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Research Article

FORMULATION, DEVELOPMENT AND EVALUATION OF DOCOSAHEXAENOIC ACID TABLETS

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Abstract: The objective of the study was to develop fast immediate release tablets of Docosahexaenoic acid. Tablets were prepared by using different excipients by direct compression method. The powder mixture was evaluated for angle of repose, bulk density, compressibility index and hausnors ratio. The tablets were subjected to thickness, hardness, friability, dissolution test and in *vitro* release studies. The powder mixture showed satisfactory pharmacotechnical properties and compiled with in-house specifications for tested parameters. The results of in *vitro* dissolution studies indicated that formulation F7 is the most successful formulation of the study and exhibited highest drug release. Applying exponential equation, the formulations showed diffusion-dominated drug release and followed zero-order kinetics.

Key**words:** Conventional immediate release tablet, Docosahexaenoic acid.

INTRODUCTION

DHA is a fatty acid with 22 carbon atoms and 6 *cis* double bonds (all-*cis*-4, 7, 10, 13, 16, 19-docosahexaenoic acid) .DHA is found in high proportions in membrane glycerophospholipids in the brain, retina and sperm of humans and animals. DHA is also found in high concentrations in many species of marine algae**.** DHA can be made in mammals from alpha-linolenic acid (ALA), which is an essential fatty acid, however there is some dispute about how effective this process is in humans [2.] The other essential fatty acid is linoleic acid (LA) and both ALA and LA were discovered in the 1930s LA is the parent or precursor for the omega-6 series of PUFA, with arachidonic acid (AA) being the other main omega-6 PUFA. ALA is the parent fatty acid for the omega-3 series of PUFA, with eicosapentaenoic acid (EPA) and DHA being the other two main members. All fatty acids in the LA (omega-6) PUFA family have their first double bond 6 carbons from the terminal methyl end of the molecule. All fatty acids in the ALA (omega-3) PUFA family have their first double bond 3 carbons from the methyl end. The essential fatty acids are like vitamins, in that they must be obtained from the diet. The reason for their essentiality is that mammals, unlike plants, lack the enzymes to insert double bonds in 18-carbon PUFA between the methyl end and the middle of the molecule. [1]

A number of clinical and research studies suggest that fats containing omega-3 fatty acids, in particular DHA, are vital for cognitive function, especially as we get older and our faculties tend to decline. Beyond cognition (the ability to perceive and interpret information correctly), DHA is essential for many other important brain functions. It has a significant impact on behavior and learning and is critical for proper neural development in infants and children. So coldwater fish really *are* brain food. DHA is essential for cognition and many other important brain functions. It has a significant impact on behavior and learning and is critical for proper neural development in infants and children.

DHA is "conditionally essential," which means that, although it can be synthesized in the body, most of it must be obtained from food or dietary supplements. That's because the body's method of synthesis is inefficient, and it takes a long time to produce enough to satisfy cellular needs. By contrast, DHA from food or supplements can readily be incorporated into cells on an as-needed basis to promote improved health. [2]

Epidemiological studies have shown that high fish consumption is associated with less risk of dementia and Alzheimer disease. And increased gray matter volume in hippocampus, anterior Cingulate cortex, and amygdale, areas involved in cognitive processes. Studies have reported that when the animals were fed diets deficient in DHA/ omega- 3 polyunsaturated fatty acids, there is reduction in the level of DHA in the brain and changes in many brain functions including learning and memory. DHA is one of the major component of the neuronal membrane cytoskeleton Neuronal degradation in specific area of brain is associated with cognitive impairment. Such data and new brain research support the role of DHA supplementation in diet is maintaining optimal brain function and cognitive activity. [3]

Additional research revealed that low serum DHA levels are a significant risk factor for the development of Alzheimer's disease (AD). This is consistent with the observation that the brains of AD patients typically have a lower DHA content than the brains of normal elderly adults. The researchers also found that low serum DHA levels

appear to be common in other types of dementia and cognitive impairment related to aging.

