WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 5.210

Volume 4, Issue 12, 1314-1328 Research Article ISSN 2278 – 4357

OPTIMIZATION OF NANO-EMULSION FORMULATIONS FOR CERTAIN EMOLLIENT EFFECT

Ahmed A. H. Abdellatif[1] and Heba A. Abou-Taleb[2*]

[1]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Assuit, Egypt.

[2]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Nahda University, Benisuef, Egypt.

Article Received on
24 Oct 2015,

Revised on 15 Nov 2015,
Accepted on 06 Dec 2015

***Correspondence for Author**
Dr. Heba A. Abou-Taleb
Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Assuit, Egypt.

ABSTRACT

Nano-emulsions are isotropic dispersed systems of two immiscible liquids, normally consisting of an oily system dispersed in an aqueous system, or an aqueous system dispersed in an oily system. The aim of this study was to formulate Nano-emulsion that is capable of penetrating the skin easily depending on its size or its homogeneity. Nano-emulsion was prepared using co-surfactants, Oleic acid as an oily phase, (Water) as an aqueous phase, Tween 20 as surfactant. The formulations were prepared, containing of (Vitamin A, Vitamin E, Lactic Acid, and Hydroquinone) to help in decreasing the signs and symptoms of skin dryness as emollient and bleaching agent of the skin. The formulations were characterized for zeta potential, particle size analysis. The results showed that the formulated nano-emulsions were of a uniform particle size of 178.9 ± 0.5 nm and with acceptable polydespersity index (PDI) value of 0.25 which indicate that the particle size distribution falls within a narrow range. The UV Spectrophotometric analysis showed red shift more than the plain formula, also scanning electron microscopy confirmed the spherecity of particles. We conclude that the nano-emulsion containing Vitamin A, Vitamin E, Lactic Acid, and Hydroquinone may serve as an emollient agent in many skin diseases e.g. age.

KEYWORDS: Nano-emulsion, Transdermal delivery, Vitamin A, Vitamin E, Lactic Acid, and Hydroquinone.

INTRODUCTION

Nano-emulsion is dispersed particles used as vehicles for pharmaceutical preparation and seems to be promising for the future of cosmetics, diagnosis, drug therapies and biotechnologies.[1] Nano-emulsions are transparent or translucent systems containing droplets with a mean diameter of 100–900 nm, and they are thermodynamically unstable, but they are kinetically stable.[2-3] Nano-emulsions can solubilize hydrophobic and hydrophilic drugs, enhance the permeation and bioavailability of the drugs due to high kinetic stability so they can be formulated into different dosage forms with ease of manufacture and scale up as well as used for taste masking so improve patient compliance and acceptance.[4-8] Nano-emulsions can be utilized for the production of pharmaceutical preparations e.g. sterile injection and infusion solutions; solutions, especially oral liquids, eye drops and nose drops.[9] Recently nano-emulsions are becoming increasingly important as potential vehicles for the controlled delivery of cosmetics and for optimized dispersion of active ingredients into skin. Nano-emulsion gain increasing interest due to their own bioactive effects. Nano-emulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation or coalescence that observed with macro emulsions. Nano-emulsions are used as vehicle in cancer chemotherapy for prolonging the rate of drug release after intramuscular and intratumoral injection (W/O systems). It also enhances the transport of anti-cancer drugs via lymphatic system.[10]

Vitamin A is available in several forms such as (retinaldehyde, retinol and retinyl esters such as retinyl palmitate). Retinyl palmitate is a combination of palmitic acid and ester of retinol.21 Retinaldehyde by retinaldehyde oxidase can oxidized to retinoic acid. Retinol can be converted to retinaldehyde by retinol dehydrogenase and via esterases enzymes retinol is produced from retinyl esters. All of these forms are converted to its biologically active form trans-retinoic acid in the skin.[11] The anti-aging effect come from thickness of skin to relief the presence of wrinkles, and pigmentation. It also leads to epidermal proliferation, keratinization, peeling, fibroplast proliferation, increase epidermal ground substances production such as glycosminoglycans that bind water particles and improvement of extracellular matrix production of substances such as collagen. It also inhibits the collagenase production.[12] Vitamin E is a lipophilic antioxidant that naturally present in skin. It is forms in the cosmetics are tocotrienols and tocopherols. Vitamin E prevents free radical ability to damage lipid cell membrane. In case of antioxidant vitamins combination, it gives synergistic effect. Vitamin E regenerated from its oxidized form by vitamin C, lead to enhancement of

antioxidant effect.[13] Hydroquinone mechanism of action is the inhibition of melanin synthesis. Hydroquinone leads to reversible depigmentation of the skin which achieved thought inhibition of the tyrosinase enzyme, this enzyme catalyzes the oxidation of tyrosine to 3, 4-dihydroxyphenylalanine. It also increases the melanosomes degradation or decreases the melanosomes formation and it inhibits the RNA and DNA melanocytes synthesis.[14]

