WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 134-144. Research Article ISSN 2277– 7105

FORMULATION OF TETRACYCLINE NANO-EMULSION

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Article Received on
28 Jan 2015,

Revised on 19 Feb 2015,
Accepted on 09 Mar 2015

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ABSTRACT

Nano-emulsions are one of the various forms of nano-pharmaceuticals that have been used in the process of development. Nano-emulsions are isotropically clear dispersion of two immiscible liquids such as water and oil which is thermodynamically stable and stabilized by an interfacial film of surfactant molecules. Typically the dispersion phase of the nano-emulsion consists of small particles or droplets that have size range of 5 nm to 200 nm. Antimicrobial nano-emulsion has a broad spectrum activity against bacteria, enveloped viruses, fungi and spores. Antimicrobial nano-emulsion has the droplet sizes that range from 200 nm to 600 nm and usually in the form of oil-in-water nano-emulsion. Oil-in-water nano-emulsion can easily permeate into the skin. The active ingredient in oil phase of oil-in-water nano-emulsion is protected from hydrolysis and oxidation as they are not exposed to attack by water and air. The aim of this study was conduct to do formulation of tetracycline nano-emulsion. It was done by constructing the phase diagram as the first step and followed by the preparation of nano-emulsion by using the method of phase inversion of emulsion. As to produce nano-emulsion, a reasonable concentration of surfactant, water and oil concentration was used. Formulation 8 which consists of 10% of oil concentration, 70% of water concentration and 20% of Smix (surfactant and co-surfactant) had a creamy appearance but producing a size range of 405 nm. The droplets of formulation 8 are in between the droplets size of antimicrobial nano-emulsion which the sizes range from 200 nm to 600 nm. In different temperature, the viscosity of the

formulation 8 is varied. The calculated P value for the viscosity for the three formulations is 0.7523. This means that the temperature do affect the viscosity. For formulation 8, the viscosity increased in low temperature and whilst in high temperature the viscosity decreased.

KEYWORDS: Nano-emulsion, Tetracycline, Droplet size, Viscosity and Surfactants.

INTRODUCTION

Over the last few years, the use of nano-technology in pharmaceuticals and medicine has grown. Nano-pharmaceuticals are the termed that are used for the pharmaceuticals that developed on the basis of nanotechnology. Currently, there are various forms of nano-pharmaceuticals being used or in the process of developed for instance Nano-emulsions (Sharma and Sarangdevot, 2012). Nano-emulsions are isotropically clear dispersion of two immiscible liquids such as water and oil which is thermodynamically stable and stabilized by an interfacial film of surfactant molecules (Devarajan and Ravichandran, 2011).

Typically, nano-emulsion dispersion phase consists of small particles or droplets that have size range of 5 nm to 200 nm. The dispersed phase also has very low oil/water interfacial tension. Physically, the appearance of nano-emulsion is transparent due to the droplet size that is less than 25% of the wavelength of visible light. Depending upon the composition which it is formed, Nano-emulsion can be classified into three types which are; oil in water nano-emulsions, water in oil nano-emulsions and bi-continuous nano-emulsion.

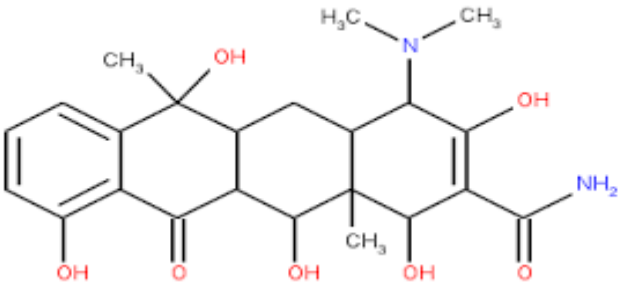

Figure 1 Structure of Tetracycline

Principally, nano-emulsions can be used to deliver drugs to the patients via several routes. Currently the administrations of drugs via topical application of nano-emulsions have gained increasing interest. Nano-emulsions can increase the rate of absorption and eliminate variability in absorption. Lipophilic drugs can also be solubilized by nano-emulsions.

Bioavailability of the drugs in the nano-emulsions can also be increased. The permeability of drug in the skin may be affected by nano-emulsions.

The aim of this study was to develop the drug formulation of tetracycline nano-emulsion for the treatment of acne; a disease of the pilosebaceous units in the skin. In the study of Nelson (1998), it was stated that tetracycline are broad-spectrum antibiotics with tetracycline naphthacene carboxamide ring. Tetracyclines also have been used clinically for rheumatoid arthritis and various skin disorders such as inflammatory acne, rosacea, bullous dermatoses and neutrophilic dermatoses.

