficiency [57, 74-78]. In many studies in the literature, it has been reported that nanoemulsion formulations have a suitable surface property that provides an easier contact area with microorganisms owing to their nano-sized droplets. Thus, it was observed that the antimicrobial activities of bioactive contents formulated as NEs increased [28,68,69].

The bacteria cell membrane contains charged lipids with high binding efficiency and electrostatic attraction potential [70,71]. On the other hand, nanoemulsions have a structure containing charged lipids in the outer shell layers and bioactive compounds in the core. NEs are colloidal solutions consisting of nanosized droplets and have physicochemical properties that remain stable for a long time [57]. NEs interact with lipids of bacteria to cause bacterial death [72]. Majeed et al., (2016) developed a clove oil nanoemulsions and evaluated its antibacterial effect on gram positive bacteria (*L. monocytogenes* ve *S. aureus*) and gram negative bacteria (*E. coli*) [73]. The results reported that negatively charged clove oil nanoemulsion were effective against *L.*

monocytogenes and *S. aureus*, but the effect was low against *E. coli*. Dilen et al., (2008) reported that positively charged polymeric nanoparticles interact with the lipopolysaccharide of gram-negative bacterial cell walls [74]. The results of the studies clearly show that the nanomaterial load plays an important role in the antibacterial activity on gram positive and gram negative bacteria. In our study, the negatively charged CA-NE formulation may have interacted well with the LPS of the gram-positive bacterial cell wall. Therefore, it may have shown antibacterial activity on *S. aureus* strains.

Electrostatic attraction increases their possibility of combining with charges on the bacterial surface. When NEs bind with bacteria, they empty their internal contents resulting in cell lysis [69,75]. Therefore, the NE formulation of bioactive content shows a more effective antibacterial activity than the free bioactive content. In this study, when the inhibition results were examined, it was determined that the CA NE formulation provided a more effective bacterial inhibition against *S. aureus* compared to free CA. Our study overlaps with the articles reporting that the bioactive content formulated as a NE has a more effective bacterial inhibition than the free bioactive content [16,76-81].

CONCLUSION

NEs have been widely used in the fabrication of bioactive content loaded nanocarriers for topical applications. These formulations are not only guaranteeing a controlled release profile but also high penetration with small droplet size and large surface area for an efficient topical application.

Based on overall our findings, it was concluded that CA-NE formulation had an effective inhibition property on *S. aureus* with the nano-size formulated and controlled release of CA.

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AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

REFERENCES

[1] Dréno B, Araviiskaia E, Berardesca E, Gontijo G, Sanchez Viera M, Xiang L, et al. Microbiome in healthy skin, update for dermatologists. J Eur Acad Dermatol Venereol 2016;30:2038–2047. [CrossRef]

[2] Moran GJ, Krishnadasan A, Gorwitz RJ, Fosheim GE, McDougal LK, Carey RB, et al. Methicillin-resistant S. aureus infections among patients in the emergency department. N Eng J Med 2006;355:666–674. [CrossRef]

[3] McCaig LF, McDonald LC, Mandal S, Jernigan DB. Staphylococcus aureus-associated skin and soft tissue infections in ambulatory care. Emerg Infect Dis 2006;12:1715. [CrossRef]

[4] Diaz JH, Lopez FA. Skin soft tissue and systemic bacterial infections following aquatic injuries and exposures. Am J Med Sci 2015;349:269–275. [CrossRef]

[5] Robbins JR, Bakardjiev AI. Pathogens and the placental fortress. Curr Opin Microbiol 2012;15:36–43. [CrossRef]

[6] Lowy FD. Staphylococcus aureus infections. N Eng J Med 1998;339:520–532. [CrossRef]

[7] Lehar SM, Pillow T, Xu M, Staben L, Kajihara KK, Vandlen R, et al. Novel antibody-antibiotic conjugate eliminates intracellular S. aureus. Nature 2015;527:323–328. [CrossRef]

[8] Dhanalakshmi V, Nimal T, Sabitha M, Biswas R, Jayakumar R. Skin and muscle permeating antibacterial nanoparticles for treating Staphylococcus aureus infected wounds. J Biomed Mater Res Part B 2016;104:797–807. [CrossRef]

[9] Sully EK, Geller BL. Antisense antimicrobial therapeutics. Curr Opin Microbiol 2016;33:47–55. [CrossRef]

