IJPSR (2015), Vol. 6, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 September, 2014; received in revised form, 22 November, 2014; accepted, 06 January, 2015; published 01 May, 2015

FORMULATION OF CLINDAMYCIN NANO-EMULSION

K. Jiyauddin [1]*, A. Fadli [1], J. C. Wei [1], A. Jawad [1], A. D. Samer [1], M. Kaleemullah[1], S. Budiasih[1], S. Rasha [1], M. R. Rasny [1], Y. K. Sung [1], A. H. Junainah [1], H. Todo [2] and Y. Eddy [1]

School of Pharmacy [1], Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia.
Faculty of Pharmaceutical Sciences [2], Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan.

Keywords:

Nano-emulsion, Clindamycin,Tween®80, Span®20 and Olive oil.

Correspondence to Author:
K. Jiyauddin

School of Pharmacy, Management & Science University, 40100 Shah Alam, Selangor Darul Ehsan, Malaysia

E-mail: jiyauddin_khan@msu.edu.my

ABSTRACT: Nano-emulsions consist of fine oil-in-water or water-in-oil dispersions, having droplets covering the size range of 10 - 600 nm. The aim of this study is to formulate nano-emulsion of Clindamycin by using Emulsion Phase Inversion method and olive oil as the oil phase. Pseudo ternary phase diagram was first developed by using distilled water, olive oil and mixture of surfactants (Tween®80 and Span®20) at a ratio of 1:1. Then, appearance test and microscopic examination were done for all the pre-formulation. Three potential pre-formulation were then selected and incorporated with the Clindamycin Phosphate and Methyl Paraben. The mean droplet size and stability studies were done for these three formulations. Clindamycin Nano-emulsions were not obtained using the Emulsion Phase Inversion (EPI) method in this study, whereby the mean droplet sizes were in micro-range. However, out of all the three formulations which undergone extensive studies which include the heating-cooling cycle, whereby the formulation F8 and F17 were found to be physically stable. Significant differences were identified on the pH value and viscosity measurement for all the three formulations which undergone the heating-cooling cycle; except for the pH in F17. Furthermore, the formulation F8 had the smallest droplet size of 0.92 µm. Future research on this topic is needed to reduce the droplet size of the formulation.

INTRODUCTION: Nano-emulsions consist of fine oil-in-water or water-in-oil dispersions, having droplets covering the size range of 10-600 nm. Nano-emulsion is a group of dispersed particles used for pharmaceuticals and biomedical aids. These vehicles show great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies. The terms sub-micron emulsion (SME), mini-emulsion and ultra-fine emulsion are used as synonyms. Nano-emulsion is a heterogeneous mixture of lipid and aqueous phase and stability are achieved by using suitable material known as emulsifying agents.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(5).1845-54

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(5).1845-54

Nano-emulsion is a translucent system compare to ordinary emulsion or some time micro-emulsion. It has been demonstrated that with the help of nano-emulsion as a delivery system retention time of a drug in the body can be increased, so low amount of drug is required for the therapeutic action.

Past studies show the utilization of nano-emulsion technology for the enhancement of bioavailability of lipophilic drug. Acne vulgaris is a very common skin disease, which causes a high degree of psychosocial suffering [1] and has a detrimental effect on the quality of life of the patients irrespective of age or gender [2, 3, 4]. Acne vulgaris affects up to 85% of adolescent population in the United Kingdom [5, 6]. In Malaysia, the prevalence of facial acne vulgaris among teenagers was 67.5% [7]. The condition was more common among males (71.1%) compared to females (64.6%) [8]. Pathophysiology of acne primarily includes a complex interaction between the presence and

activity of *Propionibacterium acnes*, inflammation and hyperkeratinization [9]. Treatment of acne is principally directed towards these known pathogenic factors. Clindamycin and erythromycin are the commonly prescribed topical antibiotics for acne vulgaris with anti-inflammatory properties, among which the efficacy of Clindamycin has remained better over a period of time [10, 11].

The effectiveness of acne treatments has been limited by their relative inability to permeate into the pilosebaceous unit, the site of acne formation. Nano-emulsions are oil-in-water high energy emulsions with nanometer-sized droplets that fuse with bacteria to cause membrane disruption and lysis.

Clindamycin is a chlorine-substituted derivative of lincomycin, an antibiotic that is elaborated by *Streptomyces lincolnensis*.

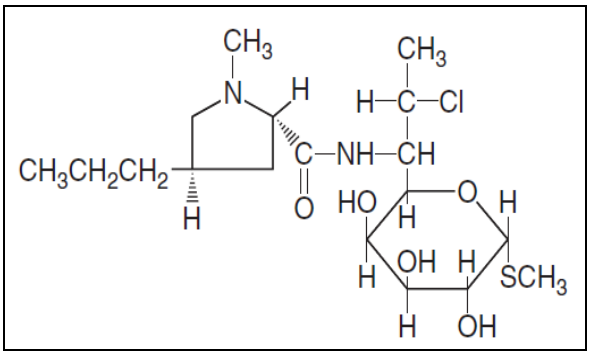

FIGURE 1: CLINDAMYCIN

Clindamycin binds to the 50S subunit of bacterial ribosomes and suppresses protein synthesis [12]. Topical clindamycin reduces free fatty acid concentrations on the skin and suppresses the growth of *Propionibacterium acnes*, an anaerobe found in sebaceous glands and follicles.

