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FORMULATION AND EVALUATION OF DOCOSAHEXAENOIC ACID MICROEMULSION FOR OPHTHALMIC DRUG DELIVERY

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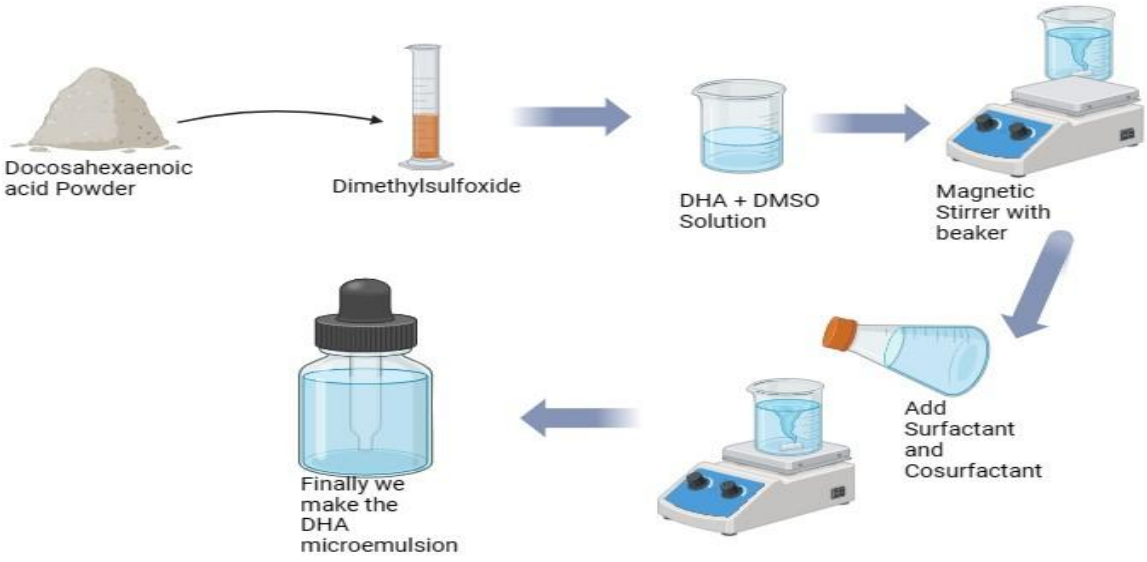
ABSTRACT:

The current research was carried to estimate and validate the formulation of a Docosahexaenoic acid ophthalmic emulsion, intended for ocular medication delivery. Certain ophthalmic diseases such as dry eye syndrome which leads to ophthalmic inflammation is affecting ocular health very drastically so in search of effective cure docosahexaenoic acid microemulsion has withstood all the analysis. Many times, when formulation is taken as capsules, less of the medication reaches the area of the eye that is inflamed; therefore, a higher dose is needed, yet the inflammation remains dangerous. Because of this, DHA can be produced as eye drops for direct ocular administration. Taking a source of docosahexaenoic acid, thin layer chromatography was used to identify the isolated chemical by comparing it to standard DHA. The separated chemicals were subjected to additional examination using gas chromatography. The emulsion had a white color, 0.4 micrometer is the particle size, with viscosity of 30.41 cp, and was steady at a temperature range of 25 - 40 ºC. The created emulsion was also shown to have the same physical characteristics. The produced micro emulsions were assessed for zeta potential, pH, viscosity, and drug content. The optimized micro emulsion formulation had the highest in-vitro drug penetration (85%), a pH - 7.1 and a viscosity of 30.4 cp. Overall the novel DHA containing microemulsion shows promising effects on eye health.

Keywords: Docosahexaenoic acid, ophthalmic microemulsion, dry eye syndrome.