[10] Wang F, Fang RH, Luk BT, Hu CMJ, Thamphiwatana S, Dehaini D, et al. Nanoparticle-Based antivirulence vaccine for the management of methicillin-resistant Staphylococcus aureus Skin Infection. Adv Funct Mater 2016;26:1628–1635. [CrossRef]

[11] Nannini E, Murray BE, Arias CA. Resistance or decreased susceptibility to glycopeptides, daptomycin, and linezolid in methicillin-resistant Staphylococcus aureus. Curr Opin Pharmacol 2010;10:516–521. [CrossRef]

[12] Song M, Zeng Q, Xiang Y, Gao L, Huang J, Huang J, et al. The antibacterial effect of topical ozone on the treatment of MRSA skin infection. Mol Med Rep 2018;17:2449–2455. [CrossRef]

[13] Hanson B, Dressler A, Harper A, Scheibel R, Wardyn S, Roberts L, et al. Prevalence of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) on retail meat in Iowa. J Infect Public Health 2011;4:169–174. [CrossRef]

[14] Morehead MS, Scarbrough C. Emergence of global antibiotic resistance. Prim Care 2018;45:467–484. [CrossRef]
[15] Girish VM, Liang H, Aguilan JT, Nosanchuk JD, Friedman JM, Nacharaju P. Anti-biofilm activity of garlic extract loaded nanoparticles. Nanomed: Nanotechnol Biol Med 2019;20:102009. [CrossRef]
[16] Rajendran R, Radhai R, Kotresh T, Csiszar E. Development of antimicrobial cotton fabrics using herb loaded nanoparticles. Carbohydrate Polym 2013;91:613–617. [CrossRef]
[17] Almasi H, Azizi S, Amjadi S. Development and characterization of pectin films activated by nanoemulsion and Pickering emulsion stabilized marjoram (Origanum majorana L.) essential oil. Food Hydrocolloids 2020;99:105338. [CrossRef]
[18] Harrison JJ, Turner RJ, Joo DA, Stan MA, Chan CS, Allan ND, et al. Copper and quaternary ammonium cations exert synergistic bactericidal and antibiofilm activity against Pseudomonas aeruginosa. Antimicrobial Agents Chemother 2008;52:2870–2881. [CrossRef]
[19] Karunanidhi A, Thomas R, Van Belkum A, Neela V. In vitro antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of Stenotrophomonas maltophilia including the trimethoprim/sulfamethoxazole resistant strain. Bio Med Res Int 2013;2013:392058. [CrossRef]
[20] Lou Z, Wang H, Zhu, S, Ma C, Wang Z. Antibacterial activity and mechanism of action of chlorogenic acid. J Food Sci 2011;76:M398–M403. [CrossRef]
[21] Ayaz FA, Hayırlıoglu-Ayaz S, Alpay-Karaoglu S, Grúz J, Valentová K, Ulrichová J, et al. Phenolic acid contents of kale (Brassica oleraceae L. var. acephala DC.) extracts and their antioxidant and antibacterial activities. Food Chem 2008;107:19–25. [CrossRef]
[22] Fattouch S, Caboni P, Coroneo V, Tuberoso CI, Angioni A, Dessi S, et al. Antimicrobial activity of Tunisian quince (Cydonia oblonga Miller) pulp and peel polyphenolic extracts. J Agricultural Food Chem 2007;55:963–969. [CrossRef]
[23] Li G, Wang X, Xu Y, Zhang B, Xia X. Antimicrobial effect and mode of action of chlorogenic acid on Staphylococcus aureus. Eur Food Res Technol 2014;238:589–596. [CrossRef]
[24] Hussain A, Altamimi MA, Alshehri S, Imam SS, Shakeel F, Singh SK. Novel approach for transdermal delivery of rifampicin to induce synergistic antimycobacterial effects against cutaneous and systemic tuberculosis using a cationic nanoemulsion gel. Int J Nanomed 2020;15:1073. [CrossRef]
[25] Caon T, Campos CEM, Simões CMO, Silva MAS. Novel perspectives in the tuberculosis treatment: Administration of isoniazid through the skin. Int J Pharm 2015;494:463–470. [CrossRef]
[26] Chen S, Han Y, Yu D, Huo F, Wang F, Li Y, et al. Transdermal delivery of isoniazid and rifampin in guinea pigs by electro-phonophoresis. Drug Deliv 2017;24:467–470. [CrossRef]
[27] Hamouda T, Baker Jr J. Antimicrobial mechanism of action of surfactant lipid preparations in enteric Gram-negative bacilli. J Appl Microbiol 2000;89:397–403. [CrossRef]
[28] My A, Vanhecke T, Landers JJ, Hamouda T, Baker JR. The fungicidal activity of novel nanoemulsion (X8W 60 PC) against clinically important yeast and filamentous fungi. Mycopathologia 2003;155:195–201. [CrossRef]
[29] Pannu J, McCarthy A, Martin A, Hamouda T, Ciotti S, Ma L, et al. In vitro antibacterial activity of NB-003 against Propionibacterium acnes. Antimicrobial Agents Chemother 2011; 55:4211–4217. [CrossRef]
[30] Sugumar S, Ghosh V, Nirmala MJ, Mukherjee A, Chandrasekaran N. Ultrasonic emulsification of eucalyptus oil nanoemulsion: antibacterial activity against Staphylococcus aureus and wound healing activity in Wistar rats. Ultrasonics Sonochem 2014;21:1044–1049. [CrossRef]
[31] Meral R, Ceylan Z, Kose S. Limitation of microbial spoilage of rainbow trout fillets using characterized thyme oil antibacterial nanoemulsions. J Food Safety 2019;39:e12644. [CrossRef]
[32] Ceylan Z, Meral R, Kose S, Sengor G, Akinay Y, Durmus M, et al. Characterized nano-size curcumin and rosemary oil for the limitation microbial spoilage of rainbow trout fillets. LWT 2020;134:109965. [CrossRef]
[33] Chua SK, Fu JY, Zulfakar MH, Ng MH, Hasan ZAA, et al. Optimisation and biological evaluation of palm glyceryl monocaprylate antimicrobial nanoemulsion for combating S. aureus wound infection. J Mater Res Technol 2020;9:12804–12817. [CrossRef]
[34] Alvarado H, Abrego G, Souto E, Garduno-Ramirez M, Clares B, Garcia M, et al. Nanoemulsions for dermal controlled release of oleanolic and ursolic acids: In vitro, ex vivo and in vivo characterization. Colloids Surfaces B: Biointerfaces 2015;130:40–47. [CrossRef]
[35] Clares B, Calpena AC, Parra A, Abrego G, Alvarado H, Fangueiro JF, et al. Nanoemulsions (NEs), liposomes (LPs) and solid lipid nanoparticles (SLNs) for retinyl palmitate: Effect on skin permeation. Int J Pharm 2014;473:591–598. [CrossRef]
[36] Tadros T, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. Adv Colloid Interface Sci 2004;108:303–318. [CrossRef]
[37] Mason TG, Wilking JN, Meleson K, Chang CB, Graves SM. Nanoemulsions: formation, structure, and physical properties. J Physics Condensed Matter 2006;18:R635. [CrossRef]

