Development and Evaluation of Nanoemulsions for Phenytoin Drug Loading

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To understand about the nanoemulsion types and the process formation of spontaneous emulsification method by phase inversion. Then to test the different combinations of Oil, Surfactants and Co-surfactants for formation of suitable nanoemulsions for phenytoin drug loading.

Study Design: Spontaneous emulsification method by phase inversion used to form the nanoemulsions.

Place and Duration of Study: Department of Pharmaceutical Sciences, Kumaun University, Nainital, Uttarakhand, India.

Methodology: Phenytoin is a widely used drug in anticonvulsants class for epilepsy which comes under BCS Class II of drug category. Phenytoin has high permeability property but it also shows low solubility property which makes it difficult to absorb from GI tract hence make a poor penetration into the brain to target disease in the CNS. To overcome the situation of poor delivery of phenytoin, the requirement of nanoparticulate drug delivery as an innovative and effective drug

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delivery system from nose to brain raised. The objective of our study was to find the best combination of oil and $S_{na}$ (surfactant and co-surfactant mixture) to form o/w (Oil in Water) nanoemulsions suitable for loading phenytoin drug using spontaneous emulsification method for brain targeting.

**Results:** Based on different compositions of oil (sunflower), surfactants (Tween-20), and cosurfactants (Transcutol P), forty-five test mixtures were made, water titration technique was employed for preparing the pseudo-ternary-phase diagrams. On the basis of these phase diagrams twenty-five phenytoin loaded nanoemulsions were formulated and further examined. After physicochemical characterization of these formulations the viscosity, pH, RI and % transmittance was found \((6.149 \pm 0.084 \text{ to } 9.114 \pm 0.027)\), \((6.546 \pm 0.018 \text{ to } 6.656 \pm 0.017)\), \((1.395 \pm 0.003 \text{ to } 1.41 \pm 0.005)\) and \((94.53 \pm 1.4\% \text{ to } 95.58 \pm 1.2\%)\) respectively. The release rate of phenytoin was found very satisfactory i.e., 98.51 ± 0.25 % to 99.82 ± 0.28 % after 24 hrs. The four formulations showed best release rate had further taken for particle size analysis. The particle size analysis showed that all the properties were in the desired range i.e., droplet size \((18.9 \text{ to } 21.9)\), zeta potential \((-12.4 \text{ to } -28.8)\), PDI \((0.334 \text{ to } 0.363)\). The study shows that the phenytoin loaded nanoemulsion is possible to make by water titration method and shall have a good drug release rate.

**Conclusion:** The nanoemulsion formulations passed through stress testing had also showed good release rate of phenytoin. Also, the other parameters like viscosity, pH, RI and percentage transmittance were in a quit satisfactory range to proceed further with these formulations. The particle size analysis confirms the formation of nanoemulsions which had very good drug release rates.

**Keywords:** CNS; Nanoemulsion; Phenyoit; pseudoternary-phase diagram; Spontaneous emulsification; particle size; zetapotential; TEM.

1. **INTRODUCTION**

Epilepsy One of most common neurological disorder which affects people of all ages is epilepsy. It is a chronic disorder, the characteristic of which is recurrent, unprovoked seizures. Human brain is the basis of human epilepsy [1]. Epilepsy can develop as a result of brain damage from other disorders including brain tumors, alcoholism, Alzheimer's disease, strokes, and heart attacks [2].

Phenytoin is a widely used drug in anticonvulsants class for epilepsy which comes under BCS Class II of drug category i.e., Phenytoin has property of high permeability but low solubility. Due to this behavior, phenytoin is hard to absorb from GI tract and has poor penetration into the brain to target disease in the CNS [3]. The requirement of delivering drug to brain so that it can properly act on CNS is a big challenge. Accordingly, the requirement of enhancing the solubility of Phenytoin raised [4].

The blood–brain barrier (BBB) plays a fundamental role in protecting the brain from toxic substances and therefore also controls and restricts the entry of therapeutic agents. The nasal administration of drugs using the nose-to-brain pathway allows direct drug targeting into the brain, avoiding the first-pass effect and bypassing the BBB. Through the nasal route, the drug can access the brain directly along the trigeminal and olfactory nerves, which are located in the upper part of the nasal cavity [5].

The central nervous system (CNS) is the part of the nervous system consisting of the brain and spinal cord. Retina and the optic nerve (2nd cranial nerve), as well as the olfactory nerve (1st) and olfactory epithelium are considered as parts of the CNS, synapsing directly on brain tissue without intermediate ganglia [6]. The olfactory epithelium, membranous tissue located inside the nasal cavity is the only central nervous tissue in direct contact with the environment, which opens up for therapeutic treatments [7]. Here the nanoemulsion for nano particulate drug delivery of phenytoin comes in picture as a novel system for nose to brain drug delivery.