INTRODUCTION:

Since the human eye is such a delicate organ, it is shielded from harm by a number of mechanisms that maintain its structural and functional integrity. But these obstacles also provide serious difficulties for efficient medication distribution, especially when it comes to treating posterior region disorders. Conventional ocular formulations, which include suspensions, ointments, and solutions, sometimes have drawbacks such as low bioavailability, quick pre-corneal clearance, and low patient adherence because of frequent dosage requirements. Research is being done on enhanced medication delivery strategies with the goal of improving patient happiness and treatment efficacy in an attempt to overcome these obstacles [1]. The creation of ophthalmic emulsions, biphasic systems made up of two immiscible liquids with one dispersed as tiny droplet within the other, is one such achievement. Improved drug solubilization, prolonged drug release, and the capacity to integrate both hydrophilic and lipophilic medicines are just a few advantages of using emulsions. Docosahexaenoic acid, an Omega 3 fatty acid, is one of the lipids being researched for ophthalmic emulsions; it has garnered attention because of its inherent anti-inflammatory qualities and function in preserving retinal health [2]. DHA is an important fatty acid required for the development and function of cell membranes, especially those found in the retina and central nervous system. Its capacity to regulate the inflammatory response by affecting the synthesis of eicosanoid and the metabolism of arachidonic acid is thought to be the source of its anti-inflammatory properties [3]. In order to gradually develop a regular and stable system, this makes DHA a viable treatment for inflammatory ocular disorders including dry eye syndrome, which is characterized by instability of the tear film and inflammation of the ocular surface. To accurately assess the active component and its role in sustaining retinal health, advanced techniques like gas chromatography are usually used to test the DHA content inside the

emulsion. To summarize, choosing the appropriate oil, surfactant, and co-surfactant carefully is necessary for the creation of an ocular emulsion of DHA. Part of the evaluation procedure involves determining the physicochemical properties of the emulsion, including pH, globule size, refractive index, viscosity, and drug content [4]. These qualities need to be confirmed in order to ensure the consistency and reliability of the emulsion for therapeutic use [5-7]. A stable emulsion shouldn't have phase separation or experience significant changes in its properties. By utilizing the therapeutic potential of DHA and the advantages of emulsion-based delivery methods, researchers hope to develop a formulation that can effectively deliver medications to the eye, reduce inflammation, and improve the quality of life for patients with ocular disorders (Tandem Teal.). The study aimed to validate a gas chromatographic method for analyzing eicosapentaenoic and docosahexaenoic acid as vigorous ingredients in pharmaceuticals. The method's findings indicate a relationship between the active ingredient's label claim and established reference standards. When comparing the response factors of DHA and EPA ethyl esters to the internal standard [8]. The anti-inflammatory action is due to a compound called arachidonic acid which then gets in contact with the ocular membrane produces a substrate which further increases the vascular permeability and boosts the cell activity hence gives an anti-inflammatory action. Research has demonstrated its potential therapeutic agent for chronic inflammation. Comparative studies have shown that DHA can protect the brain against neural inflammations and reduce brain ageing by stimulating anti-oxidant stress responses. Dry eye syndrome usually results from defects in one or more of physiologically intricate periocular tear film's constituent parts. This happens when the eye is either evaporating too quickly or is not secreting enough tears. Different parts of the eye produce each layer; the lacrimal layer, for instance, is produced by the lacrimal gland. As a result, dry eyes can be caused by issues with any area of the eye. DHA has shown an improvement in the eyes oil film production by the meibomian glands, which helps to maintain tear quality and reduce dry eye syndrome [9-12]. It has been demonstrated that the recently approved topical cyclosporine A reduces inflammation of the ocular surface, increased tear production, and ameliorates dry eye symptoms. In conclusion DHA has been proven to work efficiently in cure on the inflammation on the ocular surface. This article is aimed to give an comprehensive overview of Docosahexaenoic acid its underlined benefits and its potential application in various health conditions DHA fatty acids like EPA may help to improve dry eye syndrome and is likely helps to improve condition and signs by reducing inflammation in the eyes.

In summary DHA have proven benefits for dry eye syndrome.

MATERIALS AND METHOD:

Docosahexaenoic acid was obtained from AYUSH AGRO FOODS NEW DELHI, and other chemicals were obtained from university.