Small amounts of clindamycin may be absorbed after topical application to the skin; bioavailability from topical preparations of the hydrochloride and phosphate (the former in an extemporaneous formulation) has been reported to be about 7.5% and 2% respectively [13].

Topical formulations containing clindamycin phosphate equivalent to 1% of clindamycin are used for the treatment of acne. The hydrochloride may be applied similarly, but systemic absorption may be greater. Clindamycin phosphate is also available in combination preparations with benzoyl peroxide and tretinoin [13].

The aim of this work was to formulate the Clindamycin nano-emulsion by using Emulsion Phase Inversion method and olive oil as the oil phase to increase the effectiveness of acne treatment through the increase the penetration of the active compounds inside the lipophilic environment of the pilosebaceous unit.

MATERIALS AND METHODS:

Materials:

Clindamycin phosphate provided by Y.S.P. Industries (M) Sdn. Bhd., CIRIO® olive oil from local supermarket, polyethylene glycol sorbitan monooleate (Tween®80), sorbitan monolaurate (Span®20), methyl paraben, and distilled water. (All chemicals were obtained from the Management and Science University, Pharmacy Laboratory).

Apparatus/ equipments:

Laser diffractomer Mastersizer 2000 with the Hydro 2000SM module (Malvern Instruments, UK), Brookfield RS Portable rheometer with Coxial CC3-14 Spindle, hand homogenizer, digital pH meter, digital weighing scale, magnetic stirrer, oven, cool room, beakers, spatula, glass rod, measuring cylinder, urine containers, weighing tray, disposable pipette, volumetric flask, aluminum foil.

Method of Preparation:

Pre-formulation Studies:

Pseudo-ternary Phase Diagram:

Surfactant (Tween®) and co-surfactant (Span®20) was mixed at fixed mass ratio (1:1) which was then labeled as mixture of Surfactant (Smix). For the phase diagram, oil, distilled water and Smix at a specific ratio was mixed thoroughly at different mass ratios as stated in the table. Twenty one different combinations of oil, distilled water and Smix were made so that maximum ratios will be covered for the study.

The physical state of the nano-emulsion was marked on a pseudo-three-component phase diagram with one axis representing the aqueous

phase, the second one representing oil and the third representing a mixture of surfactant and co-surfactant at a fixed mass ratio.

TABLE 1: COMPOSITION OF PRE-FORMULATION

Formulation Code	Olive Oil (%)	Water (%)	Smix (%)
F_1	80	10	10
F_2	60	30	10
F_3	40	50	10
F_4	20	70	10
F_5	70	10	20
F_6	50	30	20
F_7	30	50	20
F_8	10	70	20
F_9	60	10	30
F_{10}	40	30	30
F_{11}	20	50	30
F_{12}	50	10	40
F_{13}	30	30	40
F_{14}	10	50	40
F_{15}	40	10	50
F_{16}	20	30	50
F_{17}	30	10	60
F_{18}	10	30	60
F_{19}	20	10	70
F_{20}	20	10	70
F_{21}	10	10	80

Appearance Test:
The appearance test was carried out visually, whereby it will include the observation of physical character of the nano-emulsion such as color and homogeneity of the nano-emulsion.

Microscopic Examination of Emulsion:
The types of emulsions formed were confirmed by microscopic examination of emulsion stained with methylene blue which is known as the water soluble dye method.

Preparation of Nano-emulsion:
All emulsions was prepared according the Emulsion Phase Inversion (EPI) method, where the water and oil phases was heated separately at 75°C, the water phase was added into the oil phase (Olive oil, Clindamycin phosphate and mixture of surfactants) while stirred at 600 rpm, and the mixture was then cooled to 25°C while stirring.

Evaluation of Clindamycin Nano-Emulsion:
The prepared nano-emulsions were characterized by the following techniques.