It is difficult to pinpoint how DHA exerts its protective benefits for cognition and memory. One potential benefit, however, may be the ability of DHA to decrease damage to the cardiovascular system. For example, localized cerebral infarcts (regions of dead tissue caused by an insufficient blood supply) may occur in the brain as a result of atherosclerosis (plaque deposits) and thrombosis (blood clots) in cerebral arteries. These infarcts can damage neurons involved in learning, memory, and cognition. The beneficial effects of omega-3 fatty acids on heart health may help prevent these kinds of problems and thus preserve cognitive function as a person ages. [4]

Depression is a complex behavior, difficult to understand, but there is evidence that DHA plays an important role in this brain disorder as well. Research from a number of countries has demonstrated that depression is more common in patients with omega-3 fatty acid deficiencies. Not surprisingly, perhaps, countries with the lowest rates of fish consumption (and thus the lowest consumption of DHA) tend to have the highest rates of depression.

When depressed individuals were instructed to increase their fish intake over a 5-year study period, the incidence of depression and hostility decreased dramatically. It has been suggested that repeated periods of emotional stress may be particularly taxing on DHA brain levels and that elevation of these levels through dietary intervention or supplementation may reduce stress-related behavioral changes. [5]

DHA is a vital component of phospholipids in cellular membranes and is especially prevalent in cells of the brain and the retina of the eye. Not surprisingly, DHA is important for neural and retinal development in infants. The importance of this compound is reinforced by the fact that it is the most abundant of the omega-3 fatty acids in human breast milk. This may help to explain the observation that breast-fed infants routinely score better on visual acuity and intelligence tests than those who were formula-fed.

It is also essential during fetal development in pregnant wom en. Maternal DHA is capable of crossing over into the fetal circulation; it preferentially enters the developing fetus from the mother to participate in neural and retinal development. An English study has demonstrated that women who routinely ate fish during their pregnancy produced children whose visual acuity was better than that of formula-fed infants. [6]

While the benefits of DHA and other omega-3 fatty acids for cognitive development and other aspects of brain function are impressive, their role in supporting good cardiovascular health has received even more press lately. Omega-3 fatty acids from coldwater fish are associated with several benefits to cardiovascular health:

Improving lipid profiles by reducing serum triglyceride levels

- Increasing the HDL/LDL ratio (the ratio of "good cholesterol" to "bad cholesterol")
- Stabilizing heart rhythm (by inhibiting cardiac arrhythmias)
- Inhibiting platelet aggregation (thereby reducing the risk of thrombosis)
- Reducing the risk of fatal heart attacks

Research on this topic, published recently in the New England Journal of Medicine, has revealed that men with the highest serum levels of omega-3 fatty acids had a reduced risk of sudden death from heart disease. Importantly, these men were all healthy at the beginning of the study and had no previous history of heart disease. [7] Clinical trials employing omega-3 fatty acid supplementation or increased coldwater fish intake provide compelling evidence that increased consumption of omega-3 fatty acids reduces the occurrence of nonfatal and fatal heart attacks and also reduces the risk of stroke. Clinical trials provide compelling evidence that increased consumption of omega-3 fatty acids reduces the occurrence of nonfatal and fatal heart attacks, and also reduces the risk of stroke. [7]

The research on omega-3 fatty acids and DHA in particular, is impressive. In addition to improving brain and heart function, there is evidence that DHA can reduce the symptoms of cancer and of inflammatory diseases such as rheumatoid arthritis, asthma and Crohn's disease. It is speculated that DHA may be able to moderate the production of inflammation-producing compounds and thus decrease the incidence of inflammation that leads to these debilitating diseases.