Preparation of drug loaded microemulsion:

For the preparation of 1 gram of DHA is mixed with 10 ml dimethyl sulfoxide, as indicated in table no.1, and was stirred at a low speed for approximately 20 minutes using magnetic stirred, now oleic acid is mixed along with it is a constant rate of surfactant (Tween 20) and Cosurfactant (Ethanol) and now the mixture was allowed to rotate at 450 rpm at magnetic stirred. **Table no. 1 Formulation code.**

	F1	F2	F3	F4	F5	F6
DHA	1g	1g	1g	1g	1g	1g
DMSO	10ml	10ml	10ml	10ml	10ml	10ml
Castor oil	50	30	30	-	-	-
Oleic acid	-	-	-	50ml	30ml	30ml
Tween 20	20ml	20ml	40ml	20ml	40ml	20ml
Ethanol	20ml	40ml	20ml	20ml	40ml	20ml

EVALUATION OF MICROEMULSION:

Determination of pH:

A crucial factor in determining the effectiveness of an ophthalmic microemulsion is its pH level, which needs to match the natural pH of the eye to prevent irritation or pain during application. Ophthalmic solutions usually have an optimal pH range of 6.9-7.4, which is similar to the pH of natural ophthalmic tears. DHA microemulsions' pH is tested and changed as necessary to guarantee formulation stability and ocular tolerability [11]. The micro emulsion's potential for a prolonged shelf life and resistance to microbial contamination are also indicated by a steady pH. A digital pH meter is used to determine the pH of the formulation. In this microemulsion 1gm of drug was added and dissolved in 100 ml of distilled water for about 2 hrs. and the pH was analyzed [12].

Drug content:

The concentration of the therapeutic agent in the formulation is indicated by the drug content of a microemulsion. It is crucial to guarantee that the microemulsion contains a precise and stable concentration of the medication. Gas chromatography is used as an analytical method which is frequently used to measure the amount of medication in microemulsions [13]. To satisfy quality control requirements, the medication content must fall within an acceptable range of the specified

amount. For determination,0.1N HCL was taken in 100 ml and drug (100gm) is dissolved in it, now 1 ml of sample is taken in volumetric flask of 50 ml and diluted then subjected to UV spectrophotometer.

Viscosity:

Viscosity, a fluid's resistance to flow, is a crucial factor to consider when designing ocular microemulsions since it affects how long a medicine stays in the eye and, in turn, how much bioavailable it is. Viscosity-appropriate ophthalmic formulations can extend the duration of the drug's contact with the ocular surface without unduly impairing vision [14-16]. Rheological investigations are carried out to ascertain the microemulsion viscosity and verify that it demonstrates appropriate flow characteristics for topical application. The formulation was subjected to 63 spindles and the data was collected.

Particle size distribution:

The particle size of the preparation was determined by using differential light scattering. The micro preparation was watered down with pure water and a zetasizer is used for analysis [17-18]. As shown in Figure 3 and Figure 4 the Z- Average is 675 with a peak 1 of 654.23 having an intensity of 89% similarly Z-Average is 5672 with a peak 1 of 674.9 of intensity 65.1%.

In-Vitro permeation study:

Research on in vitro permeation is done to evaluate the ability of the microemulsion to deliver the drug through the tissues of the eyes. Drug release investigations using a dialysis membrane were carried out using Franz diffusion cells [19-20]. In this case, the tissue was clamped with the receptor compartment between the donor and the receptor after being dipped in phosphate buffer 7.4. Preparation was in the donor compartment and buffer 7.4 (phosphate) was inside the receptor. A sample of around 1 milliliter was taken out and examined with a UV visible spectrophotometer.

RESULTS AND DISCUSSION:

The pH of all the preparation is found to be between (6.9-7.4) as shown in “table no.2” that is similar to the PH range of the human eyes. The similarity in the pH is considered to be an important aspect for making a less irritant and more sustainable formulation. The viscosity of all the formulation is found to be around 30cp.

As shown in “figure 1” the graph plotted has shown shear thinning fluid flow.

Table no 2: pH, Viscosity and drug content of prepared microemulsion.