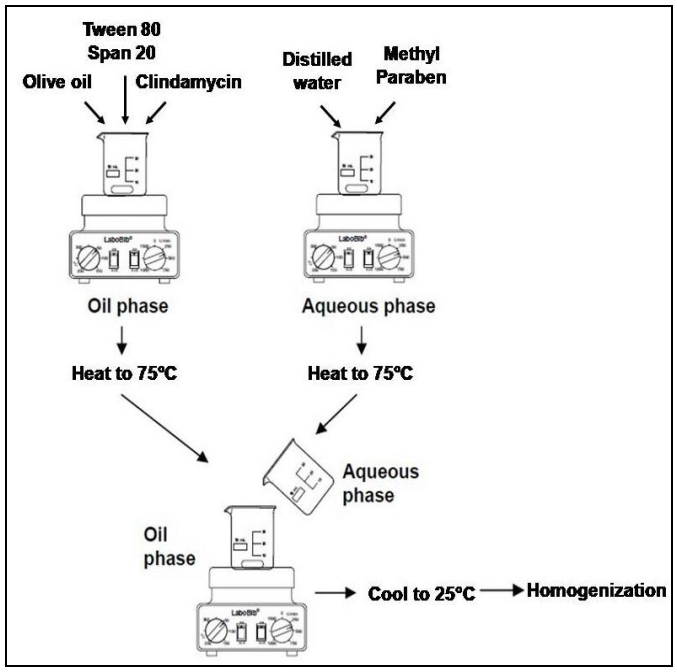

FIG. 2: FLOW FOR PREPARATION OF NANO-EMULSION USING EMULSION PHASE INVERSION (EPI) METHOD.

Nano-emulsion Droplet Size Analysis:
The nano-emulsion particle size was analyzed by using laser diffractormeter Mastersizer 2000 with the Hydro 2000SM module (Malvern Instruments, UK).

Dispersion Stability Studies:
To overcome the problem of metastable formulation, dispersion stability tests will be performed. Selected formulations will be taken for the heating and cooling cycle. Six cycles between the refrigerator temperature 4°C and 45°C with storage at each temperature for not less than 48 hours will be done. The Organoleptic characteristics, viscosity and pH will be recorded.

Organoleptic Characteristics:
Clindamycin nano-emulsion was investigated organoleptically such as liquefaction, color and phase separation. Organoleptic characteristics of Clindamycin nano-emulsion kept at different storage conditions were noted at various intervals such as day 0, day 2, day 4, day 6, day 8, day 10, and day 12.

Viscosity Determination:
The viscosity of the formulations (0.5g) will be determined as such without dilution using a Brookfield DV III ultra V6.0 RV cone and plate

rheometer (Brookfield Engineering Laboratories, USA) using spindle # CPE40 at 25 ± 0.3°C.

pH Determination:

The pH value of the prepared Clindamycin Nano-emulsion kept at different storage conditions were determined by a digital pH-meter. pH measurements were repeated for Clindamycin Nano-emulsion after 0 day, 2 days, 4 days, 6 days, 8 days, 10 days, and 12 days.

Statistical analysis:

The data obtained from different formulations were analyzed by one-way analysis of variance (ANOVA) procedure using the Statistical Package for the Social Science (SPSS) program (SPSS Statistics 22.0). When there was a statistically significant difference, a post-hoc Tukey test was then conducted to detect the differences among the pairs. A statistically significant difference was considered at $p < 0.05$.

RESULTS AND DISSCUSSION:

Pre-formulation Studies:

Pseudo-ternary Phase Diagram:

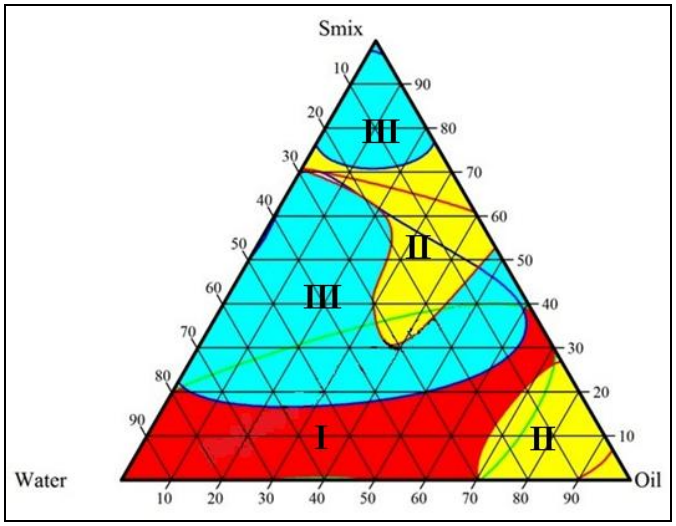

FIG.3: PSEUDO TERNARY PHASE DIAGRAM WITH OLIVE OIL, WATER AND MIXTURE OF SURFACTANT (TWEEN® 80 AND SPAN® 20). REGION I: NON-TRANSPARENT EMULSION, II: TRANSPARENT GEL, III: VISCOUS REGION.

Where;

Red is non-transparent emulsion, Yellow is transparent gel and Cyan is Viscous Region.

The construction of the pseudo ternary phase diagram was done with the 21 selected formulations with different percentage of Smix, Water and Olive Oil. The pseudo ternary phase diagram was categorized into three region which is the non-transparent emulsion region, transparent gel region, and viscous region.