[38] Severino P, Fangueiro J, Ferreira S, Basso R, Chaud M, Santana M, et al. Nanoemulsions and nanoparticles for non-melanoma skin cancer: effects of lipid materials. Clin Transl Oncol 2013;15:417–424. [CrossRef]

[39] Macedo AS, Quelhas S, Silva AM, Souto EB. Nanoemulsions for delivery of flavonoids: formulation and in vitro release of rutin as model drug. Pharmaceutical Dev Technol 2014;19:677–680. [CrossRef]

[40] Teixeira M, Severino P, Andreani T, Boonme P, Santini A, Silva A, et al. D-α-tocopherol nanoemulsions: Size properties, rheological behavior, surface tension, osmolarity and cytotoxicity. Saudi Pharmaceutical J 2017;25:231–235. [CrossRef]

[41] Nasir A. Nanotechnology and dermatology: Part II-risks of nanotechnology. Clin Dermatol 2010;28:581–588. [CrossRef]

[42] Soriano-Ruiz JL, Calpena-Capmany AC, Cañadas-Enrich C, Bozal-de Febrer N, Suñer-Carbó J, Souto EB, et al. Biopharmaceutical profile of a clotrimazole nanoemulsion: Evaluation on skin and mucosae as anticandidal agent. Int J Pharm 2019;554:105–115. [CrossRef]

[43] Ghosh V, Saranya S, Mukherjee A, Chandrasekaran N. Cinnamon oil nanoemulsion formulation by ultrasonic emulsification: investigation of its bactericidal activity. J Nanosci Nanotechnol 2013;13:114–122. [CrossRef]

[44] Adjonu R, Doran G, Torley P, Agboola S. Whey protein peptides as components of nanoemulsions: A review of emulsifying and biological functionalities. J Food Eng 2014;122:15–27. [CrossRef]