Nanoemulsion is a thermodynamically stable formulation with a size range up to 100 nm, [8] Nanoemulsion have the properties of encapsulating a variety of drugs in it, [9] being a nano dosage form it can go deeper into to the nasal cavity because it is composed of oils, surfactant and co-surfactants and also the overall properties of the nanoemulsion makes it perfect to cross the barriers in nasal absorption like low
bioavailability and low membrane transport [10].

The objective of our study was to understand about the nanoemulsion types and the process formation of spontaneous emulsification method by phase inversion. Then to test the different combinations of Oil, Surfactants and Co-surfactants for formation of suitable nanoemulsions for phenytoin drug loading. At last, In-Vitro drug release studies of suitable nanoemulsion formulations.

2. MATERIALS AND METHODS

2.1 Materials

Phenytoin was obtained from M/s Sigma Aldrich, Mumbai and caprylcaproyl macrogol glycerides (Labrasol), Capryol 90 (propylene glycol monocaprylate), diethylene glycol monoethyl ether (Transcutol P) were obtained as a gift sample by M/s Gattefosse (Mumbai). Tween 20, Tween 80, propylene glycol, PEG 200, glycerol, Isopropyl myristate, sunflower oil, linseed oil and olive oil were made available by Department of pharmaceutical science, Kumaun university Nainital. All chemicals were of analytical grade. Milli Q water was used during the whole experiment.

2.1.1 Nanoemulsion and its types

The word “Nanoemulsion” refers to the thermodynamically stable and isotropically clear dispersion of nano particle of a fluid such as oil, stabilized by an interfacial film of surface-active agent molecules, into other unmixable fluid such as water. There are mainly two types of nanoemulsions, oil particles dispersed in aqueous phase (o/w) and water particles dispersed in oil phase (w/o). The phenytoin has poor solubility in water so it should be loaded in oil phase and accordingly o/w type nanoemulsion shall be prepared. The o/w nanoemulsion is characterized as the mean droplet diameters ranges within 5 to 200 nm [11].

2.1.2 Spontaneous emulsification method by phase inversion

There are many techniques to form nano emulsions. The spontaneous phase inversion technique is the best out of them as it requires minimum free energy to form a nanoemulsion. So, the nanoemulsion formed by this technique shall have better stability as compare to others. To form the desired o/w nano emulsion the predefined combinations of oil and $S_{mix}$ (a mixture having certain concentrations of surfactant and co-surfactant) are mixed well. The mixtures of oil and $S_{mix}$ were titrated with water slowly. Water forms separate bubbles in oil and $S_{mix}$ mixture and a two-phase system was clearly visible. After the water exceeded a certain amount there happened a spontaneous phase inversion and the both phases i.e., oil and water mixed spontaneously and formed a clear mixture which is known as o/w nano emulsion system [12,13,14].

2.2 Screening of Excipients

The solubility of drug in different oils, surfactants and co-surfactants was the criteria for optimum combination selection.

2.2.1 Screening of oil for nanoemulsion

To determine the maximum solubility of Phenyoitn in different oils such as Capryol 90, Isopropyl myristate, Olive oil, Sunflower oil, Linseed Oil, a surplus drug amount was mixed in 2 mL of the oils in a 5 mL capacity stopper vials using a vortex mixer. After that, vials containing mixture were placed in an isothermal shaker for three days between 24 to 26 °C to achieve the equilibrium. After three days the samples were centrifuged for 15 minutes at 3,000 rpm. After centrifugation the supernatant was filtered through a 0.22-μm membrane filter. The concentration of phenytoin drug in filtered supernatant was determined by UV spectrophotometer at 263 nm and for that 10 µL of supernatant oil was taken [15].

2.2.2 Screening of surfactant and co-surfactant for nanoemulsion

Similarly, to determine the best surfactant and co-surfactant for nanoemulsion formulation the solubility of Phenytoin was determined in different surfactants including Tween 20, Tween 80, Labrasol and co-surfactants such as Polyethylene glycol 200, diethylene glycol monoethyl ether (Transcutol-P), glycerol, Propylene glycol.

2.2.3 Phase studies and Selection of nanoemulsions

The screened oil, surfactant and co-surfactant were further taken for phase study to achieve their optimum ratios for nano emulsions formation. The water used for aqueous phase
formation was Milli Q. The pseudo ternary phase diagrams were constructed to determine the nanoemulsion region formed using different concentrations oil and S\textsubscript{mix} (combination of surfactant and co-surfactant) by means of water titration method (spontaneous emulsification method). The S\textsubscript{mix} was formed in different volume combinations of surfactant and co-surfactant like 1:1, 1:2, 1:3, 2:1 & 3:1. For each phase diagram, oil and specific S\textsubscript{mix} were mixed well in different ratios of increasing S\textsubscript{mix} from 1 to 9 and decreasing Oil ratios from 9 to 1. Accordingly, total nine combinations of S\textsubscript{mix} and oil (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1) were tried to incorporate a wide range for nanoemulsion region [16]. All the combinations of S\textsubscript{mix} and oil mixture were slowly titrated with water under medium stirring, and visual observation was done for checking phase inversion / separation with transparency / opaqueness and easily flowable nanoemulsion.