Lactic acid is from alpha hydroxyacids (AHAs) which is act by reducing the coenocyte that is the dead layer of skin surface, leading to thin the stratum corneum and thus speeding up the process of exfoliation and skin regeneration. They also reduce the amount of calcium in the epidermis that promotes cell growth and slow cell differentiation that leads to look younger of the skin. Additionally, AHAs can increase the dermis and epidermal thickness, through improving the elastic fiber quality and increasing the density of collagen. Moreover, AHAs increase hyaluronic acid and collagen gene expression in epidermis and dermis. Additionally, they have the ability to decrease wrinkles and improve smoothness of the skin, and the anti-aging effect is directly proportional with concentration and duration of treatment. They can decrease roughness, hyper-pigmentation and sallowness.[15-18]

The aim of this study was to formulate nano-emulsion of (Vitamin A, Vitamin E, Lactic Acid, and Hydroquinone) that can improve the signs and symptoms of skin dryness as emollient and bleaching agent of the skin and as promising formula for decreasing the age signs. The formulations were characterized for zeta potential, particle size analysis, polydespersity index (PDI), UV Spectrophotometric analysis and transmission electron microscopy. The formula is very promising and may serve as an emollient agent in many skin diseases e.g. age in future work.

MATERIALS AND METHODS

2.1 Materials and Instruments

Oleic acid was purchased from Oxford Co., (Egypt). Tween 80 and Lactic acid were purchased from Al Nasr pharmaceutical chemicals Co., ADWIC (Egypt). Ethanol was obtained from Pio Chemical Co., (Egypt). Transcutol was kindly gifted by Dr. Hala (T.A at October University for Modern Science and Arts), (Egypt). Butanol was purchased from Al Gomhorya Co., (Egypt). Vitamin A and Vitamin C were gifted from El-Kahira Pharmaceutical Industrial Co., (Egypt). Hydroquinone was kindly gifted from Sedico CO., Egypt. Malvern zetasizer nano 6.01 (Malvern Instruments GmbH, Herrenberg, Germany), Sonicator UH-100B ultrasonic processor, (Tianjin Automatic Science Instrument 1td, China),

Homogenizer Branson 450 (Emerson industrial sutomation, USA) and SHIMADZU UV spectrophotometer (SHIMADZU co., Japan).

2.3 Preparation of nano-emulsion

For the preparation of nano-emulsion, several oils were evaluated to find out the most suitable oil for a stable nano-emulsion formulation by applying the solubility studies to mineral oil, olive oil and oleic acid. Also 2 different surfactants have been used for the formulation of nano-emulsion, e.g., Tween 20 & 80. The surfactant with the maximum nano-emulsion formulation in phase diagram was selected. Two different co-surfactants were also used such as glycerin, propylene glycol butanol and transcutol and ethanol. Nano-emulsion was prepared according to the previously reported method.[19-21] For preparation of plain nano-emulsion formulations, mixture of oils, surfactants and co-surfactants were used. Oleic acid was used as the oily phase; Tween 80 was used as surfactant. Glycerin, polypropylene glycol, ethanol, butanol and transcutol were also used as different co-surfactants.

Pseudo ternary phase diagram was required to find out the concentration components that provide large nano-emulsion zone.[22-23] The ratios of the oil to the mixture of surfactant and co-surfactant (oil/S, CoS) were 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The mixture of surfactant to co-surfactant (S/CoS) ratio was 1:1, 2:1 and 3:1. The mixture was then titrated with distilled water until first turbidity under stirring. The volume of water was recorded, and then the titration method was repeated until the last drop before turbidity.

2.5 Preparation of medicated nano-emulsion

For preparation of nano-emulsion loaded with drugs, the drugs were loaded to the prepared plain nano-emulsion. 20 % Vitamin E and 20 % Vitamin A were added to the oily phase with oleic acid, surfactant and co-surfactant. 5 % lactic acid and 5 % hydroquinone that added to the aqueous phase then follow the previous procedures. Finally, the nano-emulsion droplet that composed of lipid core comprising a mixture of oil dispersed in aqueous phase was formed.

2.6 Characterization of prepared nano-emulsion

2.6.1 Thermodynamic stability of nano-emulsion

In order to find out the stable nano-emulsion and to discard the unstable or metastable nano-emulsions, the placebo nano-emulsions were subjected to following thermodynamic stability studies.[24] Freeze thaw cycle: Nano-emulsions were kept in deep freezer (at - 20 ^{o}C) for 24

hrs then nano-emulsions were removed and kept at room temperature. The thermodynamically stable nano-emulsions returned to their original form within 2-3 minutes. 2-3 such cycles were repeated. Heating cooling cycle: Six cycles between refrigerator temperature (4 ^{0}C) and 40 ^{0}C with storage of 48 hours were performed. Those formulations which were stable at these temperatures will be, subjected to further study.