[45] Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, et al. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J Nanobiotechnol 2011;9:1–9. [CrossRef]

[46] Ehlers C, Ivens U, Møller M, Senderovitz T, Serup J. Females have lower skin surface pH than men: a study on the influence of gender, forearm site variation, right/left difference and time of the day on the skin surface pH. Skin Res Technol 2001;7:90–94. [CrossRef]

[47] Lin C-Y, Chen L-W. Comparison of fuel properties and emission characteristics of two-and three-phase emulsions prepared by ultrasonically vibrating and mechanically homogenizing emulsification methods. Fuel 2008;87:2154–2161. [CrossRef]

[48] Gosh V, Mukherjee A, Chandrasekaran N. Ultrasonic emulsifivation of food-grade nanoemulsion formulation and evulation of its bactericial activity. Ultrasonic Sonochem 2013;20:338–344. [CrossRef]

[49] Sugumar S, Nirmala J, Ghosh V, Anjali H, Mukherjee A, Chandrasekaran N. Bio-based nanoemulsion formulation, characterization and antibacterial activity against food-borne pathogens. J Basic Microbiol 2013;53:677–685. [CrossRef]

[50] Moghimi R, Aliahmadi A, McClements DJ, Rafati H. Investigations of the effectiveness of nanoemulsions from sage oil as antibacterial agents on some food borne pathogens. LWT-Food Sci Technol 2016;71:69–76. [CrossRef]

[51] Garzoli S, Petralito S, Ovidi E, Turchetti G, Masci VL, Tiezzi A, et al. Lavandula x intermedia essential oil and hydrolate: Evaluation of chemical composition and antibacterial activity before and after formulation in nanoemulsion. Industrial Crops Products 2020;145:112068. [CrossRef]

[52] Nirmala MJ, Durai L, Gopakumar V, Nagarajan R. Preparation of celery essential oil-based nanoemulsion by ultrasonication and evaluation of its potential anticancer and antibacterial activity. Int J Nanomed 2020;15:7651. [CrossRef]

[53] Kumari S, Kumaraswamy R, Choudhary RC, Sharma S, Pal A, Raliya R, et al. Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. Sci Rep 2018;8:1–12. [CrossRef]

[54] de Oca-Ávalos JMM, Candal RJ, Herrera ML. Nanoemulsions: stability and physical properties. Curr Opin Food Sci 2017;16:1–6. [CrossRef]

[55] Nishi T, Garima G, Sharma P, Nitin K. Nanoemulsions: a Review on Various Pharmaceutical Applications. Global J Pharmacol 2012;6:222–225.

[56] Kildaci L, Budama-Kilinc Y, Kecel-Gunduz S, Altuntas E. Linseed oil nanoemulsions for treatment of atopic dermatitis disease: formulation, characterization, in vitro and in silico evaluations. J Drug Deliv Sci Technol 2021;64:102652. [CrossRef]

[57] Müller R. Zeta Potential and Particle Charge in Laboratory Usage. 1996.

[58] Karl B, Alkhatib Y, Beekmann U, Bellmann T, Blume G, Steiniger F, et al. Development and characterization of bacterial nanocellulose loaded with Boswellia serrata extract containing nanoemulsions as natural dressing for skin diseases. Int J Pharm 2020;587:119635. [CrossRef]

[59] Ribeiro RC, Barreto SMAG, Ostrosky EA, Rocha-Filho PA, Veríssimo LM, Ferrari M. Production and characterization of cosmetic nanoemulsions containing Opuntia ficus-indica (L.) Mill extract as moisturizing agent. Molecules 2015;20:2492–2509. [CrossRef]

[60] Ibrahim N, Raman I, Yusop MR. Effects of functional group of non-ionic surfactants on the stability of emulsion. Malaysian J Anal Sci 2015;19:261–267.