2.2.4 Development of phenytoin loaded nanoemulsions

The predetermined ratio of oil and S\textsubscript{mix} are mixed and 1% (w/v) of phenytoin drug was then incorporated into that mixture. The mixture is blended until the complete dissolution of the drug. Then the aqueous phase was added dropwise under continuous stirring to achieve transparent mixture after spontaneous phase inversion [17].

2.3 Physicochemical Characterization

Selected drug loaded nanoemulsion formulations were characterized for the various parameters:

2.3.1 Thermodynamic stability testing of nanoemulsions

The thermodynamic stability studies incorporated three stress tests, Heating-cooling cycles (from 4 °C to 45 °C, six times with storage of 03 days), freeze-thaw cycles (deep freeze at -21°C for one day and after that back to 25°C) and centrifugation studies (3500 rpm for half hour) were conducted to examine the physical stability of phenytoin drug loaded nanoemulsion formulations and to reject the unstable or metastable nanoemulsion formulations [18,19].

2.3.2 Viscosity

The viscosity of nanoemulsion is a very important parameter which directly related with stability of nanoemulsion as well as release of drug from it. Brookfield DVIII viscometer (Brookfield Engineering Laboratories Inc., USA) was used to measure the viscosity of nanoemulsions. The rheogram plotted the shear stress (dynes/cm²) against the shear rate (s⁻¹). The speed of spindle no. 4 was maintained to 150 rpm at 25.0 ± 0.5°C [20, 21].

2.3.3 pH measurement

The pH of samples was determined at 25 °C in triplicate using Mettler Toledo, Switzerland pH meter. [22] The pH should be in accordance of body part where nanoemulsion is applied [23].

2.3.4 Refractive index

The refractive index represents the isotropic nature of formulation. The nanoemulsions were tested in triplicate at 25 °C for measurement of refractive index. Abbe-type refractometer (Shanghai Optical Instrument Factory, China) is used for the same.

2.4 Percentage Transmittance

The life of nanoemulsion will be less if it will absorb more light. So, the nanoemulsion should be transparent. UV/Vis spectrophotometer (UV-6100 PC, EMC lab, Germany) was used to determine the transmittance of each NE systems in triplicate [24]. The absorbance is inversely proportional to transmittance.

\[
\text{Percent Transmittance} = 10(\text{-absorbance}) \times 100 \%
\]

\[
\text{Absorbance} = -\log \left( \frac{\text{Percent Transmittance}}{100} \right)
\]

2.4.1 In-vitro drug release studies

The release behavior of phenytoin from nanoemulsion formulations was studied using dialysis bag method. A cellulose dialysis bag (MW cut-off 12,400 Da) was washed with distilled water at 4 °C and filled with 1 ml of each nanoemulsion formulation. The bag was attached with a paddle of USP apparatus rotating at a speed of 50 rpm and dipped into 900 ml of phosphate buffer release medium (pH=7.4) [25]. For drug release analysis 2 ml of samples after 15min, 30 min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 12hr and 24hr were taken out and replaced with fresh buffer release medium. The taken-out samples were analyzed by UV spectrophotometry at 205 nm to measure the amount of Phenytoin.
released from NEs, accordingly the percentage of release efficiency (RE) of nanoemulsions were calculated [26].

2.4.2 Particle size, polydispersity index and zeta potential

To confirm that the size of particles in formulations were in nano the Malvern Zetasizer NanoZS (Malvern, United Kingdom) was used. The equipment also provided information about the uniformity of droplet size i.e., polydispersity index (PDI) and electrostatic repulsion between the particles i.e., zeta potential. For this testing the Phenytoin NEs were diluted to 1:49 v/v in HPLC grade water [27].

2.5 Transmission Electron Microscopy (TEM)

In order to verify the nano-droplet formation of formulations, the transmission electron microscopy (TEM) of best NE formulations was also done using Morgagni 268D electron microscope (Fei Company, Netherlands) 70 kV. The TEM captures high resolution images increasing magnification to identify the form and size of droplets and their distribution in nanoemulsion. TEM image analysis gives the true radiuses which is more precise as compare to hydrodynamic radius provided by Zetasizer [28].

3. RESULTS AND DISCUSSION

To form the o/w phase nanoemulsions the different combinations of oil and S mix tried for their mixture and closely observed the water titration process.