MATERIALS AND METHODS

Materials

The olive oil was obtained from the supermarket, organic solvent (Ethanol) was obtained from Kimmimas Enterprise SDN. BHD (Selangor, Malaysia). Surfactant and co-surfactant (Tween 80 and Span 20) were ordered from Sigma-Aldrich. The active ingredient for the nano-emulsion, tetracycline was obtained from Pharmaniaga Berhad (PNB Bangi, Malaysia).

Apparatus/ equipments

Laser diffractometer Mastersizer 200 with the Hydrosizer 2000MU module (borrowed from UiTM, Malaysia), IKA T-18 Basic Homogenizer 115 VAC (Cole-Parmer, USA), DV-II+Pro Viscometer (Moncon.co.za) and QA9010X - Hot Plate Stirrer, Ceramic Surface (QIS, Netherlands).

Method of Formulation

Preparation of Nano-emulsion

Three formulations from three different phases were selected to prepare for the final product of nano-emulsion. A method of phase inversion emulsification was used to produce the nano-emulsion. 1% of Tetracycline was first dissolved in ethanol and was mixed together in olive oil. Homogenizer was used to homogenize the solutions. The solutions were homogenized for 15 minutes with speed of 24,000 rotations per minute.

Analysis of Droplet Size and Viscosity of the Formulations

After the preparation of nano-emulsion, the samples were sent to laboratory of University Teknologi Mara (UiTM) in Shah Alam, Selangor. The droplet size were analysed by using the Laser diffractormeter Mastersizer 200 with the Hydrosizer 2000MU module. For the

stability study by measuring the viscosity of the samples, the selected formulations were placed in different temperature and were measured its viscosity every one week for one month. The viscosity was measured by using Rheometer R/S+ which is rotational viscometer.

Statistical Analysis

The viscosities of the formulations were expressed as the average viscosity in different temperature. By using ANOVA one-way, F value was calculated and was compared by using calculated P value. A statistically significant difference was considered at $p < 0.05$.

RESULTS AND DISSCUSSION

Analysis of region of formulations

Based on the pseudo ternary phase diagram of Smix (Tween 80 and Span 20), oil and water as shown in Figure 2, the region of transparent without any phase separation was produced in the concentration range of 60% - 90% of Smix and 10% - 40% of oil. The region which produced creamy appearance was in the concentration of 10% - 40% of Smix and 10% - 60% of oil concentration. For cloudy creamy appearance, it was produced in the concentration of 50% - 80% of Smix and 10% - 20% of oil. Phase separation was occurred at the range of 10% - 40% of Smix and 60% - 80% of oil concentration.

Table 1 Composition of Formulation 8, 17 and 18

Formulation code	Smix (%)	Oil (%)	Water (%)	Average size (nm)	Uniformity
F8	20	10	70	0.405	0.346
F17	60	30	10	28.060	17.9
F18	60	10	30	47.482	0.598

Three formulations were selected for final product as shown in the table 1. Among them only formulation 8 produced mean droplet size of antimicrobial nano-emulsion which is in the range of 200 nm to 600 nm. Formulation 8 consists of a single droplet size distribution with two peaks at the top that lies between 200 nm to 600 nm (Fig. 3). For formulation 17, it consists of more than two peaks that were in the micrometer size (Fig. 4). Meanwhile for formulation 18, it consists of single peak at the range of micrometer size (Fig. 5).