Formulation code	pH	Viscosity	Drug content
F1	7.4±0.12	30.12	95.56±0.23
F2	7.0±0.09	30.16	91.89±0.98
F3	7.1±0.02	30.21	89.76±0.83
F4	7.1±0.02	30.04	85.91±0.75
F5	7.3±0.04	30.41	80.55±0.33
F6	6.9±0.10	30.04	75.56±0.23

Figure 1: Viscosity of formulation F1-F6

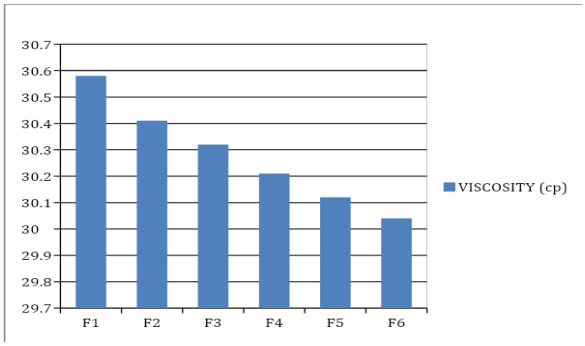


Figure 2: Drug content of formulation F1-F6

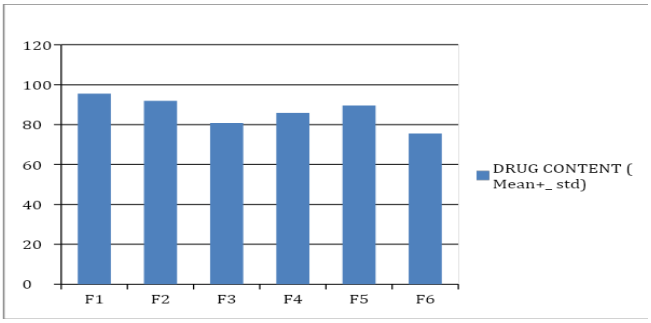


Figure 3: Particle size distribution by intensity F1

			Size (d. nm):	% Intensity:	St Dev. (d.n...
Z-Average (d. nm):	675	Peak 1:	654.23	89.1	168.4
PdI:	0.185	Peak 2:	45.85	12.4	7.563
Intercept:	0.867	Peak 3:	68.44	5.5	13.51
Result quality	Good				

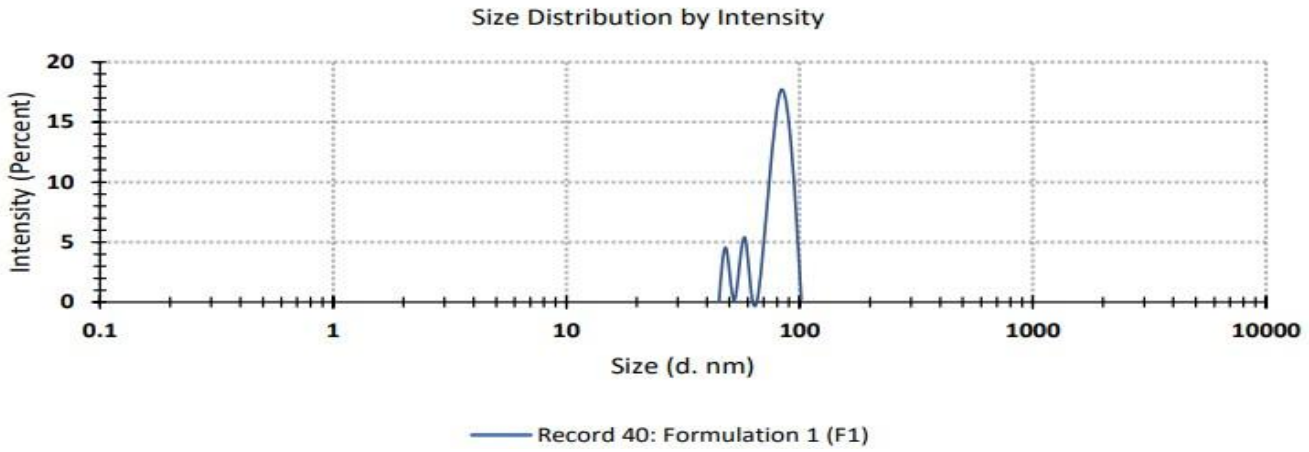
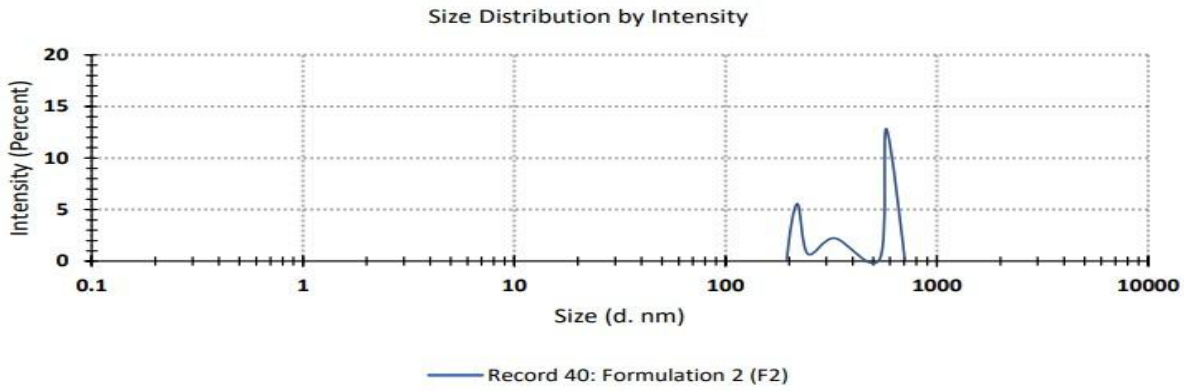