3.1 Spontaneous Phase Inversion and Nanoemulsion Formation

The surfactant chosen for nanoemulsion formulation had a high HLB value which means surfactant is more reactive with water as compare to oil. So, a certain concentration of surfactant initially required more amount of oil to form a bond in mixture as compare to water to be mixed after that by titration method. When titration by aqueous phase was done the surfactant, particles were attracted towards water particles. After a certain water concentration, the surfactant breaks its bonds with oil and joins their head with water particles but this time oil was also available in mixture so the tail of surfactant covers to oil particles and form a layer between oil and aqueous phase. As the surfactant breaks its bonding with oil and forms a bonding with water the volume of oil as compare to volume of water and surfactant suddenly decreased. In the meantime, co-surfactant acted as an agent to reduce the surface tension of aqueous phase and oil particles started penetration inside the aqueous phase in nano particle form covered by surfactant tails. As the particle size should lie in nano range so the oil particles should be fine enough to see by human eyes results a transparent nanoemulsions [29,30].

3.2 Screening of Excipients

3.2.1 Screening of oil

In the development of drug loaded nanoemulsion system the main factor is to test the solubility of drug in a variety of formulation components. Better the solubility means the volume of drug loaded nanoemulsion will also be reduces and provide better therapeutic dose.

The solubility test graph of phenytoin drug in different oils is shown in Fig-1. It depicts the solubility of phenytoin was found highest in sunflower oil (7.4±0.048 mg/ml) in comparison with others. Studies shows that the higher the concentration of surfactant may increase toxicity of the system. So, it is preferred to have oil having high solubility of phenytoin as less oil in formulation will also require less surfactant [31].

3.2.2 Screening criteria for surfactants

As per industry standards the o/w nanoemulsion formulations shall have a good in-vivo stability. Therefore, it become more important to select a proper surfactant. The HLB value of surfactant should also be chosen properly as in o/w base nanoemulsions higher HLB value of surfactant is required so that during the mixing of water phase it may easily break its bonding with oil phase and join with aqueous phase results a phase inversion [32].

After analyzing it was found that out of three surfactants Tween-20 showed the best solubility of phenytoin drug i.e., 23.2±0.062 mg/ml (Fig. 1).

3.2.3 Screening of co-surfactants

Co surfactants are used to reduce the boundary tension and increase the fluidity of interface. These are generally medium-chain alcohols (C3-C8). The co surfactants are also allow better
penetration of oil by increasing the mobility of the hydrocarbon tails.

As depicted in Fig-1 the PEG 200 showed the best solubility of phenytoin drug i.e., 38.6±0.091 mg/ml.

3.2.4 Preparation of pseudo ternary phase diagram

The ternary phase diagram between oil, S<sub>mix</sub> and water is plotted to obtain the nanoemulsion region on the basis of results obtained in Table No. -1. On phase diagram an o/w nanoemulsion region was observed on high water ratio side. Beyond the nanoemulsion reason there was a phase separation and turbid mixture found for high and low oil concentrations respectively. The equal concentration of surfactant and co-surfactant gave a good nanoemulsion reason (Fig. 2a), which further increased by increasing co-surfactant concentration to double of surfactant (Fig. 2b) but again increase co-surfactant concentration to triple of surfactant showed reduction in nanoemulsion region (Fig. 2c). So, no further concentration of co-surfactant was increased. Similarly, the nanoemulsion region was also increase for increasing the surfactant ration to double by co-surfactant (Fig. 2d) but again decreased for further increase (Fig. 2e). Therefore, there was no need to attempt more test with further increase in surfactant concentration.

3.3 Loading of Phenytoin in Selected Nanoemulsion

On the basis of visual observation twenty optimized combinations of nanoemulsions out of total forty-five were selected. The oil and S<sub>mix</sub> as per those appropriate predetermined combinations were mixed and loading of 1% (w/v) of phenytoin drug was done. The drug was solubilized in oil and form a mixture which was titrated by water to achieve spontaneous phase inversion and formation of phenytoin loaded nano emulsions.

3.3.1 Physicochemical characterization

The selected formulations were tested for physicochemical characterization.

3.3.2 Thermodynamic stability testing of nanoemulsions

In bar diagram all three stability tests were represented by different color bars. Any formulation succeeded in any test shall contain that color bar. So, the formulations having all three-color bars means got succeeded in all three tests and were thermodynamically stable. After Heating-cooling cycles, freeze-thaw cycles and centrifugation studies only eight formulations out of twenty were passed the stress testing (Fig. 3).