2.6.2 Size and zeta potential measurements by dynamic light scattering (DLS)

To determine the size, count rate and zeta potential of nano-emulsion, the samples were kept at to 25 ^{o}C and laser light scattering analysis was performed with an incident laser beam of 633 nm at a scattering angle of 90^o using the Malvern zetasizer nano 6.01 (Malvern Instruments GmbH, Herrenberg, Germany).[25]

2.6.3 UV Spectrophotometry

UV-Visible Spectrophotometer, Shimadzu UV-1800 connected to compatible computer. The software was UVPC personal spectroscopy software version 2.32 (Shimadzu). The absorption spectra of the reference and test solutions were carried out in a 1 cm quartz cells.[25]

RESULTS AND DISCUSSION

3.1 Preparation of nano-emulsion

The most important criteria for selection of all the nano-emulsion components is that all the excipients should be pharmaceutically acceptable for topical application, depending upon the requirement and generally regarded as safe category. After performing the solubility studies for different oils, it was found that lactic acid, Vitamin A and Vitamin E have high solubility in olive oil but the nano-emulsion cannot be obtained with this oil. Mineral oil has high viscosity and high molecular weight so that it was excluded. Oleic acid was found to be the most suitable one. Oleic acid showed many benefits, it has good enhancing properties for transdermal drug delivery and it increases the fluidity of the intercellular lipid barriers in the stratum corneum by forming separate domains that interfere with the continuity of the multilamellar stratum corneum and induce high permeable pathways in the stratum corneum.

The main problem related to the nano-emulsion-based systems is the toxicity of the components.[26-28] Large amounts of surfactants may cause skin irritation when administered topically. Therefore, the proper selection of surfactants is very important. Therefore, the surfactant concentration was determined properly and the minimum concentration was used in the formulation. Nonionic surfactants are relatively less toxic than their ionic counterparts and typically have lower CMCs.

Moreover, O/W nano-emulsion dosage was formed for topical uses based on nonionic surfactants are likely to offer *in vivo* stability. Therefore, proper selection of surfactants becomes a crucial factor. Hydrophilic surfactant and co-surfactant were considered to prefer the interface and to lower the necessary energy to form the nano-emulsions, as a result improving the stability. After selection of oil phase, the main aim was to identify the surfactant that has the highest solubilization capacity for the oil. In the present study, two surfactants, namely, tween 20 and 80 were used.

Nonionic surfactants were selected since they are known to be less affected by pH and changes in ionic strength, are generally regarded as safe, and are biocompatible.[29-30] Ionic surfactants were excluded from the study due to toxicological concerns. Here, we have selected the surfactant through the maximum nano-emulsion formulation in phase diagram such as is tween 80. The role of co-surfactant is to reduce further interfacial tension to make nano-emulsion more stable by providing flexible film.[31-32] Ethanol was selected as a co-surfactant because it is used for topical delivery and acts as a penetration enhancer and it comes under GRAS category.[33-34]

3.2 Preparation of pseudo ternary phase diagram

Formulations were carefully observed so that the metastable systems were not selected, although the free energy required to form a nano-emulsion is very low and the formation is thermodynamically spontaneous. On the basis of pseudoternary phase diagram tween 80 and isopropyl alcohol at (1:1) ratio was selected for further study because this combination of surfactant and co-surfactant was able to produce maximum nano-emulsion area. When co-surfactant was added with surfactant in equal amounts, a higher nano-emulsion region was observed, perhaps because of further reduction of the interfacial tension and increased fluidity of the interface at S/CoS and mix 1:1. For better results, the ratio of the oil to the mixture of surfactant and co-surfactant (S/CoS) 3:7 and the ratio for the mixture of surfactant and co-surfactant (S/CoS) was 1:1 were selected.

3.3 Characterization of the prepared Nano-emulsion

3.3.1 Thermodynamic stability tests of Nano-emulsion

In order to exclude the possibility of meta-stable formulations, stress testing is required. Some representative formulations were taken from the o/w nano-emulsion region of the phase diagram constructed at S/CoS mix 1:1 for tween 80 and ethanol, and were subjected to the thermodynamic stability tests such as heating cooling cycle, freeze thaw cycle, and

centrifugation. Thermodynamic stability test confers long-term stability to the nano-emulsion as compared to ordinary emulsions.

3.3.2 Size measurements by DLS

Size (hydrodynamic diameter), Polydispersity index (PDI) and zeta potential of particles are parameters that indicate the stability of nano-emulsion.[21, 35] PDI is an index that can indicates stability, since it represents the size distribution range in colloidal solution. High PDI indicates the heterogenity of the particle size in suspension, while smaller PDI values indicate the homogeneity of the particle size in suspension. Polydispersity indices lower than 0.7 are ideal, because they indicate that the particle size distribution falls within a narrow range of sizes.[21, 25] The results showed a good, small and uniform nano-emulsion were obtained using spontaneous emulsification method. The formulated nano-emulsion was of a non aggregated particle size of 178.9 ± 0.5 nm **(Fig. 1 and 6).** PDI value of 0.25 which indicates that the particle size distribution falls within a narrow range of sizes. The DLS showed skewed peak which means (asymmetrical particle size distribution curve).