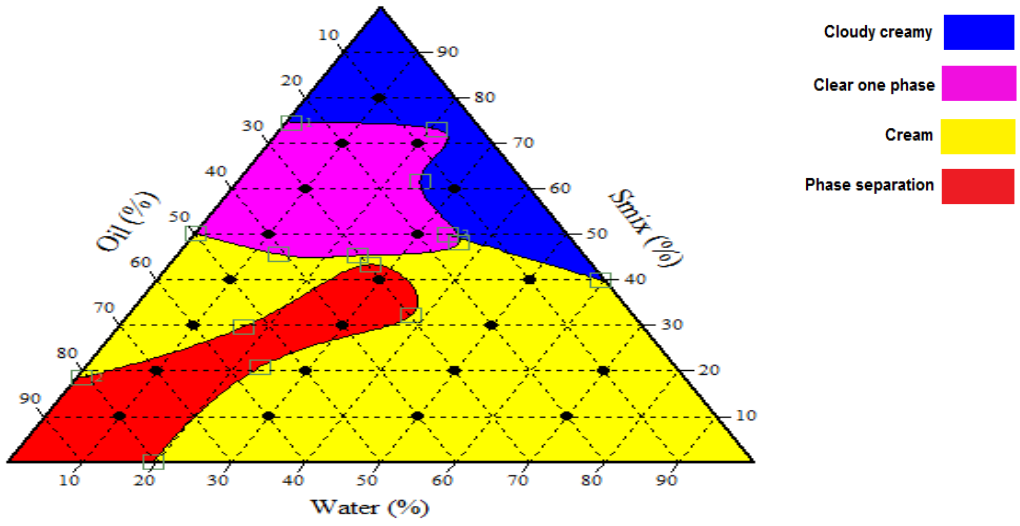

Figure 2 Analysis of region of pre nano-emulsion for Smix, oil and water

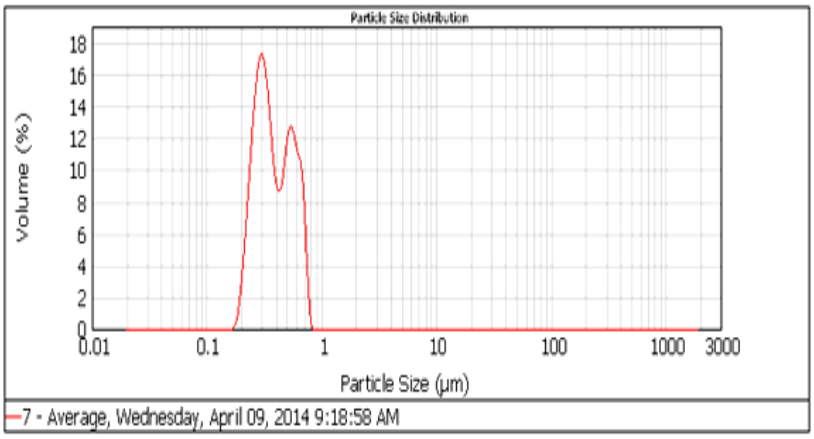

Figure 3 Droplet size distribution produced by F8

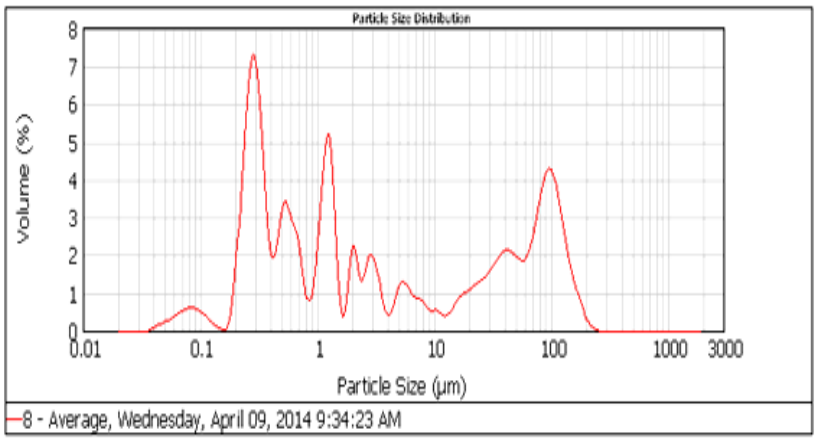

Figure 4 Droplet size distribution produced by F17

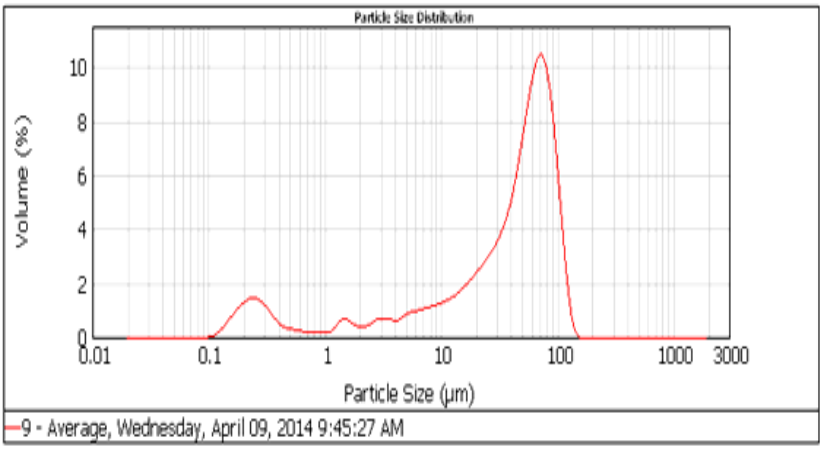

Figure 5 Droplet size distribution produced by F18

Effect of temperature on its viscosity

Table 2 shows the viscosity values of the three selected formulations in the three different temperature places; hot oven, room temperature and cold room. Regards with high concentration of surfactant in formulation 18, they produced high viscosity compared to that of formulation 8. Formulation 17 has low viscosity among the three formulations (Fig. 6).