![Fig. 1. Solubility of phenytoin in different oils, surfactants & co-surfactants at 25°C (mean ± SD, n=3)](image-url)
Table 1. Nanoemulsion formulations by different combinations of Oil, \( S_{\text{mix}} \), & Water

| S. No. | \( S_{\text{mix}} \) Ratio | Formulation No. | Drug (% w/v) | OIL | \( S_{\text{mix}} \) | Water | Transparency |
|--------|--------------------------|----------------|-------------|-----|----------------|-------|--------------|
| 1      | 1:1                      | FPHT1          | 1           | 43.80 | 29.20 | 27.01 | Clear       |
| 2      | 1:1                      | FPHT2          | 1           | 36.23 | 36.23 | 27.54 | Clear       |
| 3      | 1:1                      | FPHT3          | 1           | 28.37 | 42.55 | 29.08 | Clear       |
| 4      | 1:1                      | FPHT4          | 1           | 17.86 | 41.67 | 40.48 | Clear       |
| 5      | 1:2                      | FPHT5          | 1           | 48.00 | 32.00 | 20.00 | Turbid      |
| 6      | 1:2                      | FPHT6          | 1           | 34.25 | 34.25 | 31.51 | Clear       |
| 7      | 1:2                      | FPHT7          | 1           | 26.49 | 39.74 | 33.77 | Clear       |
| 8      | 1:2                      | FPHT8          | 1           | 17.86 | 41.67 | 40.48 | Clear       |
| 9      | 1:3                      | FPHT9          | 1           | 44.44 | 29.63 | 25.93 | Clear       |
| 10     | 1:3                      | FPHT10         | 1           | 33.56 | 33.56 | 32.89 | Clear       |
| 11     | 1:3                      | FPHT11         | 1           | 25.32 | 37.97 | 36.71 | Clear       |
| 12     | 1:3                      | FPHT12         | 1           | 17.05 | 39.77 | 43.18 | Turbid      |
| 13     | 2:1                      | FPHT13         | 1           | 48.39 | 32.26 | 19.35 | Clear       |
| 14     | 2:1                      | FPHT14         | 1           | 37.88 | 37.88 | 24.24 | Clear       |
| 15     | 2:1                      | FPHT15         | 1           | 29.63 | 44.44 | 25.93 | Clear       |
| 16     | 2:1                      | FPHT16         | 1           | 19.23 | 44.87 | 35.90 | Clear       |
| 17     | 3:1                      | FPHT17         | 1           | 48.00 | 32.00 | 20.00 | Turbid      |
| 18     | 3:1                      | FPHT18         | 1           | 39.68 | 39.68 | 20.63 | Clear       |
| 19     | 3:1                      | FPHT19         | 1           | 28.99 | 43.48 | 27.54 | Clear       |
| 20     | 3:1                      | FPHT20         | 1           | 20.55 | 47.95 | 31.51 | Clear       |

\( \% \)=Percentage; w/v = weight per volume; \( S_{\text{mix}} \)=Surfactant: Co-surfactant; FPHT=Phenytoin formulation
3.3.3 Viscosity

The viscosity of nanoemulsion formulations was found between 6.149 ± 0.084 cps to 9.114 ± 0.027 cps (Fig 4). Which will give more hold time to drug on nasal mucosa results better drug delivery [33].

3.3.4 pH measurement

The pH of nanoemulsions was found between 6.546 ± 0.018 to 6.656 ± 0.017 (Fig. 4). In general, the pH of nose mucosal ranges between 5.5 to 6.5 but it increases in the range of 7.2 to 8.3 with condition of rhinitis (nasal obstruction or congestion situation). [34] Study shows that the children allergic with rhinitis have 76% increase risk chances of epilepsy. [35] Accordingly, the pH of nose mucosal for study was considered in the range of 5.5 to 7.8.

3.3.5 Refractive index

The RI of nano formulations were found between 1.395 ± 0.003 to 1.41 ± 0.005 (Fig. 4). This shows isotropic nature of the formulations and RI closer to water i.e., 1.334 shows high water content used in formulation and safer in terms of toxicity. [35]

3.4 Percentage Transmittance

The percentage transmittance of formulations found between 94.53 ± 1.4% to 95.58 ± 1.2%. This represents that there was less absorption of light and similarly, the absorbance varied...
between 0.0196 ± 0.0055 to 0.0244 ± 0.0064. It shows the better stability of nanoemulsions.

3.4.1 In-vitro drug release studies

The results of in-vitro drug release profile of all eight selected formulations are shown in Fig.5. It was observed that initially within 6 hours the release of drug from all formulations were very high and ranged between 75.20 ± 0.15 % to 95.77 ± 0.14 %. After that from 6 to 12 hours the release rate was slightly decreased and 88.33 ± 0.64 % to 98.53 ± 0.36 %. After 24 hrs the range of release rate of formulations was found between 98.51 ± 0.25 % to 99.82 ± 0.28 % which is a very good release rate of drug from nanoemulsion. Formulations having top four release statics had been taken further for their droplet size analysis.