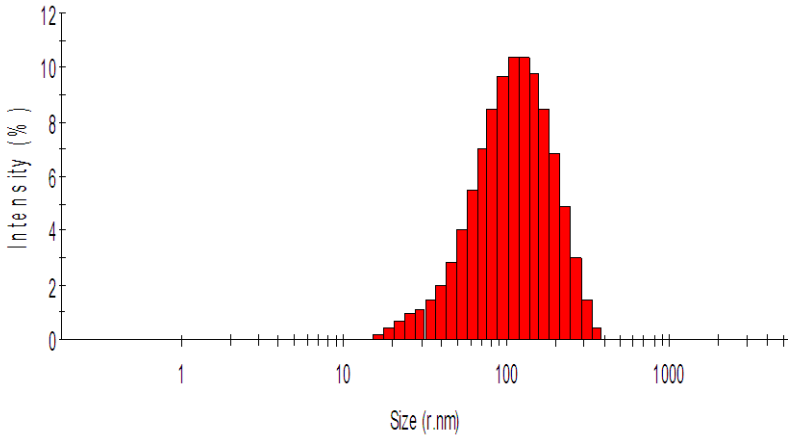

Figure 1. Particle size distribution of nano-emulsion measured using dynamic light scattering using ethanol as co-surfactant.

The plain nano-emulsion was formulated before the addition of the drug to confirm the effect of the drug on the formulation. Plain nano-emulsion have smaller particle size than the medicated one which is 142.4 nm. PDI of the plain nano-emulsion was also smaller than the medicated 0.115 which indicates that the particle size distribution falls within a narrower range than the medicated formulation **(Fig. 2 and 6)**. These results indicated that the increase in size is due to entrapped drug in transfersomes.

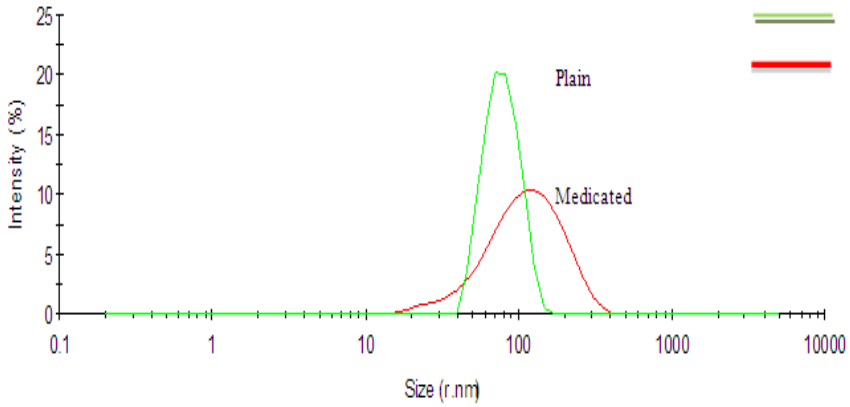

Figure 2. Particle size distribution of medicated and non-medicated nano-emulsions containing ethanol as co-surfactant using dynamic light scattering.

3.3.3 Nano-emulsion contaning transcutol as co-surfactant

The results showed a good, small and uniform nano-emulsion were obtained using spontaneous emulsification method with the medicated nano-emulsion formulation. The uniform particle size is 217.1 nm and the PDI is 0.335 which means that the particle size distribution falls with in the narrow range (**Fig. 3 and 6**). The nanoparticles formed using transcutol showed a better normal distribution curve (symmetrical particle size distribution) which indicated the transcutol is better as co-sufactant.

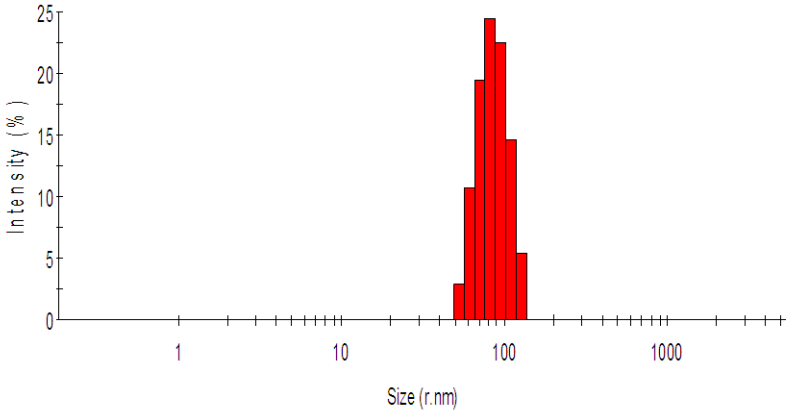

Figure 3. Particle size distribution of nano-emulsion containing transcutol as co-surfactant measured using dynamic light scattering.

The plain nano-emulsion was obtained and compared with the medicated one to know the effect on the drug on the preparation. The plain nano-emulsion showed a larger particle size than the medicated one which is 2214 nm and PDI 1.000 which is an indication for the heterogeneity of the particle size in suspension (**Fig. 4 and 6**).