Figure 4: Particle size distribution by intensity F2

			Size (d. nm):	% Intensity:	St Dev. (d.n...
Z-Average (d. nm):	5672	Peak 1:	674.9	65.1	81.4
PdI:	0.114	Peak 2:	73.65	8.5	8.65
Intercept:	0.689	Peak 3:	71.84	9.7	8.22
Result quality	Good				



concentration is measured by employing various methods and it was discovered that the drug concentration of formulation has ranged from 75.56%-95.56%. Differential scattering of light is encountered to determine the particle size distribution. A zetasizer is used where the micro preparation is dissolved in ultrapure water and analyzed. Taking in vitro studies in consideration Franz diffusion cell is used which have a dialysis tissue, initially the tissue was soaked in (phosphate buffer) of 7.4 and was then clamped with the donor and the receptor. UV visible spectrophotometer is used to analyze the formulation. The development and evaluation of DHA microemulsion for ophthalmic drug delivery systems can provide a promising approach to enhance the solubility, bioavailability and therapeutic efficacy of the formulation in the treatment of various ophthalmic conditions. The comprehensive evaluation of the formulation physicochemical and drug delivery properties as well as its ocular tolerance and biocompatibility is crucial for the successful development of this ophthalmic drug

delivery system. According to the conclusions we have got we can say that the ophthalmic microemulsion formulation has proven a major role and impact on the research and development for many ophthalmic complications. In addition to this the microemulsion formulation has also opened many possibilities for further investigations and use of microemulsions for better and more efficient ophthalmic preparations. Complications related to ocular membranes has become a challenging task to counter and its effects such as dry eyes and inflammation would cause trouble in many cases, in these cases use of a DHA ophthalmic microemulsion has proven its grounds it's because of its physicochemical properties that its pH is similar to that of ocular tears and due to its good viscosity properties, its contact time with the eye has make it more efficient and non-irritant ophthalmic drug delivery system. In nutshell we can say that the use of Docosahexaenoic acid microemulsion has proven to be more reliable and sustainable preparation for ophthalmic complications and it is believed to enhance the bioavailability of the formulation used for ophthalmic complications.

CONCLUSION:

The experimental investigation has led to the conclusion that docosahexaenoic acid micro emulsion has proven to validate its benefits to cure ophthalmic disease. The studies have suggested that DHA as micro emulsion can be used for formulation of ophthalmic preparations with its anti-inflammatory and antimicrobial activity, in addition to its better absorption and more contact time leads to more rapid and successful treatment. The versatility of these ophthalmic emulations has been demonstrated to treat various ophthalmic complications. Stability studies have shown that these formulations are stable at a temperature range of 25ºC - 45ºC and has a in-vitro penetration of 85%. So, by the experimental justifications we can say that the micro emulsion has withstand all the studies and its efficiency, potency is analyzed to avail its benefits for preparation of ophthalmic formulations for various ocular complications.

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