DHA has the potential to improve a number of serious health problems that afflict Americans. Our diets, however, are typically deficient in fish that are rich in omega-3 fatty acids, so we don't consume very much of these health-promoting compounds; in fact, we ingest only about 0.1-0.2 gram of omega-3 fatty acids daily, on average. This is significantly less than the recommended amount, which can be obtained by eating two to three servings of coldwater fish per week. For most people, however, that is an unreliable and expensive way to get their omega-3 fatty acids. Supplementation is much more reliable and convenient for those who seek DHA as a means to improve their cognitive function, heart health, mood, and more. [8]

MATERIALS AND METHODS

Materials

Docosahexaenoic acid (DHA) powder was obtain as a gift sample from Central India Pharmaceuticals M.I.D.C Nagpur. All other ingredients used throughout the study were of analytical grade and were used as received.

Methods

Method of preparation of conventional tablet of Docosahexaenoic acid

Different tablet formulations were prepared by direct compression method. Firstly accurately weighed DHA and other excipients were mixed in mortar and pestle to uniform size. The powder was compressed into tablet by single punch compress machine using 9 mm diameter punch. [9]

Evaluation DHA tablets

Tablets were evaluated for both its pre-compression parameters like bulk density, tapped density, Carr's index, Hausner ratio, angle of repose as well as their post compression parameters tablet thickness, hardness, friability, uniformity of weight and content uniformity of drug and release rate of drug.

Precompression Parameters

I. Bulk density and Tapped density

Both bulk density (BD) and tapped density (TD) was determined. A quantity of 2 g of powder blend from each formula, previously shaken to break any agglomerates formed, was introduced into 10 ml measuring cylinder. After that the initial volume was noted and the cylinder was allowed to fall under its own weight on to a hard surface from the height of 2.5 cm at second intervals. Tapping was continued until no further change in volume was noted. BD and TD were calculated using the following equations. [10]

Bulk density = W/V_0
Tapped density = W/V_f
Where, W - wt. of powder, V_0 - initial volume, W - wt. of powder, V_f - final volume.

II. Compressibility index and Hausner ratio

The compressibility index and Hausner ratio are measures of the propensity of a powder to be compressed. As such, they are measures of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Compressibility Index and the Hausner Ratio. The compressibility index and Hausner ratio may be calculated using measured values for bulk density (D_b) and tapped density (D_t) as follows: [10]

Compressibility index = $D_t - D_b/D_t$ X 100
Hausner ratio = D_t / D_b
Where D_b- Bulk density, D_t - Tapped density

III. Angle of repose

Angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizontal plane. The angle of repose of powder blend was determined by the funnel method. The accurately weight powder blend were taken in the funnel. The height of the funnel was adjusted in such a way the tip of the funnel just touched the apex of the powder blend. The powder blend was allowed to flow through the funnel freely on to the surface. The diameter of the powder cone was measured and angle of repose was calculated.

$\text{Tan } \theta = h/r$ or $\Theta = \tan^{-1}(h/r)$
Where h = height of pile, r = radius of the base of the pile, Θ= angle of repose. [10]

Post- compression parameters

I. Tablet Hardness

The crushing strength ($kg/cm^{2)}$ of prepared tablets was determined for tablets of each batch by using Monsanto tablet hardness tester. Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. [11]

II. Tablet Thickness

The thickness of the tablets was determined by using vernier caliper. Five tablets were used, and average values were calculated [11].

III. Weight variation test

Twenty tablets were selected randomly from each batch and weighed individually. The average weight of each batch of tablet was calculated. Individual weights of the tablets were compared with the average weight. Since the tablets weighed over 250 mg, I.P. specifies that the tablets pass the test if not more than two of the individual weights deviate from the average weight by more than 5 % [12].

Table 1: Percentage Deviation Allowed Under Weight Variation Test As Per I.P.