As labeled in the diagram with color and roman number, red color region (I) was the non-transparent emulsion region, in which the sample's visual inspection showed white milky semi-solid products. While the yellow color region (II) was the transparent gel region, in which the sample's visual inspection showed the gold color gel. On the other hand, the cyan color region (III) was the viscous region, in which the sample's visual inspection showed the thick oily semi-solid products.

In this study, the appearance of the formulations changed from cloudy to transparent gel followed by viscous semi-solid as the mixture of surfactant (Smix) concentration increase.

Appearance Test:

Appearance test was carried out visually. This is an observation of physical character of the nano-emulsion. Its include color and homogeneity of the nano-emulsion.

TABLE 2: OBSERVATION OF PHYSICAL CHARACTERISTIC OF THE PRE-FORMULATION

Formulation Code	Color	Texture	Homogenity
F_1	Yellow, Cloudy	Soft	2 phase
F_2	White, Creamy	Soft	Homogen
F_3	White, Creamy	Soft	2 phase
F_4	White, Creamy	Soft	Homogen
F_5	Yellow, Cloudy	Soft	2 phase
F_6	Yellow, Creamy	Thick	Homogen
F_7	White, Creamy	Soft	2 phase
F_8	White, Creamy	Soft	Homogen
F_9	White, Creamy	Soft	Homogen
F_{10}	Yellow, Clear	Soft	2 Phase
F_{11}	Yellow, Creamy	Thick	2 Phase

F_{12}	Yellow, Creamy	Thick	Homogen
F_{13}	Yellow, Clear	Soft	2 Phase
F_{14}	Yellow, Creamy	Thick	Homogen
F_{15}	Yellow, Clear	Soft	Homogen
F_{16}	Yellow, Creamy	Thick	Homogen
F_{17}	Yellow, Clear	Soft	Homogen
F_{18}	Yellow, Creamy	Thick	Homogen
F_{19}	Yellow, Clear	Soft	Homogen
F_{20}	Yellow, Clear	Soft	Homogen
F_{21}	Yellow, Creamy	Thick	Homogen

Based on the appearance test, Formulation 1 and 5 showed the same characteristics, whereby the color of the formulation was yellow and cloudy, the texture was soft and showed phase separation. White and creamy semi-solid where found in the Formulation 2, 3, 4, 7, 8, and 9 with soft texture but Formulation 3 and 7 showed phase separation. On the other hand, Formulation 6, 11, 12, 14, 16, 18 and 21 showed the same colour which was yellow and creamy with thick texture, and only Formulation 11 showed phase separation. The other six formulations (Formulation 10, 13, 15, 17, 19, and 20) showed yellow and clear gel with soft texture, and only Formulation 10 and 13 showed phase separation.

Microscopic Examination of Pre-formulation Micro-emulsion:

Microscopic slides had been prepared for all the 21 formulations with the use of methylene blue stain, but only 6 were able to be observed under the light microscope. The Formulation 2, 4, 10, 15, and 20 showed the formation of the oil in water emulsion, while Formulation 7 showed the formation of water in oil emulsion.

Evaluation of Clindamycin Nano-emulsion:

Mean Droplet Size Analysis of Clindamycin Nano-emulsion:

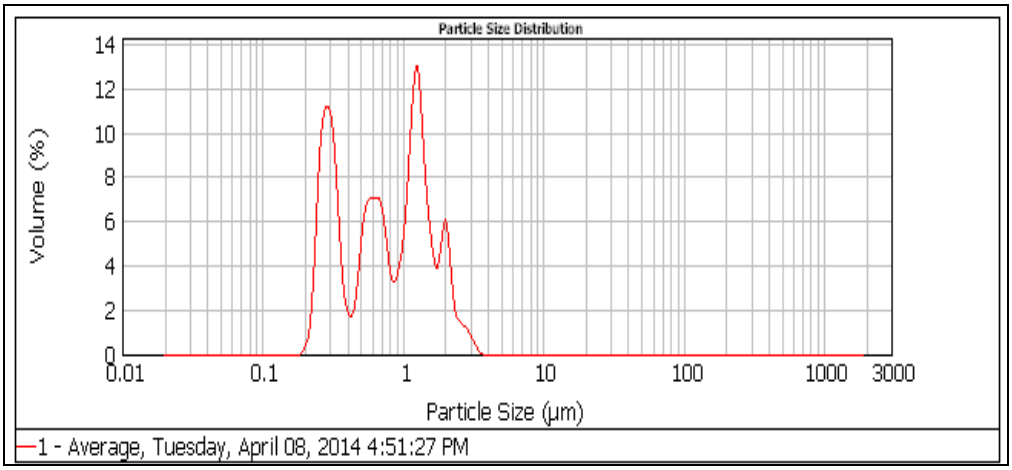

FIG.4: DROPLET SIZE DISTRIBUTION OF FORMULATION F8

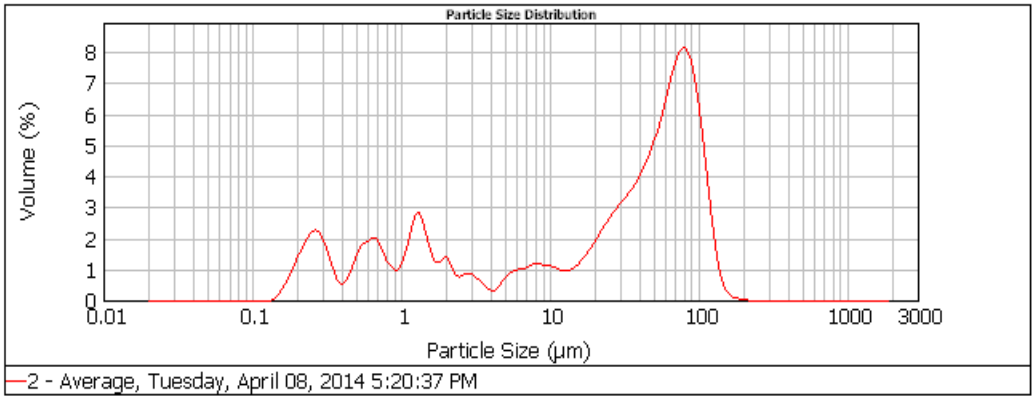

FIG. 5: DROPLET SIZE DISTRIBUTION OF FORMULATION F17

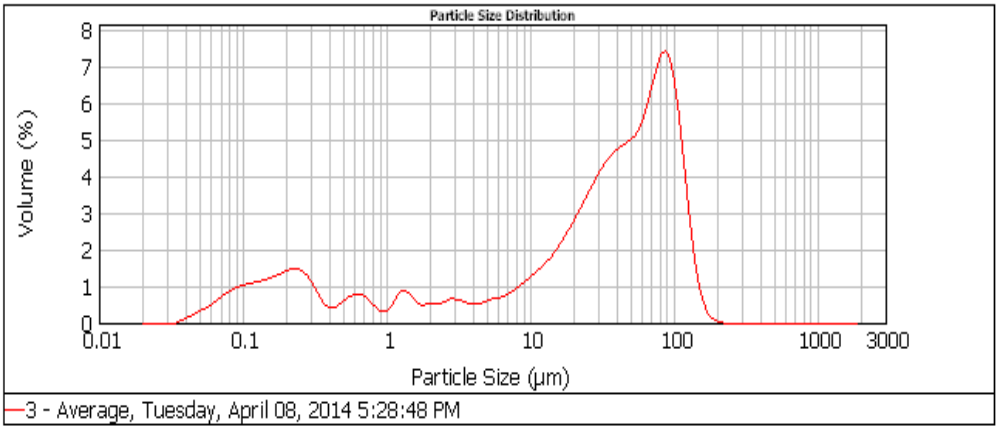

FIG.6: DROPLET SIZE DISTRIBUTION OF FORMULATION F18

From the results, all the three formulations (F8, F17 and F18) produced mean droplet size in micrometer range with all mean droplet size below 45 µm. Mean droplet size of micro-emulsion produced was between 0.920 µm to 44.695 µm. The average size of F8 is 0.920 µm, F17 is 41.647 µm and the F18 had the largest average size which is 44.695 µm.

Organoleptic Characteristics:

Liquefaction:

No liquefaction was observed in the Formulation 8, 17, and 18 of Clindamycin micro-emulsion. This shows that Clindamycin micro-emulsions were stable at different storage conditions up to 12 days.

Color:

No changes in color were observed in the Formulation 8, 17, and 18 of Clindamycin micro-emulsion. This shows that Clindamycin micro-emulsions were stable at different storage conditions up to 12 days.

Phase Separation:

Formulation 8 and 17 were stable under the 4°C (Cool room), 25°C (Room temperature) and 40°C (Oven), but the Formulation 18 was found not stable under the 40°C (Oven).

Viscosity determination:

Formulation 8:

The mean viscosity of the sample stored at 4°C (Cool room) was 395.943 mPas. On the other hand, the viscosity of the sample stored at 25°C (Room temperature) was 586.912 mPas. While the mean viscosity of the sample stored at 40°C (Oven) was 407.585 mPas.

Data obtained was evaluated statistically using one-way ANOVA test. It was found by the one-way ANOVA test at the 5% significant level that the changes in viscosity of the sample were significant at different levels of time and temperature ($p < 0.05$). It means that three storage conditions of the samples were significantly variants from the control group.

Formulation17:

The mean viscosity of the sample stored at 4°C (Cool room) was 395.943 mPas. On the other hand, the viscosity of the sample stored at 25°C (Room temperature) was 586.912 mPas. While the mean viscosity of the sample stored at 40°C (Oven) was 407.585 mPas.