3.4.2 Particle size, polydispersity index and zeta potential

Table-3 shows the results of particle size, polydispersity index and zeta potential values of top four formulations having best drug release percentage. It was observed that all the four nanoemulsion formulations had nano sized droplets. [36] The PDI value of all these formulations are less than 0.5 which indicated that each formulation has droplets spread uniformity. [37] Also, the zetapotential of formulation were found from -28.8 to -12.4 which showed good repulsion force between nanoparticles results a good stability property of nanodroplets in aqueous phase. [38,39]
Table 2. Viscosity, pH measurements, Refractive index, % transmittance and absorbance

| S. No. | Formulation | Viscosity (cps) ± SD | pH ± SD | Refractive Index ± SD | Transmittance (%) | Absorbance |
|--------|-------------|----------------------|---------|-----------------------|-------------------|------------|
| 1      | FPHT4       | 6.946 ± 0.072        | 6.567 ± 0.011 | 1.399 ± 0.007 | 95.29 ± 0.8 | 0.021 ± 0.0036 |
| 2      | FPHT6       | 9.114 ± 0.027        | 6.652 ± 0.019 | 1.41 ± 0.005  | 94.53 ± 1.4 | 0.0244 ± 0.0064 |
| 3      | FPHT7       | 8.856 ± 0.018        | 6.594 ± 0.014 | 1.407 ± 0.008 | 94.73 ± 0.4 | 0.0235 ± 0.0018 |
| 4      | FPHT8       | 6.946 ± 0.032        | 6.567 ± 0.021 | 1.398 ± 0.02 | 95.32 ± 0.7 | 0.0208 ± 0.0032 |
| 5      | FPHT10      | 8.526 ± 0.049        | 6.656 ± 0.017 | 1.408 ± 0.009 | 94.66 ± 1.1 | 0.0238 ± 0.0050 |
| 6      | FPHT11      | 7.695 ± 0.041        | 6.606 ± 0.013 | 1.403 ± 0.005 | 95.01 ± 1.5 | 0.0222 ± 0.0069 |
| 7      | FPHT12      | 6.149 ± 0.084        | 6.579 ± 0.022 | 1.395 ± 0.003 | 95.58 ± 1.2 | 0.0196 ± 0.0055 |
| 8      | FPHT16      | 8.631 ± 0.094        | 6.546 ± 0.018 | 1.406 ± 0.006 | 94.84 ± 0.3 | 0.023 ± 0.0014 |

SD=Standard Deviation; %=Percentage; Cps=Centi poise; Values are mean ± SD; (n=40); (p < 0.001)

Table 3. Particle size, polydispersity index and zeta potential of nanoemulsion

| S. No. | Formulation code | Droplet Size (d.nm) | Polydispersity Index | Zeta Potential (mV) |
|--------|------------------|---------------------|----------------------|---------------------|
| 1      | FPHT6            | 21.9                | 0.345                | -12.4               |
| 2      | FPHT7            | 21.5                | 0.334                | -27.0               |
| 3      | FPHT10           | 19.5                | 0.342                | -13.1               |
| 4      | FPHT16           | 18.9                | 0.363                | -28.8               |

Fig. 6. TEM image of optimized nanoemulsion FPHT16
3.5 Transmission Electron Microscopy

In order to further confirm the nano-droplet formation of formulations, the morphology of the best selected NE formulation (FPHT16) was determined by TEM (Fig. 6). The size distribution analysis in figure shows the uniformity of particles size. The image obtained from TEM was in accordance with the results obtained from MDS.