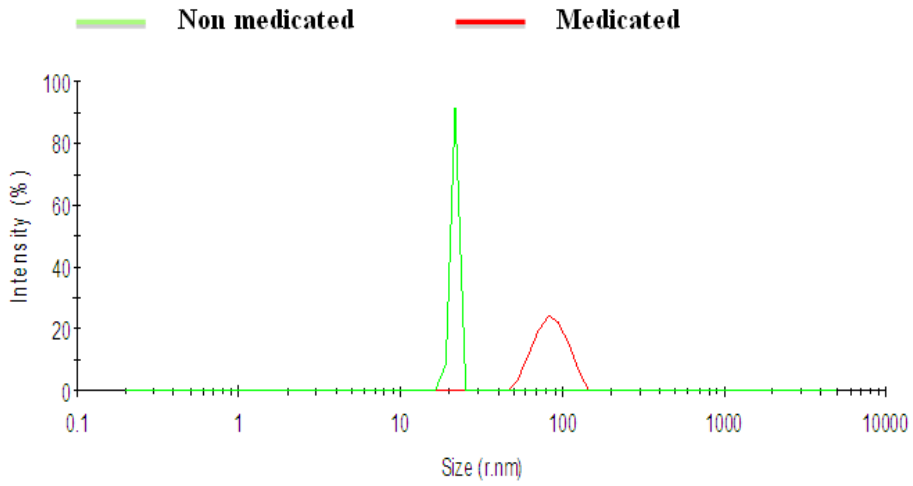

Figure 4. Particle size distribution of medicated and non-medicated nano-emulsions containing transcutol co-surfactant measured using dynamic light scattering.

3.3.4 Nano-emulsion contaning butanol as co-surfactant

The nano-emulsion containing butanol have large particle size in both plain and medicated formulations in comparison with the plain nano-emulsion only that have particle size of 4037 nm and the medicated nano-emulsion have particle size of 7070 nm. PDI of the plain formulation is 0.200 which is ideal and indicates the homogeneity of the particle size. But the medicated formulation has high PDI which show the heterogeneity of the particle size (**Fig. 5 and 6**).

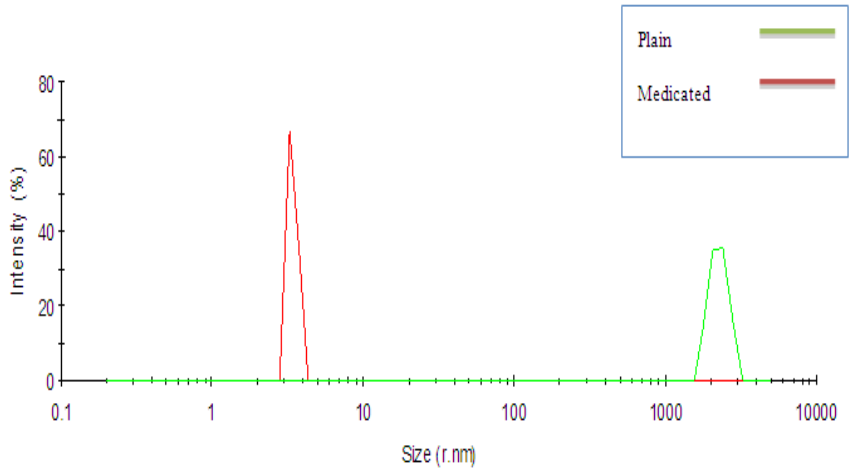

Figure 5. Particle size distribution of medicated and non-medicated nano-emulsions containg butanol co-surfactant measured using dynamic light scattering.

Finally, The results obtained by DLS indicated that a successful formulation of the nano-emulsion using Ethanol and Transcutol were prepared. The average diameter of the nanoparticles prepared using Ethanol and Transcutol were 178 and 207 nm respectively, while the average diameter of the NPs using butanol was 7070 nm. DLS results yielded a reasonable PDI of Ethanol and Transcutol, both below 0.3, while NPs prepared using Butanol showed high PDI.

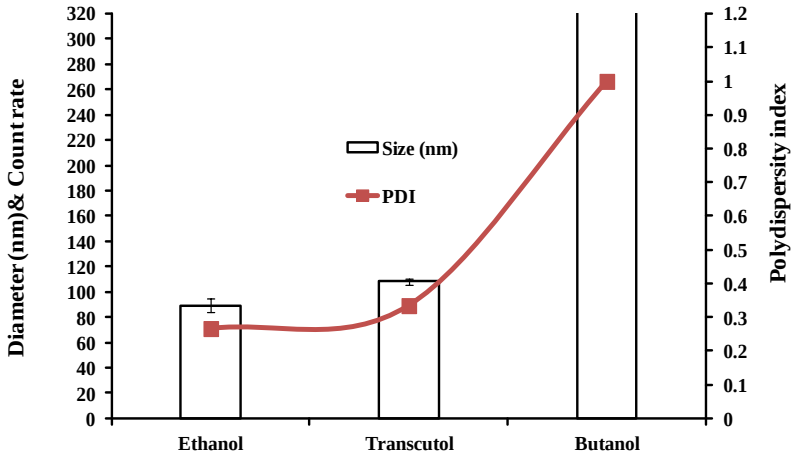

Figure 6. Particle size distribution, count rate and poly dispersing index nano-emulsion containing of ethanol, transcutol and butanol.

