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Mohsina Khan
Oriental College of Pharmacy and Research, Oriental University, Indore (M. P.)

Gulfisha Shaikh
Oriental College of Pharmacy and Research, Oriental University, Indore (M. P.)

M K Gupta
Oriental College of Pharmacy and Research, Oriental University, Indore (M. P.)

Gurdeep Singh
Oriental College of Pharmacy and Research, Oriental University, Indore (M. P.)

Correspondence

Ms. Mohsina Khan
Research Scholar, Department of Pharmaceutics, Oriental College of Pharmacy and Research

Email: *mohsinanisarkhan@gmail.com*

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Development and Evaluation of Nanoemulsion of Primaquine for Prevention of Relapsing Malaria

Mohsina Khan, Gulfisha Shaikh, M K Gupta, Gurdeep Singh

ABSTRACT

Malaria relapsing refers to the reactivation of the infection via Relapse, when symptoms reappear after the parasites have been eliminated from blood but persist as dormant hypnozoites in liver cells. Malaria relapse commonly occurs between 8–24 w and is commonly seen with P. Vivax and P. Ovale infections. Primaquine (PQ) is one of the most widely used antimalarial and is the only available drug till date to combat relapsing form of malaria especially in case of Plasmodium Vivax and Plasmodium Ovale. Primaquine acts specifically on the pre-erythrocytic schizonts which are concentrated predominantly in the liver and causes relapse after multiplication but one of the major drawback of this drug is that it dissolves in less proportion in systemic circulation to show an active effect. So to reduce these effects, Primaquine incorporated into oral lipid nanoemulsion having particle size in the range of 10–200 nm. The absorbtion capacity of primaquine is significantly increased as nanoemulsion of Primaquine used. The drug is readily absorbed by the liver 45% more than before. So the results declared the successful absorbtion of primaquine by the liver in its nenoemulsion form as it will be used further in the treatment of malaria because it is less toxic.

Keywords: Relapsing malaria, Nanoemulsion, Primaquine, Pre-erythrocytic schizonts

1. INTRODUCTION

Malaria, a very fatal disease, it has killed millions of people over 30 y. In this duration of time, the death state have been increased[1]. It is being caused by the parasites termed as Plasmodium. The major deaths due to malaria are being caused by Plasmodium falciparum and plasmodium vivax. However, malaria is transferred by the bite of female anopheles mosquito in humans which in turn produces multiple parasites into the body. The growing rate of this disease is known all over the world but in some parts of the world, it is growing at a tremendous rate. The global occurrence of this disease has led to the discovery of new drugs in the past few years.[2] One of the recent trends in curing malaria is chemotherapy. In chemotherapy, the main parasite is directly targeted so it can be eliminated from the human body. One of the most accurate cure or treatment of malaria is targeting the parasite for elimination from the body while keeping the other cells safe it can only be possible by nanotechnology.

Primaquine (PQ), a liver schizonticide, is the only drug available against the relapsing form of malaria and it specifically acts on the latent hepatic forms of the parasite.[3] The new drug Malarone® (atovaquone and proguanil hydrochloride tablet) acts against early liver stage, but it is not clear whether it can fight against latent tissue form malaria in the liver as there has been a case of vivax malarial infection in spite of Malarone® prophylaxis).[4]

Nanoemulsion comes under the category of novel drug delivery systems. It is used to increase the efficacy of a less water-soluble drug once it enters in the systemic circulation of a body.[5] Nanoemulsions are formed by the mixture of two immiscible liquids in which one phase is dispersed in another phase. Their size typically ranges from 50-200 nm. Mostly nanoemulsions consist of oil molecules or droplets dispersed in a watery phase of water droplets dispersed in an oily phase depending on their method of preparation. Nanoemulsions show the separation of two different layers within them that is mostly termed as phase separation. Not all of them show phase separation if they show greater stability.[6] Nanoemulsion are considered as the most adaptable method for drug delivery.[7]

2. MATERIALS AND METHODS

2.1 Materials

Primaquine diphosphate was obtained from ITL Labs, Indore as a gift sample. Tween 80 was procured by RFCL Limited, Faridabad, Span 80 was procured by CDH Limited, New Delhi, Methyl Red Solution was obtained by Fischer Scientific, Mumbai, Castor Oil was procured by Search Creations, Ujjain, Sodium Alginate and Sodium Hydroxide procured by CDH Limited, Delhi, Potassium Dihydrogen orthophosphate procured by Qualigen fine chemicals, Mumbai.