Average weight of tablet (X mg)	Percentage deviation
$X \leq 80$ mg	10
$80 < X < 250$ mg	7.5
$X \geq 250$ mg	5

IV. Friability Test:

The friability of tablets was determined using Roche friabilator. It is expressed in percentage (%). Ten tablets were initially weighed (W_0) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 min or run up to 100 revolutions. [1] The tablets were weighed again (W_f). The % friability was then calculated by

% Friability = $(1-W_f / W_0) \times 100$

Where, W_0 -Weight of tablet before test, W_f -Weight of tablet after test. [13]

V. Drug content uniformity:

The tablets were triturated to form a fine powder. It was transferred to 100 ml volumetric flask to dissolve in dichloromethane. The volume was made upto 100 ml to get a stock solution. 1 ml of this solution was taken in 100 ml volumetric flask and diluted with dichloromethane. Again 1 ml of solution was pipetted out and made upto the volume of 100 ml. absorbance of this solution was measured at λmax using UV spectrophotometer and on the basis of absorbance drug content was calculated. [14]

VI. *In vitro* Drug release studies

In vitro dissolution studies of DHA tablets were studied in USP XXIII dissolution type II apparatus using paddles at 50 rpm. 900 ml of pH 1.2 buffer was used as a dissolution

medium. The temperature of the dissolution medium was previously warmed to 37±0.50 C and was maintained throughout the experiment. One tablet was used in each test. 5 ml of the sample of dissolution medium was withdrawn by means of syringe fitted with a prefiter at known intervals of time (15 min). The volume withdrawn at each interval was replaced with same quantity of fresh dissolution medium. The sample was analyzed for drug release by measuring the absorbance at 232 nm using UV-visible spectrophotometer after suitable dilutions. All the studies were conducted in triplicate. [15]

Table-2: Composition Of Dha Tablet

Sr. No.	Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	DHA	200	200	200	200	200	200	200	200	200
2	Microcrystalline cellulose	160	110	60	160	110	60	160	110	60
3	Di calcium Phosphate	100	150	200						
4	Starch				100	150	200			
5	Lactose							100	150	200
6	Crospovidone	25	25	25	25	25	25	25	25	25
7	Magnesium stearate	15	15	15	15	15	15	15	15	15

All quantities in mg

Table 3: Precompression Parameters For Formulations F1-F9

Sr. No.	Batch	Angle of Repose(^{0})	Bulk density (g/ml)	Tap density (g/ml)	Carr's Index	Hausner Ratio
1	F1	30.34 ± 0.040	0.475 ± 0.020	0.586± 0.020	18.94 ± 0.030	1.236± 0.200
2	F2	28.58 ± 0.020	0.432 ± 0.030	0.502± 0.140	16.29± 0.020	1.903± 0.230
3	F3	27.24 ± 0.050	0.421 ± 0.022	0.515± 0.028	16.78± 0.140	1.190± 0.002
4	F4	25.56 ± 0.030	0.452 ± 0.028	0.567± 0.208	15.17 ± 0.210	1.192± 0.004
5	F5	26.32 ± 0.900	0.488 ± 0.130	0.539± 0.022	16.18 ± 0.231	1.204± 0.222
6	F6	25.01 ± 0.910	0.456 ± 0.281	0.553± 0.030	14.19 ± 0.003	1.173± 0.042
7	F7	35.32 ± 0.032	0.469 ± 0.132	0.585± 0.120	19.82 ± 0.004	1.24± 0.210
8	F8	27.12 ± 0.412	0.441 ± 0.030	0.572± 0.003	22.80 ± 0.231	1.30± 0.302
9	F9	31.53 ± 0.060	0.522 ± 0.048	0.663± 0.170	21.26 ± 0.080	1.27± 0.260

* All values are expressed as mean ± SD(n=5)

