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# "A FORMULATION AND EVALUATION OF PRIMAQUINE LOADED NANO EMULSION OF ANTIMALARIAL DRUG"

Pharmaceuticals

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## ABSTRACT

Malaria, a very fatal disease that is killing millions of people for over 30 years. It is being caused by the parasites termed Plasmodium. Each species of this parasite has its own different characteristics but the major deaths due to malaria are being caused by Plasmodium falciparum and plasmodium vivax. Nano emulsions are formed by the mixture of two immiscible liquids in which one phase is dispersed in another phase. Mostly nano-emulsions 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. Nano emulsions show the separation of two different layers within them that is mostly termed phase separation. Primaquine when incorporated into oral nano-emulsion having a particle size in the range of 50–200 nm showed effective antimalarial activity. Nano emulsion of primaquine exhibited improved oral bioavailability. Nanoparticle were evaluated for dye test, Filter paper test, microscopy, refractive index, in-vitro release pattern.

## KEYWORDS

Malaria, Lipid Nano Emulsion, Primaquine diphosphate.

## INTRODUCTION

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 the systemic circulation of a body [Thiagarajan P 2011 27]. 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 or water droplets dispersed in an oily phase depending on their method of preparation. In o/w nanoemulsion, the oily phase is dispersed in a watery phase while in w/o nanoemulsion the watery phase is dispersed in an oily phase. In continuous nanoemulsions, the particles of both the oil and water are interspersed in the complete system [Kumar S H 2012 30]. In the above-described nanoemulsions, the accurate mixture of surfactants and cosurfactants indicates proper stability of the interface. The major difference between the emulsions and nanoemulsions is that the exhibition of kinetic stability and thermodynamic instability. Nanoemulsion is now considered an excellent method of drug delivery in the body. Nowadays nanoemulsions are used for delivering vaccinations, antibiotics cosmetics, and topical formulations in the body through the oral, ocular, intranasal, pulmonary, and transdermal routes.

Advantages of nanoemulsions

- Reduction of bad taste.
- Enhances bioavailability.
- Enhances the absorption rate.
- Increased patient compliance.
- Requirement of lesser energy.
- Reduces the differences in absorption.
- Solubilization of drugs of lipophilic nature.
- Increased the penetration of drug molecules.
- Delivered via the oral, topical, and intravenous route.
- Water-insoluble drugs can be delivered via the use of nanoemulsions.
- Effectiveness of the drugs is improved when nanoemulsions are used as a mode of drug delivery.
- Disadvantages of nanoemulsions
- Large quantities of surfactant and cosurfactant is required for nanoemulsion preparation.
- Nontoxic surfactant is required for nanoemulsion preparation.
- The nanoemulsion stability is affected by temperature and pH.

## MATERIAL AND METHOD

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 Hydrogen orthophosphate procured by Qualigen fine chemicals, Mumbai.

### Preparation of Primaquine lipid Nanoemulsion:

First of all, Primaquine was weighed and mixed with castor oil after that made an S (mix) ratio. Then mix both, drug mixture and surfactant mixture then this mixture was placed on a magnetic stirrer with a hot plate and placed magnetic bead in them. Add distilled water in a drop-wise 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 Ultra sonicated 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 that did not show any phase separation or turbidity. The low energy method is the preferred method for preparation.

1: **Dye test:** If a water-soluble dye is added to an o/w emulsion the Nanoemulsion takes up the color 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.

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

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.

5: **pH:** The pH is measured by using a 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

6: **Refractive index:** The refractive index of the system was measured by using an Abbe refract meter 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.

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

**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 months. No change in the viscosity and drug content was observed within 3 months.

**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 at 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.

**10: Centrifugation study:** The emulsions after the freeze-thaw cycle was 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.

**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

## RESULTS AND DISCUSSION

The preparation of Nanoemulsion was made by using Primaquine and castor oil. Batches F1 to F8 were prepared by using a low energy method for the 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 the F7 batch with F3, then batch F3 showed better droplets and more intense colour. For this reason batch, the 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 an optimized batch because of its sharp droplets and more intense colour 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 months of accelerated stability studies. SLNs were successfully formed using a modified solvent evaporation method based on a w/o/w double emulsion. The particle size is small enough to allow direct absorption in the gastrointestinal tract. This coupled with a moderately positive surface charged is envisaged to allow for high absorption that could trigger an elevated concentration of PQ base in the liver. Since the drug is highly effective against hypnozoites/liver stages of malaria species, the formulation will be highly efficacious, even at reduced dosages. A prolonged drug release profile was observed which may positively impact dosage frequency and consequently result in reduced toxicity. Efficacy evaluation in mice showed that the Nano formulated PQ was 20% more effective as compared with conventional oral dose, indicating the great potential of improving antimalarial efficacy of drugs by Nano formulating them into SLNs.

Various parameters were examined to optimize nanoparticle size, change, and DL%. A particle of size <500 nm were targeted, as particles of this size are known to be absorbed in the gastrointestinal tract intact. Considering that PQ targets liver stages of malaria parasites, the targeted size was thus found to be ideal. Gives results obtained under varied parameters

**Future prospects for primaquine:** Primaquine is a well known and well-tolerated antimalarial drug. It is widely used in almost all type of malaria except for patient having G6PD deficiency. Primaquine is used as a chemoprophylaxis agent, particularly for traveller's malaria. Novel formulation of primaquine can be prepared for travellers to reduce frequent/daily doses, due to short half-life.

**Transdermal preparation of primaquine:** alone and along with another drug can be prepared in future to overcome the re-occurrence of malaria in the malaria-prone zone.