2.2 Preparation and characterization of primaquine free base

Primaquine diphosphate was converted into lipid-soluble primaquine free base by alkalinization with ammonium hydroxide at pH 12.0 and extracted twice with chloroform. The organic phase was washed twice with water and twice with a saturated solution of sodium chloride. Before evaporation, the chloroform was dried with anhydrous sodium sulphate. PQ obtained was characterized by infrared spectroscopy, UV-Spectroscopy, FTIR, DSC, Melting point and solubility.

2.3 Preparation of primaquine lipid nanoemulsion

In the formulation of emulsion 2^3 factorial designing is used. Primaquine emulsion was prepared according to the formula given in the table no.1. A total number of 8 formulations were prepared. First of all primaquine was weighed and mixed with castor oil after that made a S_{mix} ratio. Then mix both, drug mixture and surfactant mixture then this mixture was placed on a magnetic stirrer with hot plate and placed magnetic bead in them. Add distilled water in a dropwise manner with the help of a syringe along with stabilizer and flavoring agents after that continuously add water up to the volume with the help of a syringe with high rpm. When a homogeneous mixture was formed then this mixture was placed on an ultrasonicated for 30 min after this mixture is placed in vortex tube for vortexing and operate instrument for 15 min at 2500 rpm. Then this sample is subjected to centrifugation at 5000 rpm in a centrifuge to achieve a stable formulation which did not show any phase separation or turbidity. The low energy method is the preferred method for the preparation.

Table 1. Composition of Nanoemulsions

S. No.	Emulsion ingredients (mg/ml)	Formulation							
		F1	F2	F3	F4	F5	F6	F7	F8
1.	Primaquine	30	30	30	30	30	30	30	30
2.	Castor Oil	2	2	2	4	4	1.5	3	3
3.	Surfactant (Tween 80)	0.25	0.30	0.33	0.40	0.42	0.325	0.380	0.285
4.	Co–surfactant (Span 80)	0.75	0.90	0.99	1.2	1.26	0.975	1.14	0.855
5.	Water (ml)	27	26.8	26.7	24.4	24.3	27.2	25.5	25.9
6.	Stabiliser (Sodium Alginate)	5	5	5	5	5	5	5	5
7.	Flavouring Agents (Vanilla)	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s

2.4 Stability of Primaquine lipid nanoemulsion

Stability studies of the developed primaquine nanoemulsion were performed as per ICH guidelines. The samples were kept at four different conditions of temperature and relative humidity (%RH) as 40 °C/75% RH, 30 °C/65% RH, 25 °C/60%RH and refrigeration condition. The stability was observed over a period of 3 month. The samples were evaluated for particle size, viscosity and drug content.

3. RESULTS AND DISCUSSION

3.1 Evaluation

3.1.1 Dye test

If a water-soluble dye is added in an o/w emulsion the nanoemulsion takes up the colour uniformly. Conversely, if the emulsion is w/o type and the dye being soluble in water the emulsion takes up the color only in the dispersed phase and the emulsion is not uniformly colored.

3.1.2 Filter paper test

In this method when a drop of emulsion is spread on a filter paper then it spread rapidly this proves an emulsion is o/w and if a drop of emulsion is spread on a filter paper then it will migrate slowly prove emulsion is w/o. It is examined by microscope.

3.1.3 Light microscopy

Light microscopy is a system of lenses to magnify the image of a small object. It is used for the magnification of smaller droplets which cannot be seen by naked eyes.