Data obtained was evaluated statistically using one-way ANOVA test. It was found by the one-way ANOVA test at the 5% significant level that the changes in viscosity of the sample were significant at different levels of time and temperature ($p < 0.05$). It means that three storage conditions of the samples were significantly variants from the control group.

Formulation 18:

The mean viscosity of the sample stored at 4°C (Cool room) was 2.199 mPas. On the other hand, the viscosity of the sample stored at 25°C (Room temperature) was 2.840 mPas. While the mean viscosity of the sample stored at 40°C (Oven) was 59.754 mPas.

Data obtained was evaluated statistically using one-way ANOVA test. It was found by the one-way

ANOVA test at the 5% significant level that the changes in viscosity of the sample were significant at different levels of time and temperature ($p < 0.05$). It means that three storage conditions of the samples were significantly variants from the control group.

pH Determination:

Formulation 8:

The mean pH value of the sample stored at 4°C (Cool room) was 5.61. On the other hand, the mean pH value of the sample stored at 25°C (Room temperature) was 5.49. While the mean pH value of the sample stored at 40°C (Oven) was 5.81.

Data obtained was evaluated statistically using one-way ANOVA test. It was found by the one-way ANOVA test at the 5% significant level that the changes in pH value of the sample were significant at different levels of time and temperature ($p < 0.05$). It means that three storage conditions of the samples were significantly variants from the control group.

Formulation 17:

The mean pH value of the sample stored at 4°C (Cool room) was 6.51. On the other hand, the mean pH value of the sample stored at 25°C (Room temperature) was 6.40. While the mean pH value of the sample stored at 40°C (Oven) was 6.49.

Data obtained was evaluated statistically using one-way ANOVA test. It was found by the one-way ANOVA test at the 5% significant level that the changes in pH value of the sample were significant at different levels of time and temperature (p 0.093). It means that three storage conditions of the samples were not significantly variants from the control group.

Formulation 18:

The mean pH value of the sample stored at 4°C (Cool room) was 5.92. On the other hand, the mean pH value of the sample stored at 25°C (Room temperature) was 5.81. While the mean pH value of the sample stored at 40°C (Oven) was 6.00.

Data obtained was evaluated statistically using one-way ANOVA test. It was found by the one-way ANOVA test at the 5% significant level that the changes in pH value of the sample were significant at different levels of time and temperature ($p < 0.05$). It means that three storage conditions of the samples were significantly variants from the control group.

DISCUSSION: Pharmaceutically acceptable, nonirritant and non-sensitizing to skin and fall under the Generally Regarded as Safe (GRAS) category are the important criteria for selection of materials for the nano-emulsion formulation development.

Non-ionic surfactants are less toxic than ionic surfactant. The higher solubility of the drug in the oil phase is important for the nano-emulsion to maintain the drug in solubilized form. The right blend of low and high hydrophilic lipophilic balance (HLB) surfactants leads to the formation of a stable nano-emulsion formulation [14].

In this study, we selected Tween 80 as surfactant having the HLB value 15. Transient negative interfacial tension and fluid interfacial film is rarely achieved by the use of a single surfactant, usually necessitating addition of a co-surfactant. The presence of co-surfactant decreases the bending stress of the interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form a nano-emulsion over a wide range of compositions [15]. Thus, the co-surfactant selected for the study was Span 20 with the HLB value of 8.6.

Pre-formulation Studies:

The mixing of oil, water and surfactant in different ratio, can produce different types and structures of emulsion and nano-emulsion systems whereby it's depend on the chemical nature, molecular structure and concentration of surfactant and other ingredients. Surfactant type plays a major role in determining the rheological properties and droplets size of the systems [16].

Similarly, oil type whether it is triglyceride form or long chain hydrocarbon can change the physical properties of the systems. Oil can affect the interfacial tension and surface pressure as well as the stability of the system [17, 18]. Formation of micro-emulsion system by using nonionic

surfactant or mixture of nonionic surfactants, were reported by many researchers [19, 20].

Pseudo-ternary Phase Diagrams:
Construction of pseudo-ternary phase diagrams is the best way to study all types of formulations that can originate from mixing of surfactants, water and oil and these diagrams can cover the whole probabilities of mixing ratios.

The reason behind the different appearance of formulated emulsion was due to the fact that surfactant association structures were concentration dependant. Very low concentration formed unimers, then micelle, lamellar, cylinders, hexagonal and finally stacked in a cubical structure as the concentration of surfactant increased.

These various structures finally gave rise to the various appearance of the emulsion – cloudy, translucent, and transparent. At high percentage of oil and relatively low distilled water composition, an increase in surfactant concentration would lead to phase transition into cubic isotropic phase.

Besides that, from the pseudo ternary phase diagram, transparent pre nano-emulsion gel was formed at lower surfactant composition with higher percentage of oil due to the fact that the suitable formulation where the nano-emulsion is formed at 5-10% surfactant concentration with percentage of oil about 80%. At this composition, a cubic isotropic phase would be formed due to suitable combination between surfactant, water and oil.