[61] Ismail A, Nasr M, Sammour O. Nanoemulsion as a feasible and biocompatible carrier for ocular delivery of travoprost: Improved pharmacokinetic/pharmacodynamic properties. Int J Pharm 2020;583:119402. [CrossRef]

[62] Mallick A, Gupta A, Hussain A, Aparajay P, Singh S, Singh SK, et al. Intranasal delivery of gabapentin loaded optimized nanoemulsion for augmented permeation. J Drug Deliv Sci Technol 2020;56:101606. [CrossRef]
[63] Martini É. Nanoemulsões catiônicas como sistemas de liberação de oligonucleotídeos: formulação e caracterização físico-química Master Thesis. 2005. [Spanish]
[64] Nastiti CM, Ponto T, Abd E, Grice JE, Benson HA, Roberts MS. Topical nano and microemulsions for skin delivery. Pharmaceutics 2017;9:37. [CrossRef]
[65] Jiao J, Rhodes DG, Burgess DJ. Multiple emulsion stability: pressure balance and interfacial film strength. J Colloid Interface Sci 2002;250:444–450. [CrossRef]
[66] Shahnaz G, Hartl M, Barthelmes J, Leithner K, Sarti F, Hintzen F, et al. Uptake of phenothiazines by the harvested chylomicrons ex vivo model: Influence of self-nanoemulsifying formulation design. Eur J Pharm Biopharm 2011;79:171–180. [CrossRef]
[67] Rachmawati H, Budiputra DK, Mauludin R. Curcumin nanoemulsion for transdermal application: formulation and evaluation. Drug Dev Ind Pharm 2015;41:560–566. [CrossRef]
[68] Yilmaz A, Meral R, Kabli M, Ermis E, Akman PK, Dertli E, et al. Fabrication and characterization of bioactive nanoemulsion-based delivery Systems. Emerg Mater Res 2021:1–8. [CrossRef]
[69] Donsì F, Ferrari G. Essential oil nanoemulsions as antimicrobial agents in food. J Biotechnol 2016;233:106–120. [CrossRef]
[70] Balasubramani S, Rajendhiran T, Moola AK, Diana RKB. Development of nanoemulsion from Vitex negundo L. essential oil and their efficacy of antioxidant, antimicrobial and larvicidal activities (Aedes aegypti L.). Environ Sci Pollut Res 2017;24:15125–15133. [CrossRef]
[71] Krishnamoorthy R, Athinarayanan J, Periasamy VS, Adisa AR, Al-Shuniaber MA, Gassem MA, et al. Antimicrobial activity of nanoemulsion on drug-resistant bacterial pathogens. Microbial Pathogenesis 2018;120:85–96. [CrossRef]
[72] Pathania R, Kaushik R, Khan MA. Essential oil nanoemulsions and their antimicrobial and food applications. Curr Res Nutr Food Sci J 2018;6:626–643. [CrossRef]
[73] Majeed H, Liu F, Hategekimana J, Sharif HR, Qi J, Ali B, et al. Bactericidal action mechanism of negatively charged food grade clove oil nanoemulsions. Food Chem 2016;197:75–83. [CrossRef]
[74] Dillen K, Bridts C, Van der Veken P, Cos P, Vandervoort J, Augustyns K, et al. Adhesion of PLGA or Eudragit®/PLGA nanoparticles to Staphylococcus and Pseudomonas. Int J Pharm 2008;349:234–240. [CrossRef]
[75] Ozogul Y, Boğa EK, Akyol I, Durmus M, Ucar Y, Regenstein JM, et al. Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. Food Biosci 2020;36:100635. [CrossRef]
[76] Lee KH, Lee J-S, Kim ES, Lee HG. Preparation, characterization, and food application of rosemary extract-loaded antimicrobial nanoparticle dispersions. LWT 2019;101:138–144. [CrossRef]
[77] Karimi N, Ghanbarzadeh B, Hamishehkar H, Mehramuz B, Kafil HS. Antioxidant, antimicrobial and physicochemical properties of turmeric extract-loaded nanostructured lipid carrier (NLC). Colloid Interface Sci Commun 2018;22:18–24. [CrossRef]
[78] Fernández-Campos F, Clares Naveros B, Lopez Serrano O, Alonso Merino C, Calpena Campmany A. Evaluation of novel nystatin nanoemulsion for skin candidosis infections. Mycoses 2013;56:70–81. [CrossRef]
[79] Seibert JB, Bautista-Silva JP, Amparo TR, Petit A, Pervier P, dos Santos Almeida JC, et al. Development of propolis nanoemulsion with antioxidant and antimicrobial activity for use as a potential natural preservative. Food Chem 2019;287:61–67. [CrossRef]
[80] Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q. Physical and antimicrobial properties of peppermint oil nanoemulsions. J Agric Food Chem 2012;60:7548–7555. [CrossRef]
[81] Li, ZH, Cai M, Liu Y-S, Sun P-L. Development of finger citron (Citrus medica L. var. sarcodactylis) essential oil loaded nanoemulsion and its antimicrobial activity. Food Control 2018;94:317–323. [CrossRef]