3.3.3 Zeta potential measurements

The surface charge also plays an important role in the stability of the nano-emulsion, and the magnitude of zeta potential is an indicative of the colloidal stability of the system. The highly negative zeta potential shows that the particles are electrically stabilized to resist aggregation.[21] For the nanoemulison which contains Ethanol as co-surfactant, the nanoparticles that contain Ethanol had a negative surface charge - 3.33 ± 0.5 mV for the plain formulation and 5.10 ± 0.5 mV for the medicated formulation, but for nano-emulsion which contains Transcutol as co-surfactact, the nanoparticles Transcutol had a positive surface charge of the medicated formulation mean which is 44.8 ± 0.5 mV and for the plain formulation is 6.24 ± 0.5 mV (**Fig. 7**). The higher oppesitely charged nanoparticles confirm the stabilty of nano-emulsion.

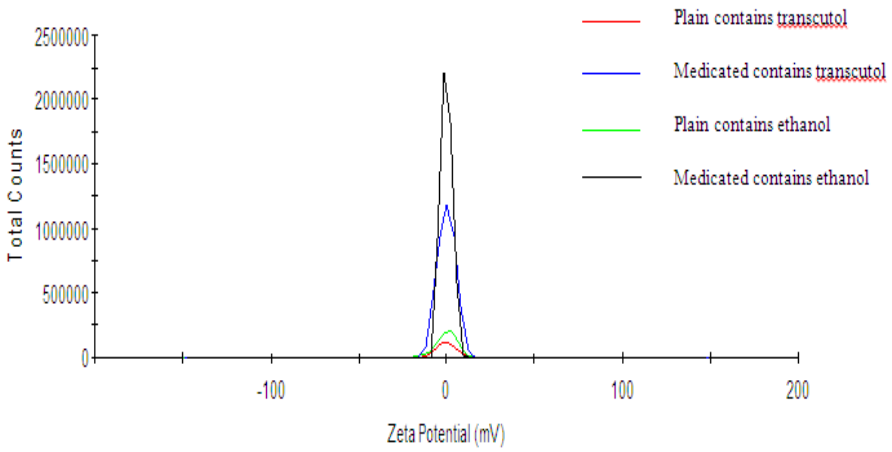

Figure 7. Zeta potential for plain and medicated nano-emulsions containing ethanol and transcutol as co-surfactant.

Particle size distributions, count rate as well as PDI of nano-emulsion were performed. The high-count rate indicates that the concentration of nanoparticles were high enough for measurements. Furthermore, the PDI was very small which indicate that the prepared nano-emulsions were uniform and in monodisperse formulation.

3.3.5 Scanning electron microscopy (SEM)

SEM confirmed the sphericity of nanoparticles, due to the particles showed spherical in shape and showed no aggregation also seem symetrical size (**Fig. 8**).

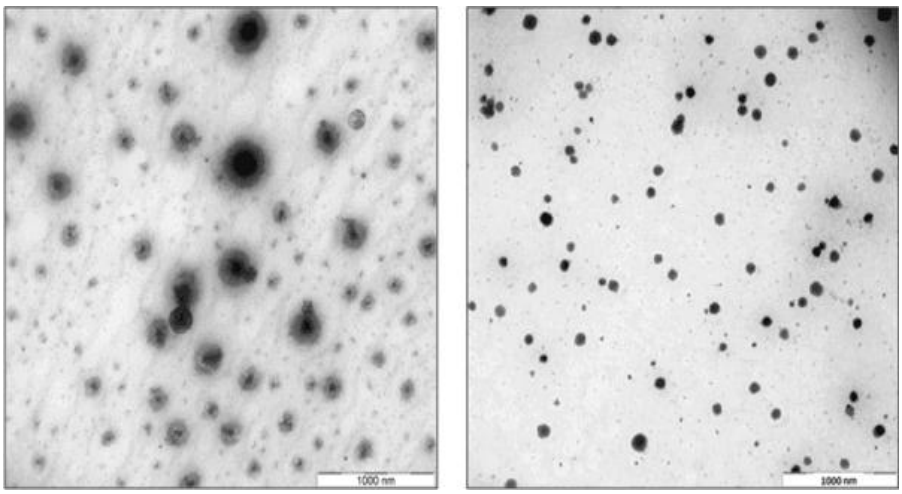

Figure 8 Scanning electron microscopy of plain and medicated nano-emulsions formulated using ethanol and transcutol as co-surfactant.