4. CONCLUSION

Phenytoin has high permeability but low solubility and it comes under BCS Class II category. Due to low release of phenytoin through GI track for brain targeting we had explored the possibility of nanoemulsion formation. As phenytoin is a lipophilic drug so during formation of its o/w nanoemulsion the surfactant was chosen in such a manner so that addition of water may easily break the bond of oil and surfactant. The nanoemulsion passed through stress testing had also showed good release rate of phenytoin. Also, the other parameters like viscosity, pH, RI and percentage transmittance were in a quit satisfactory range to proceed further with these formulations. As the objective of experiment was to formulate the nano particles of drug loaded oil dispersed in aqueous phase which was also confirmed by particle size analysis. All the formed nanoemulsion formulations had showed good drug release rate and may be considered for further studies.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pietrangelo A. Epilepsy: Causes, symptoms, treatment, and more [Internet]. Healthline.com. 2014. Accessed: 12 Jun 2018. Available: https://www.healthline.com/health/epilepsy
2. U.S. Department of Health and Human. Guidance for Industry Starting Dose in Initial Clinical Trials Guidance for Industry Estimating the Maximum Safe. Pharmacol Toxicol. 2005;1–27.
3. Devadasu Venkat Ratnam, Pran Kishore Deb, Rahul K. Maheshwari, Piyooosh Sharma, and Rakesh Tekade. “Physicochemical, Pharmaceutical, and Biological Considerations in GIT Absorption of Drugs.” Dosage Form Design Considerations. 2018;149–178. DOI:10.1016/b978-0-12-814423-7.0005-8.
4. Bonferoni MC, Rossi S, Sandri G, Ferrari F, Gavini E, Rassu G, Giunchedi P. Nanoemulsions for "Nose-to-Brain" Drug Delivery. Pharmaceutics. 2019;11(2):84. DOI:10.3390/pharmaceutics11020084. PMID: 30781585; PMCID: PMC6409749.
5. Ding J, Na L, Mao S. Chitosan and its derivatives as the carrier for intranasal drug delivery. Asian J Pharm Sci. 2012;7(6):349-61.
6. Djupesland PG, Mahmoud RA, Messina JC. Accessing the brain: the nose may know the way. Journal of Cerebral Blood Flow & Metabolism. 2013;33(5):793-4. DOI: 10.1038/jcbfm.2013.41
7. Paliwal S, Kaur G, Arya RKK, Formulation and characterization of topical nanoemulgel of terbinafine. Univers J Pharm Res, 2018;3 (6):28-37. DOI: 10.22270/ujpr.v3i6.223
8. Chandra A, Arya RKK, Tewari B, Pal GR. Formulation and Evaluation of Ginger Extract Loaded Nanoemulgel for the Treatment of Rheumatoid Arthritis, J Drug Delivery Ther, 2019;9(4):559-570. DOI: 10.22270/jddt.v9i4.3143
9. Behl CR, Pimplaskar HK, Sileno AP, Xia WJ, Gries WJ. Optimization of systemic nasal drug delivery with pharmaceutical excipients. Advanced drug delivery reviews. 1998;29(1-2):117-33. DOI: 10.1016/S0169-409X(97)00064-1
10. Shahid SM, Chowdeswari A: A review on nanoemulsion. Scandinavian Journal of
11. Akay G, Tong L. Intensive structuring: intensive processing of microstructured materials by flow induced phase inversion, Che. Eng. Technol. 2000;23:285-288. DOI: 10.1002/(SICI)1521-4125(200003)23:3.0.CO;2-O.
12. Fernandez P, Andre V, Rieger J, Kuhnle A. Nano-emulsion formation by emulsion phase inversion, Coll. Surf. A, 2004;251:53-58. DOI:10.1016/j.colsurfa.2004.09.029.
13. Galindo-Alvarez JG, Sadtler V, Choplin L, Salager J. Viscous Oil Emulsification by Catastrophic Phase Inversion: Influence of Oil Viscosity and Process Conditions. Ind. Eng. Chem. Res. 2011;50:5575-5583. DOI: 10.1021/ie102224K.
14. Kulthe VV, Chaudhari PD. “UV Spectrophotometric Estimation Of Acetazolamide By Standard Calibration Curve Method And Its Validation” Indian drugs. 2012;49(7):36-41.
15. Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, Talegaonkar S. Nanoemulsion components screening and selection: a technical note. AAPS Pharm Sci Tech. 2009;10(1):69-76. DOI: 10.1208/s12249-008-9178-x. Epub 2009 Jan 16. PMID: 19148761; PMCID: PMC2663668.
16. Ammar HO, Salama HA, Ghorab M, Mahmoud AA. Nanoemulsion as a potential ophthalmic delivery system for dorzolamide hydrochloride. AAPS Pharm Sci Tech. 2009;10:809-18.
17. Ali MS, Alam MS, Alam N, Siddiqui MR. Preparation, characterization and stability study of dutasteride loaded nanoemulsion for treatment of benign prostatic hypertrophy. Iran J Pharm Res. 2014 Fall;13(4):1125-40. PMID: 25587300; PMCID: PMC4232777.
18. Shafiq-un-Nabi S, Shakeel F, Talegaonkar S. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur. J. Pharm. Biopharm. 2007;66:227-243.
19. Eini DI, 5 BW, Rhodes CT. Micellar size, shape, and hydration of long-chain polyoxyethylene nonionic surfactants. J Colloid Interface Sci. 1976;54:348-51.
20. Barry BW, El Eini DI. Solubilization of hydrocortisone, dexamethasone, testosterone and progesterone by long-chain polyoxyethylene surfactants. J Pharm Pharmacol. 1976;28:210-8.
21. Gué E, Since M, Ropars S, Herbinet R, Le Pluart L, Malzert-Frén A. Evaluation of the versatile character of a nanoemulsion formulation. Int J Pharm. 2016;498:49-65.
22. Morsi NM, Mohamed MI, Refai H, El Sorogy HM. Nanoemulsion as a novel ophthalmic delivery system for acetazolamide. Int J Pharm Pharm Sci. 2014;6:227-36.
23. Nasr AM, Gardouh AR, Ghonaim HM, Ghorab MM. Design, formulation and in-vitro characterization of Irbesartan solid self-nanoemulsifying drug delivery system (S-SNEDDS) prepared using spray drying technique. J Chem Pharm Res. 2016;8(2):159-183.
24. Miyazaki S, Suzuki S, Kawasaki N, Endo K, Takahashi A, Attwood D. In situ gelling xyloglucan formulations for sustained release ocular delivery of pilocarpine hydrochloride. Int J Pharm. 2001;229:29-36.
25. Shen J, Burgess DJ. In Vitro Dissolution Testing Strategies for Nanoparticulate Drug Delivery Systems: Recent Developments and Challenges. Drug Deliv Transl Res. 2013;3(5):409-415. DOI: 10.1007/s13346-013-0129-z. PMID: 24069580; PMCID: PMC3779615.
26. Mahboobian MM, Seyfoddin A, Rupenthal ID, Aboofazeli R, Foroutan SM. Formulation Development and Evaluation of the Therapeutic Efficacy of Brinzolamide Containing Nanoemulsions. Iran J Pharm Res. 2017Summer;16(3):847-857. PMID: 29201076; PMCID: PMC5610741.
27. Savadekar P, Bajaj A. Nanoemulsions- A Review International Journal Of Research In Pharmacy And Chemistry 2781-IRJPC. 2016;6(2):312-322. ISSN: 2231-2781.
28. Anonymous; Surfactants & critical micelle concentration (CMC) - DataPhysics Instruments [Internet]. Dataphysics-instruments.com. Accessed: 19 Mar 2019. Available:https://www.dataphysics-instruments.com/knowledge/understanding-interfaces/surfactants-cmc.
29. Furse S. The lipid Chronicles - bubbles, bubbles, everywhere, but not a drop to drink.;2011. www.samuelfurse.com/lipids.
30. Chen RQ. Explore the ecological problems of pre-treatment chemicals (I), (II). Shanghai Dye. 2001;(6):36-42.
31. Chang Y, McClements DJ. Optimization of orange oil nanoemulsion formation by
isothermal low-energy methods: Influence of the oil phase, surfactant, and temperature. J. Agric. Food Chem. 2014;62:2306–2312.