3.1.4 Viscosity

Viscosity of emulsion was measured by using brook field viscometer (DLV-E). Operation viscometer on 100 rpm at by using spindle 70. When the sample reaches equilibrium the reading is taken. The sample is repeating 3 times. It should lie between 37-43cP

3.1.5 pH

The pH is measured by using pH meter. Firstly calibrate the instrument by buffer capsules of pH 4.0, 7.0 and pH 9. Then read out the reading in 3 sets at 25 °C pH should lie between 5–6.5.

3.1.6 Refractive index

The refractive index of the system was measured by using Abbe refractometer by placing a drop of the sample on the slide at 25 °C. The sample is repeating 3 times. It should lie between 1.450-1.570.

3.1.7 Zeta Potential

The zeta potential of the prepared emulsion is determined by zeta sizer. The sample was placed in cuvette after rinsing with water. The charge on droplets and their zeta potential values were obtained. It should lie between 2 to 6 mV.

Table 2. Evaluation parameters of nanoemulsions

Formulation code	pH	Refractive Index	Viscosity (cP)	Zeta-Potential (mV)
F1	5.37±0.03	1.488±0.033	39.32±1.32	-5.23±0.63
F2	4.84±0.04	1.532±0.022	38.44±1.82	-2.98±0.11
F3	5.28±0.02	1.569±0.016	37.4±1.22	-2.33±0.09
F4	5.23±0.06	1.468±0.018	42.88±2.02	-3.68±0.14
F5	5.94±0.03	1.427±0.023	42.98±2.01	-4.82±0.21
F6	6.02±0.05	1.520±0.040	40.34±1.44	-3.39±0.19
F7	6.08±0.04	1.529±0.038	41.38±1.34	-5.38±0.23
F8	5.74±0.03	1.462±0.019	40.81±1.33	-4.97±0.13

3.1.8 Stability studies

Stability studies of primaquine lipid nanoemulsion revealed that the samples stored in refrigeration, 25 °C/60% RH and 30 °C/65% RH had stable particle size for 3 month. No change in the viscosity and drug content was observed within 3 month.

3.1.9 Freeze thaw cycle

The prepared emulsions were placed in deep freezer at 200 for about 24 h. After completion of 24 h this emulsion was removed and placed on room temperature if the emulsion is stable then it is restored in its original form within 2-3 min. 2-3 such cycles were repeated.

3.1.10 Centrifugation study

The emulsions after freeze-thaw cycle were subjected to centrifugation. The emulsion was placed in a centrifugal tube and subjected to a chamber where they were made to undergo centrifugation for about 30 min at high rpm. If the emulsion is stable, then they did not show any phase separation or turbidity.

3.1.11 Heating cooling cycle

During the study of heating cooling cycle six cycles between refrigerator temperature 40 and 400 for about 48 h.

Those formulations which are stable at this temperature are subjected to further studies which are as follows:

a. pH-5.88

b. Refractive Index–1.538

c. Viscosity–37.9cP

d. Zeta Potential-2.38±0.11mV

4. CONCLUSION

The preparation of nanoemulsion was made by using primaquine and castor oil. Batches F1 to F8 were prepared by using low energy method for preparation of stable emulsion. From the results, it was concluded that nanoemulsion containing S_{mix}in minimum concentration (batch F1, F2 and F3) exhibit better droplets compared to the remaining formulation. Batch F3 and F7 showed more soluble part of dye and sharp droplets in comparison to F1, F2, F4, F5 and F6. When we compared F7 batch with F3, then batch F3 showed better droplets and more intense color. For this reason batch, F3 batch was selected. The composition of nanoemulsion is shown in table no. 1. The nanoemulsion of all the batches were evaluated for different parameters like dye test, filter paper test, light microscopy, pH (between 5 and 6.5), viscosity (between 37 to 43 cp), refractive index (between 1.450 to 1.570) and zeta potential (between-2 to-5 mV). Among eight batches, batch F3 was selected as optimized batch because of

its sharp droplets and more intense color in comparison to formulation F4 and F5. The stability was performed on formulation F3 results for pH, viscosity and refractive index showed no appreciable change up to 3 month of accelerated stability studies.

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