3.3.4 UV Spectrophotometry

The UV Spectrophotometic analysis of nano-emulsion contains ethanol as co-surfactant shows that the medicated formula was shifted more than the plain formula. The UV Spectrophotometic analysis of nano-emulsion containing transcutol as co-surfactant shows also that the medicated formula was shifted more than the plain formula. These results confirmed that the particles of different size shifted to different red shift than plain one.

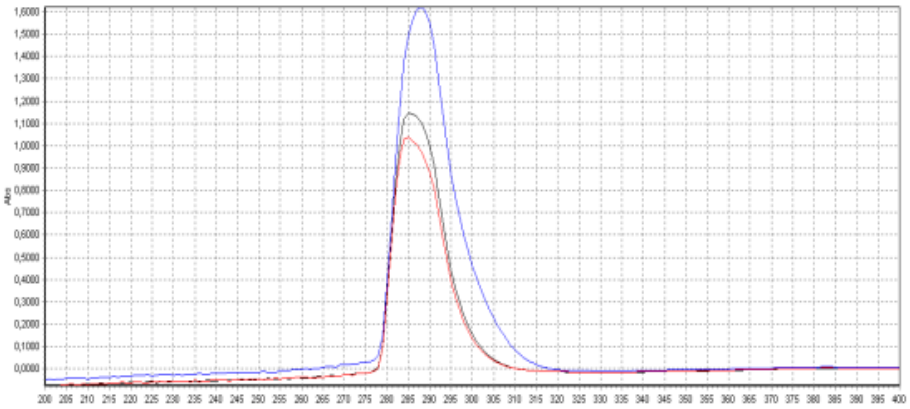

Figure 9. UV spectrophotometry of nano-emulsion containing alcohol as co-surfactant and nano-emulsion contains transcutol as co-surfactant. From above to below, nan-emulsion formulated using alcohol, transcutol and plain respectively.

CONCLUSION

The co-surfactant plays an important role in the preparation of the topical emulsion formulation with nanoparticle size range. Butanol was used as co-surfactant but it showed negative results when measured using zetasizer. Transcutol showed positive results for the medicated formulation (Nanoparticle range), but Ethanol when used as co-surfactant gave accepted results for both medicated and plain formulations. The stability of formulation may be enhanced by controlling factors such as the type and concentration of surfactant and co-surfactant, nature of oily phase, methods used, process variables as well as additives. Overall nano-emulsion may be considered as effective, safe formulation for the delivery of drugs.

REFERENCES

1. Zhou, J., et al., *Construction and evaluation of Fe(3)O(4)-based PLGA nanoparticles carrying rtPA used in the detection of thrombosis and in targeted thrombolysis.* ACS Appl Mater Interfaces, 2014; 6(8): 5566-76.

2. Zhang, Y., et al., *Enhanced anti-tumor effects of doxorubicin on glioma by entrapping in polybutylcyanoacrylate nanoparticles.* Tumour Biol, 2015.
3. Liu, R., et al., *Synthesis and characterization of tumor-targeted copolymer nanocarrier modified by transferrin.* Drug Des Devel Ther, 2015; 9: 2705-19.
4. Fornaguera, C., et al., *Design and in vitro evaluation of biocompatible dexamethasone-loaded nanoparticle dispersions, obtained from nano-emulsions, for inhalatory therapy.* Colloids Surf B Biointerfaces, 2015; 125: 58-64.
5. Laouini, A., et al., *Characterization of different vitamin E carriers intended for pulmonary drug delivery.* Int J Pharm, 2014; 471(1-2): 385-90.
6. Chen, M., et al., *[Effect of PLGA nanoparticles conjugated with anti-OX40/anti-AFP mAbs on cytotoxicity of CTL cells against hepatocellular carcinoma]*. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi, 2014; 30(4): 337-41.
7. Wakisaka, S., M. Nakanishi, and S. Gohtani, *Phase behavior and formation of o/w nano-emulsion in vegetable oil/ mixture of polyglycerol polyricinoleate and polyglycerin fatty acid ester/water systems.* J Oleo Sci, 2014; 63(3): 229-37.
8. Morral-Ruiz, G., et al., *Polyurethane and polyurea nanoparticles based on polyoxyethylene castor oil derivative surfactant suitable for endovascular applications.* Int J Pharm, 2014; 461(1-2): 1-13.
9. Fargnoli, A.S., et al., *Anti-inflammatory loaded poly-lactic glycolic acid nanoparticle formulations to enhance myocardial gene transfer: an in-vitro assessment of a drug/gene combination therapeutic approach for direct injection.* J Transl Med, 2014; 12: 171.
10. Tao, X., C. Deng, and J. Ling, *PEG-amine-initiated polymerization of sarcosine N-thiocarboxyanhydrides toward novel double-hydrophilic PEG-b-polysarcosine diblock copolymers.* Macromol Rapid Commun, 2014; 35(9): 875-81.
11. Wang, Z., et al., *Ultraviolet irradiation of human skin causes functional vitamin A deficiency, preventable by all-trans retinoic acid pre-treatment.* Nat Med, 1999; 5(4): 418-22.
12. Bosetti, M., et al., *Effect of retinoic acid and vitamin D on osteoblast differentiation and activity in aging.* J Bone Miner Metab, 2015.
13. Casoli, T., et al., *Vitamin E deficiency and aging effect on expression levels of GAP-43 and MAP-2 in selected areas of the brain.* Ann N Y Acad Sci, 2004; 1019: 37-40.
14. Nishimura, T., et al., *[Inhibitory effects of hydroquinone-alpha-glucoside on melanin synthesis]*. Yakugaku Zasshi, 1995; 115(8): 626-32.