32. Wong IY, Wong DS. Special Adjuncts to Treatment.In: Ryan SJ, Sadda SVR, et al., editors. Retina(5th ed.).vol 3.W.B. Saunders. 2013;1735-1783. DOI:10.1016/B978-1-4557-0737-9.00104-1.
Available:https://www.sciencedirect.com/science/article/pii/B9781455707379001041

33. England RJ, Homer JJ, Knight LC, Ell SR. Nasal pH measurement: a reliable and repeatable parameter. Clin Otolaryngol Allied Sci. 1999;24(1):67-8. DOI: 10.1046/j.1365-2273.1999.00223.x. PMID: 10196653.

34. England RJ, Homer JJ, Knight LC, Ell SR. Nasal pH measurement: a reliable and repeatable parameter. Clin Otolaryngol Allied Sci. 1999;24(1):67-8. DOI: 10.1046/j.1365-2273.1999.00223.x. PMID: 10196653.

35. Pan HH, Hung TW, Tsai JD, Chen HJ, Liao PF, Sheu JN. Children with allergic rhinitis and a risk of epilepsy: A nationwide cohort study. Seizure. 2020;76:64-71. DOI: 10.1016/j.seizure.2020.01.015.

36. Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, et al. Nanoemulsion: Concepts, development and applications in drug delivery. J Control Release. 2017;252:28–49. DOI: 10.1016/j.jconrel.2017.03.008

37. Biruss B, Dietl R, Valenta C. The influence of selected steroid hormones on the physicochemical behaviour of DPPC liposomes. ChemPhys Lipids. 2007;148(2):84–90. DOI: 10.1016/j.chemphyslip.2007.04.009

38. Achour B. Re: What is the meaning of Zeta potential? And for what reason is it used to investigate the efficiency of coagulation in water treatment?; 2017 [Internet]. [Cited 2021 Feb 17]. Available:https://www.researchgate.net/post/What_is_the_meaning_of_Zeta_potential_And_for_what_reason_is_it_used_to_investigate_the_efficiency_of_coagulation_in_water_treatment/59157d59ed99e15e37145bec/citation/download

39. Vleugels L. Re: How do I interpret zeta potential values?; 2017 [Internet]. [Cited 2021 Feb 21]. Available:https://www.researchgate.net/post/How_do_I_interpret_zeta_potential_values/5a017c76b0366dbc1041910e/citation/download.