Table 2 Average viscosity values at different temperature

Temperature	Average viscosity values (Pa s)		
	F8	F17	F18
Hot oven (40^{0}C)	0.5627	0.3475	1.7287
Room Temp. (24^{0}C)	0.6022	0.3895	2.3269
Cold room (4^{0}C)	0.8720	0.5352	3.4062

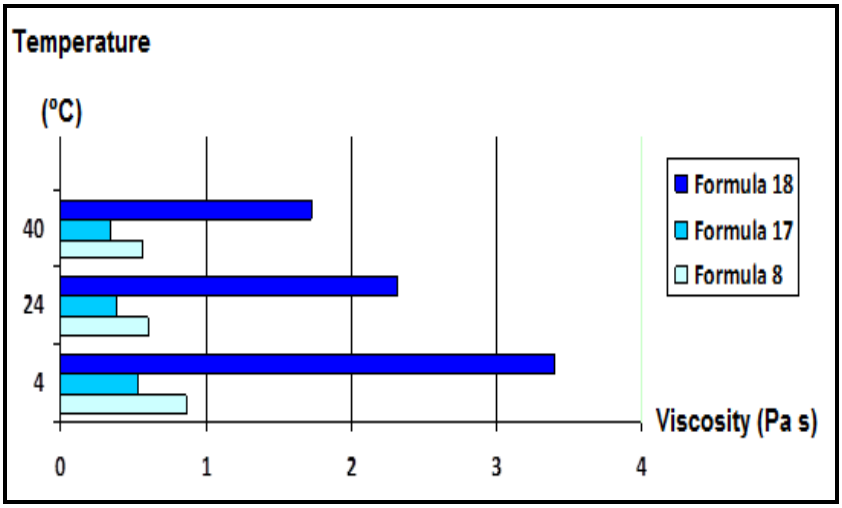

Figure 6 Trends of viscosity in different temperature

Table 3 Physical Appearance of the Formulation 8, 17 and 18 Stored in Different Temperature

Formulation code	Appearance					
	Hot oven (40^{0}C)		Room Temp. (24^{0}C)		Cold room (4^{0}C)	
	Initial storage	After one month	Initial storage	After one month	Initial storage	After one month
F8	Liquid cream	Liquid cream	Liquid cream	Liquid cream	Liquid cream	Slightly hardened
F17	Clear one phase	Clear one phase	Clear one phase	Clear one phase	Clear one phase	Hardened
F18	Viscous, two phase	Less viscous, one phase	Two phase	Two phase	Two phase	Turbid two phase

DISCUSSIONS

Based on Figure 2, the analysis showed that increasing in oil concentration and low fixed water concentration, the appearance changed from cloudy creamy to one clear phase and followed by cream and phase separation as the Smix concentration decreased. This is because of at low fixed concentration of water and increased in oil concentration that lead to phase transition of spherical micelle to cylindrical micelle and followed by hexagonal phase to cubic phase. Based on Figure 2, the formulations that showed isotropic solution are 15, 16, 17, 19 and 20 respectively. Isotropic solution was also a cubic system as the arrangement of the particles is corresponding along the three axes (Texter, J., 2001).

A phase transition from typical spherical micellar structure to a more elongated or rod-like material occurs when the concentration of the surfactant increased. The orientation and close packing of the elongated micelles into hexagonal arrays can be occurred if there is further increase in surfactant concentration. The hexagonal phase or middle phase is termed as a liquid crystalline state. The separation of a second liquid crystalline state – the neat phase or lamellar phase can be resulted in further increase of the surfactant concentration with some surfactant. The cubic phase which is another liquid crystalline state occurred between the middle and neat phases in some surfactant system (Texter, J., 2001).

This characteristic of the cubic system lead to the clear of one phase of the pre nano-emulsion. The nano-size region should be in the cubic phase. However, for formulation 17, the droplet size was not in the nano-size region. This possibly can be caused by inappropriate temperature while mixing and inappropriate time of mixing by using hand homogenizer.