15. Dong, S., et al., *Electrospun nanofibrous scaffolds of poly (L-lactic acid)-dicalcium silicate composite via ultrasonic-aging technique for bone regeneration.* Mater Sci Eng C Mater Biol Appl, 2014; 35: 426-33.
16. Fitzgerald, R. and D. Vleggaar, *Facial volume restoration of the aging face with poly-l-lactic acid.* Dermatol Ther, 2011; 24(1): 2-27.
17. Lu, Z., et al., *An economical approach for d-lactic acid production utilizing unpolished rice from aging paddy as major nutrient source.* Bioresour Technol, 2009; 100(6): 2026-31.
18. Grayhack, J.T. and K. Kropp, *Changes with Aging in Prostatic Fluid: Citric Acid, Acid Phosphatase and Lactic Dehydrogenase Concentration in Man.* J Urol, 1965; 93: 258-62.
19. Puglia, C., et al., *Nanoemulsions as vehicles for topical administration of glycyrrhetic acid: characterization and in vitro and in vivo evaluation.* Drug Deliv, 2010; 17(3): 123-9.
20. Tang, S.Y., et al., *Anti-inflammatory and analgesic activity of novel oral aspirin-loaded nanoemulsion and nano multiple emulsion formulations generated using ultrasound cavitation.* Int J Pharm, 2012; 430(1-2): 299-306.
21. Abdellatif, A.A. and H.M. Tawfeek, *Transfersomal Nanoparticles for Enhanced Transdermal Delivery of Clindamycin.* AAPS PharmSciTech, 2015.
22. Lu, X.X., et al., *[Preparation and quality evaluation of tanshinone IIA microemulsion for parenteral injection]*. Zhong Yao Cai, 2014; 37(11): 2070-6.
23. Wang, Q., et al., *[Preparation and optimization of zhitong micro-emulsion formula]*. Zhongguo Zhong Yao Za Zhi, 2014. 39(2): 222-9.
24. Anton, N. and T.F. Vandamme, *Nano-emulsions and micro-emulsions: clarifications of the critical differences.* Pharm Res, 2011; 28(5): 978-85.
25. Ahmed A. H. Abdellatif, S.A.E.R.a.S.O., *Gold Nanoparticles Decorated With Octreotide For Somatostatin Receptors Targeting.* J. Pharm. Sci. & Res., 2015; 7(1): 14-20.
26. de Campos, V.E., et al., *Polymeric nanostructured systems for liquid formulation of praziquantel: development and in vitro assessment.* Curr Drug Deliv, 2015.
27. Wulff-Perez, M., et al., *Preparation, characterization and in vivo evaluation of nanoemulsions for the controlled delivery of the antiobesity agent N-oleoylethanolamine.* Nanomedicine (Lond), 2014; 9(18): 2761-72.
28. McClements, D.J. and J. Rao, *Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity.* Crit Rev Food Sci Nutr, 2011; 51(4): 285-330.

29. Gitipour, S., et al., *Soil flushing of cresols contaminated soil: application of nonionic and ionic surfactants under different pH and concentrations.* J Environ Health Sci Eng, 2014; 12(1): 129.
30. Hari, A.C., et al., *Effects of pH and cationic and nonionic surfactants on the adsorption of pharmaceuticals to a natural aquifer material.* Environ Sci Technol, 2005. 39(8): p. 2592-8.
31. Gurram, A.K., et al., *Role of Components in the Formation of Self-microemulsifying Drug Delivery Systems.* Indian J Pharm Sci, 2015; 77(3): 249-57.
32. Nagatani, N., K. Fukuda, and T. Suzuki, *Interfacial Behavior of Mixed Systems of Glycerylether-Modified Silicone and Polyoxyethylene-Modified Silicone.* J Colloid Interface Sci, 2001; 234(2): 337-343.
33. Macedo, T.Z., et al., *The comparative advantages of ethanol and sucrose as co-substrates in the degradation of an anionic surfactant: microbial community selection.* Bioprocess Biosyst Eng, 2015; 38(10): 1835-44.
34. Chen, Y., et al., *Ethanol induced the formation of beta-sheet and amyloid-like fibrils by surfactant-like peptide A6K.* J Pept Sci, 2013; 19(11): 708-16.
35 Abdellatif A. Targeting of somatostatin receptors using quantum dots nanoparticles decorated with octreotide. *J Nanomed Nanotechnol S Nanomed Nanotechnol S* 2015;6:2.