**Table No. 1 (Concentration & absorption)**

| Concentration (µg/ml) | Absorption |
|---|---|
| 5 | 0.313 |
| 10 | 0.559 |
| 15 | 0.717 |
| 20 | 0.905 |
| 25 | 1.107 |

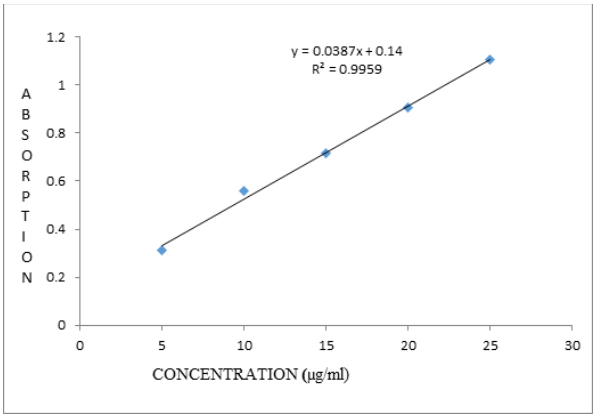
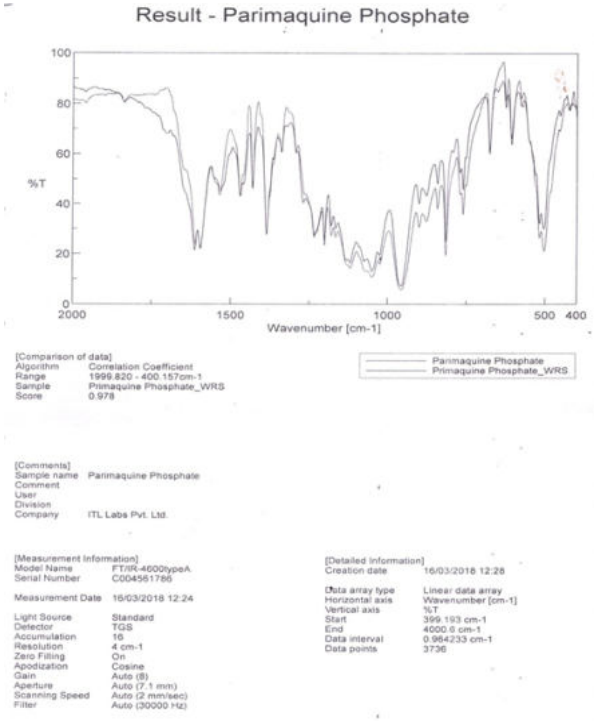



**Fig. 1 (Calibration curve of primaquine in 6.8 pH P.B.S.)**




| | |
|---|---|
| C-C-C (Out of plane bending) | 430 |
| C=C (Out of plane bending) | 460 |
| C-C (Out of plane bending) | 500 |
| C-H (Out of plane bending) | 576 |
| C-H (Out of plane bending) | 669 |
| C-H (Out of plane bending) | 757 |
| C-H (Out of plane bending) | 830 |
| P-O (Ring breathing: Expansion and contraction occurring to a ring) | 988 |
| C-C (Stretching) | 1010 |
| P-O (Asymmetric stretching) | 1058 |
| C-O (Stretching) | 1134 |
| C-O-C (Stretching) | 1156 |
| C-O (Stretching)- | 1248 |
| N-H (Bending) | 1368 |
| O-H (Bending)- | 1417 |

**(1) DSC:**

- **DSC study for drug (primaquine):**

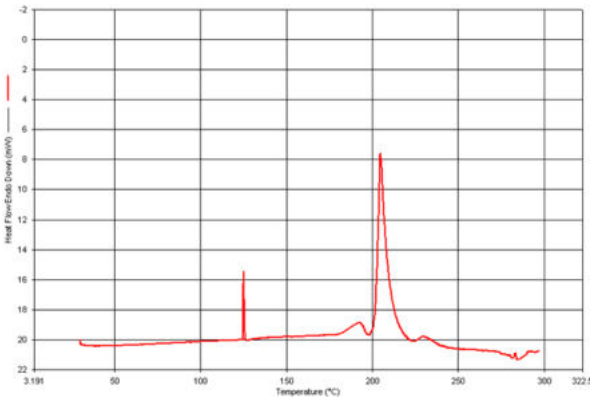



**Fig. (DSC of Primaquine)**

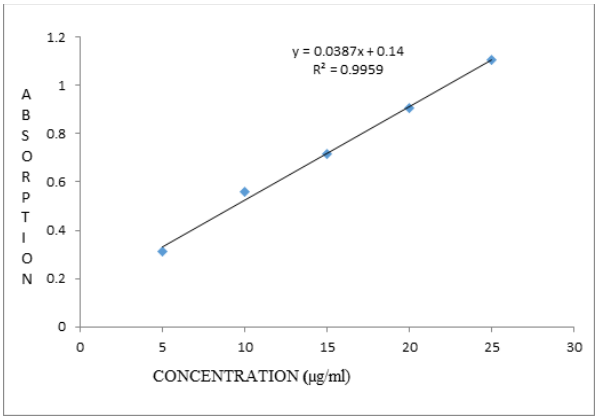



**Fig. 4 (Calibration curve of primaquine in 6.8 pH P.B.S.)**

**Table no.2: Different formulations containing varying proportions of excipients**

| S. NO | EMULSION AND INGREDIENT | Formulations | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 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. |

**Table no.3 Evaluation parameter of Nano Emulsion**

| Formulation | Ph. | Refractive Index | Viscosity | Zeta Potential |
|---|---|---|---|---|
| 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 |