Formulation 18 produced cloudy creamy appearance and not in the cubic phase since the appearance was not as clear as the formulation 17. The phase formation for formulation 18 may be in the inverted hexagonal phase. Although the selected formulations are having different phases, the studies of their mean droplet sizes were still continued. All the three selected formulations which were formulation 8, 17 and 18 are having different phases such as cream, clear one phase and phase separation respectively. Only formulation 8 was produced the antimicrobial nano-sized emulsion despite its appearance and phase formation. As stated before, the antimicrobial nano-emulsion droplet size ranges from 200 nm to 600 nm. Despite with the phase formation of formulation 17 and 18, the range of size in micrometer is perhaps results from inappropriate techniques of emulsification. According to Langmuir (2004), he stated that emulsification outcomes are depends mainly on four different types of factor which are hydro-dynamically conditions in mixing device, viscosity ratio, volume fraction of oil and water phases; and type of concentration of used emulsifier.

The phase formation of the surfactant system can be identified by measuring the properties of the system namely birefringence and viscosity. Viscosity is the one of the useful properties to identify the phase of the system. It is said that spherical micelle is often not very viscous. Formulation 8 has low viscosity as the phase formation of the surfactant system is between the cylindrical micelle and spherical micelles. If the micelles are less spherical in shape, the viscosity increased as there is increase in intermicelle interactions in the flow. There will be sudden and extreme increase in the viscosity if hexagonal phase is occurred. In the cubic phase, the surfactant system is produced highest viscosity. Despite having highest viscosity in the cubic phase, they are still liquid-like at molecular level giving rise to diffusion coefficients for water and oil species that are the same order of magnitude as that of bulk (Texter, J., 2001). However, the viscosity for the formulation 17 is lower than that of formulation 8 despite of its cubic phase. Meanwhile for formulation 18, the viscosity is highest among the three formulations.

The appearances of the selected formulations are varied after one month of storage in three different temperature conditions which in room temperature, cold room and hot oven. At room temperature, all of the appearances of the three formulations remained the same after one month of storage. At the temperature of 40^0C, which is high temperature, the three formulations become more flow able. An obvious change was seen in formulation 18 as it turned from two phase separation into one phase after one month of storage. The particles

may be speed up and spread out as the heat is added to a fluid. Volume will be increased if there is an expansion of the fluid. As the volume expands, the density is tends to decrease. As particles speed up and move apart, the viscosity will also be affected. The movements of the particles would be easy to past to each other and therefore, viscosity is decreases. Thus, the particles is tend to be loosely packed to each other. In cold room of temperature of 4^0C, formulation 8, 17 and 18 tend to have changes and become slightly hardened, hardened and turbid two phase respectively. Particles in the three formulations tend to slow down and come closer to each other as heat is taken away from a fluid. As the volume is decreasing, the fluid is tending to contract and causing the density to be increased. The movements of the particles are slowed down in cold room and they are closed to each other. It is getting harder to move past each other in the system and they tend to become hardened.

CONCLUSIONS

As the study was conducted, and these findings suggest that antimicrobial nano-emulsion is determined at the formulation 8. 10% of oil concentration, 70% of water and 20% of surfactant concentration were used in the formulation 8. Only formulation 8 produced the droplets size of 405 nm which is in that range of 200 nm to 600 nm for antimicrobial nano-emulsion. From this study, it can be concluded that after the phase inversion method, additional reduction of size can be achieved by using a high-pressure micro-fluidizer. To improve the stability of the antimicrobial nano-emulsion or to enhance the spectrum of activity, additional ingredients can be added. The final product of the formulation 8 which produced the droplets size of 405 nm, produced a milky in consistency and appearance of antimicrobial nano-emulsion. The temperature does affect the viscosity and appearance of the formulation 8 after one month of storage. Other than that, it can be concluded that to produce more stable nano-emulsion, further study is needed. Stability studies should be done in the period of one year. Other additional ingredients such as soothing agent can be added onto the formulation because tetracycline might cause skin irritation when applied locally. Since the colour of tetracycline is yellow, it might give a yellow-like-jaundice skin colour. The colour of the tetracycline should be masked in the formulation in future study.

ACKNOWLEDGEMENTS

The authors are gratefully acknowledged to the School of Pharmacy, Management & Science University, Shah Alam, Selangor Darul Ehsan, Malaysia and the Faculty of Pharmaceutical Sciences, Josai University, Japan for providing the necessary facilities utilized to carry out

the research project successfully. Further more the authors would like to extend their special thanks to Pharmaniaga Manufacturing Berhad and Pharmaniaga Lifescience Sdn. Bhd. Malaysia for their supports of supplying the raw materials during the research work.

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