Moreover, for a better formation of transparent nano-emulsion, it is recommended that the experiment was done in vacuum to avoid air incorporation that would affect the appearance of the nano-emulsion produced.

Appearance Test:
In the appearance test, all pre-formulation were observed physically. Color and homogeneity were visually observed. This characteristic is important as it show the distribution of active ingredient in the micro-emulsion. Homogeneous formulation showed greater stability and effectiveness on intact human skin [21].

Evaluation of Clindamycin Nano-emulsion:
Mean Droplet Size Analysis of Clindamycin Nano-emulsion:
The droplet size increased with the increase in oil concentration and Smix concentration in the formulations. The droplet size of formulation F8, containing 10% of oil and 20% on Smix was 0.920 µm, which was lower as compared to other formulations. There was only a marginal difference in the mean droplet size of formulations F17 and F18, which may be due to the equal concentration of Smix (60%) in both formulations. This result is in accordance with the report that the addition of surfactant to micro-emulsion systems causes the interfacial film to condense and stabilize, while the co-surfactant causes the film to expand [22]. All the formulations had droplets in the micro range.

Stability Studies:
In order to exclude the possibility of metastable formulations, stress testing is required. Thermodynamic stability confers long shelf life to the nano-emulsion as compared to ordinary emulsions. It differentiates them from emulsions that have kinetic stability and will eventually phase-separate [23].

Viscosity Determination:
Rheological measurements can be used to objectively determine skin sensation when products are applied to the skin; this may shorten research and development times [24]. Freshly prepared formulation F18 had the highest viscosity (3.067 mPas) compared to other formulations. This may be due to the lower oil content and high Smix concentration. While the freshly prepared formulation F17 had the lowest viscosity (609.347 mPas).

Studied at progressively higher temperatures, first, a viscosity enhancement was observed at intermediate temperatures, due to stronger one-dimensional growth promoted by dehydration and the consequent decrease in spontaneous curvature. However, at high temperatures, the viscosity decreased. This was attributed to the formation of branched micelles, that, due to locally negative curvature, are more effective in reducing the mean curvature of micelles than linear ones [25].

pH Determination:

pH values of skin range between 5 to 6, and 5.5 considered to be the average pH of the skin. Therefore, the formulations intended for dermal application should have a pH value around this value. Freshly prepared formulation F8 and formulation F18 had the pH value of 5.53 and 5.86 respectively, which are within the range. However, formulation F17 had the pH value of 6.34 which was slightly out of the range.

The high temperature might have destabilized the micro-emulsion by hydrolysis, but it did not affect the overall quality of the micro-emulsions because the pH values remained around 6.0, which is an acceptable, non-skin irritating pH value. Monitoring the pH value is important for determining the emulsions' stability because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Emulsions produced with vegetable oils may experience a decrease in pH due to the hydrolysis of fatty acid esters into free fatty acid degradation products [26].

CONCLUSIONS: Clindamycin Nano-emulsions were not obtained using the Emulsion Phase Inversion (EPI) method in this study. The emulsions that produced in this study were all in the micro-range. However, out of all the three formulations which undergone extensive studies which include the heating-cooling cycle, whereby the formulation F8 and F17 were found to be stable physically. Significant differences were identified on the pH value and viscosity measurement for all the three formulations which undergone the heating-cooling cycle. It is recommended that further studies on the formulation of Clindamycin Nano-emulsion with other method of emulsification being conducted.

These methods include the high-energy and low-energy emulsification methods. Formulation by using the other mixture of surfactant and co-surfactant at different ratio should also be conducted. Besides that, *in-vitro* drug release study and skin irritation test should be done for the formulations and compare it to the marketed product. The major limitations of this study are often imposed by time management, budget constraints, unavailable of the instruments and chemicals.

ACKNOWLEDGEMENTS: The authors would like to thank the School of Pharmacy and the Research Management Center, Management & Science University, Shah Alam, Selangor Darul Ehsan, Malaysia for providing the necessary facilities utilized to carry out the research project successfully.

REFERENCES:

1. Magin P., Adams, J., Heading G., Pond D. and Smith W: Psychological sequelae of acne vulgaris: Results of a qualitative study. Can Fam Physician 2006; 52:978-9.
2. Kokandi A: Evaluation of acne quality of life and clinical severity in acne female adults. Dermatol Res Pract 2012; 2012:ID571516.
3. Dunn L. K., O'neill, J. L. and Feldman, S. R: Acne in adolescents: quality of life, self-esteem, mood, and psychological disorders. Dermatol Online J. 2011; 17:1.
4. Al Robaee A. A: Assessment of general health and quality of life in patients with acne using a validated generic questionnaire. Acta Dermatovenerol Alp Panonica Adriat 2009; 18:157-64.
5. Amber V., Avinesh K. S., and Bina G: Carrier-Based Drug Delivery System for Treatment of Acne. The Scientific World Journal 2014; 260-276.
6. Shaw, L. and Kennedy, C: The treatment of acne. Peadiatr Child Health 2007; 17:385-389.
7. Hanisah, A., Khairani, O. and Shamsul, A. S: Prevalance of acne and its impact on the quality of life in school-aged adolescents in Malaysia. J Primary Health Care 2009. 1:20-25.
8. Jennifer Burris, William Rietkerk, and Kathleen Woolf: Acne: The Role of Medical Nutrition Therapy. J. Academy of Nutrition and Dietetics 2013; 113 (1): 416-430.
9. Friedlander S. F., Eichenfield, L. F., Fowler, J. F., J.R . Fried, R. G., Levy, M. L. and Webster, G. F: Acne epidemiology and pathophysiology. Semin Cutan Med Surg 2010; 29:2-4.
10. Del Rosso J. Q. and Schmidt, N. F: A review of the anti-inflammatory properties of clindamycin in the treatment of acne vulgaris. Cutis 2010; 85:15-24.
11. Simonart T. and Dramaix M: Treatment of acne with topical antibiotics: lessons from clinical studies. Br J Dermatol 2005; 153:395-403.
12. Brunton, L. L. and Goodman, L. S: Goodman & Gilman's manual of pharmacology and therapeutics, New York, McGraw-Hill Medical 2008.
13. Sweetman, S. C: Martindale : the complete drug reference, London ; Chicago, Pharmaceutical Press 2009.
14. Craig D. Q. M., Barker, S. A., Banning, D. and Booth, S. W: An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. Int J Pharm 1995; 114:103-110.
15. Kawakami K., Yoshikawa, T., Moroto, Y., Kanaoka, E., Takahashi, K., Nishihara, Y. and Masuda, K: Microemulsion formulation for enhanced absorption of poorly soluble drugs I. Prescription design. J. Control Release 2002; 81:65-74.

16. Lakatos-Szabó J. and Lakatos, I: Effect of non-ionic surfactants on interfacial rheological properties of crude oil/water systems. Prog Colloid Poly Sci 1997; 105:302-310.
17. Peltonen L., Hirvonen J. and Yliruusi J: The behavior of sorbitan surfactants at the water-oil interface: Straight-chained hydrocarbons from pentane to dodecane as an oil phase. J Colloid Int Sci 2001; 240:272-276.
18. Hayati I. N., Binche, M., Chinping, T. and Aini, I. N: Stability and rheology of concentrated O/W emulsions based on soybean oil/palm kernel olein blends. Food Res Int. 2011; 40:1051-1061.
19. Lia P., Ghosha, A., Wagnera, R. F., Krillb, S., Joshia, Y. M. and Serajuddina, A. T. M: Effect of combined use of nonionic sufactant on formation of oil-in-water micro-emulsions. Int J Pharm 2005; 288:27-34.
20. Cho Y. H., Bae, E. K., Mok, C. K. and Park, J: Formulation of co surfactant-free O/W micro-emulsion using nonionic surfactant mixture. Food Eng Phys Prop 2008; 73: E115-E121.
21. Kang L. and Jun, H. W: Formulation and efficacy studies of new topical anesthetic creams. Drug Dev Ind Pharm 2003; 29:505-12.
22. Kale N. J. and Allen, A. V: Studies on microemulsions using Brij-96 as surfactant and glycerin, ethylene glycol and propylene glycol as cosurfactants. Int J Pharm 1998; 57:87-93.
23. Jawad A., Intan F. S., Jiyauddin K., Asbi A., S. Budiasih, M. Kaleemullah and Samer A. D: Formulation and development of self micro-emulsifying drug delivery system (smedds) of flurbiprofen. World Journal of Pharmaceutical research 2014; 3(7):105-124.
24. Bekker M., Webber, G. V. and Louw, N. R: Relating rheological measurements to primary and secondary skin feeling when mineral-based and Fischer–Tropsch wax-based cosmetic emulsions and jellies are applied to the skin. International Journal of Cosmetic Science 2013; 35:354-361.
25. Tandros T. F: Self-organized Surfactant Structures. In: TANDROS, T. F. (ed.). Wiley-VCH Verlag GmbH & Co. 2011.
26. Shafiq S., Shakeel F. and Khar R: Enhanced Stability of Ramipril in Nanoemulsion Containing Cremophor-EL: A Technical Note. AAPS PharmSciTech 2008; 9:1097-1101.

How to cite this article:
Jiyauddin K, Fadli A, Wei JC, Jawad A, Samer AD, Kaleemullah M, Budiasih S, Rasha S, Rasny MR, Sung YK, Junainah AH, Todo H and Eddy Y: Formulation of Clindamycin Nano-Emulsion. Int J Pharm Sci Res 2015; 6(5): 1845-54.doi: 10.13040/IJPSR.0975-8232.6(5).1845-54.