Table 4: Post-Compression Parameters For Formulations F1-F9

Batch Code	Hardness^ (Kg/cm^2)	Thickness^ (mm)	Disintegration time (min)	Weight variation* (mg)	Friability# (%)	Content uniformity^ (%)
F1	4.5±0.09	3.5±0.04	12 min 25 sec	502±1.46	0.412±0.06	97.8± 0.55
F2	4.8±0.12	3.6±0.07	15 min 20 sec	501±1.68	0.510±0.04	98.3± 0.28
F3	4.9±0.14	3.5±0.16	14 min 18 sec	504±1.13	0.436±0.16	98.6± 1.13
F4	5.1±0.21	3.4±0.27	12 min 35 sec	501±1.86	0.430±0.54	98.0± 0.21
F5	4.2±0.16	3.6±0.22	11 min 30 sec	504±1.52	0.654±0.96	97.7± 0.26
F6	4.6±0.13	3.6±0.09	18 min 10 sec	496±1.67	0.550±0.55	98.2± 0.52
F7	5.2±0.11	3.5±0.08	10 min 22 sec	501±1.66	0.477±0.23	99.1± 0.52
F8	5.1±0.17	3.5±0.11	16 min 45 sec	501±1.38	0.410±0.09	97.9± 0.41
F9	5.0±0.19	3.5±0.18	11 min 35 sec	500±1.21	0.385±0.52	98.0± 0.38

* All values are expressed as mean ± SD (n=20).
^ All values are expressed as mean ± SD (n=5).
# All values are expressed as mean ± SD (n=10).

Table 5: *In Vitro* Drug Release Data Of Formulations F1-F5

Time (min)	Cumulative % drug release*				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
15	25.6±0.08	27.4±0.22	26.1±0.34	24.3±0.08	25.6±0.09
30	38.9±0.05	40.4±0.13	42.5±0.18	38.7±0.22	39.8±0.28
45	52.8±0.11	55.2±0.015	58.4±0.02	55.7±0.54	52.3±0.34
60	62.5±0.34	66.8±0.06	66.5±0.09	68.8±0.09	65.3±0.18
75	70.8±0.22	72.4±0.12	75.2±0.11	75.2±0.017	78.3±0.03
90	80.1±0.08	83.4±0.09	84.8±0.43	82.1±0.19	81.3±0.19
105	84.5±0.12	86.7±0.17	87.2±0.27	85.6±0.65	86.4±0.39
120	88.2±0.05	90.5±0.26	91.5±0.19	90.2±0.35	91.8±0.18

* All values are expressed as mean ± SD (n=6)

Table 6: *In Vitro* Drug Release Data Of Formulations F6-F9

Time (min)	Cumulative % drug release*			
	F6	F7	F8	F9
0	0	0	0	0
15	23.6±0.34	28.6±0.08	25.4±0.22	26.5±0.09
30	37.8±0.23	45.2±0.21	40.1±0.17	42.2±0.19
45	53.7±0.18	59.3±0.17	55.7±0.11	53.7±0.65
60	69.4±0.07	69.6±0.24	65.8±0.27	65.8±0.41
75	77.9±0.46	79.5±0.14	75.4±0.37	74.5±0.11
90	84.3±0.28	85.6±0.18	83.5±0.52	81.5±0.12
105	86.5±0.14	90.4±0.21	88.4±0.18	85.8±0.32
120	92.5±0.18	97.5±0.34	94.5±0.49	92.6±0.18

*All values are expressed as mean ± SD (n=6)

Treatment of drug release data with different kinetic equations

Different mathematical model may be applied for describing the kinetics of the drug release process from matrix tablets, the most suited being the one which best fits the experimental results. The kinetics of Lamivudine was determined by finding the best fit of the dissolution data to distinct models- Zero order, first order, Higuchi, Peppas.[16]

I. Zero Order Kinetics: A zero-order release would be predicted by the following equation.

$A_t = A_0 - K_0 t$

Where

A_t - Drug release at time‘t’

A_0 - Initial drug concentration

K_0 - Zero-order rate constant

II. First Order Kinetics: A first-order release would be predicted by the following equation.

$\text{Log } C = \text{Log } C_0 - 303.2\ K_f\ t$

Where

C - Amount of drug remained at time‘t’

C_0 - Initial amount of drug

K_f - First-order rate constant

III. Higuchi’s Model: Drug released from the matrix devices by diffusion has been described by following Higuchi’s classical diffusion equation.

$Q = K_h t^{½}$

Where

Q - Percentage of drug released at time‘t’,

K_h- Higuchi’s drug release rate constant

IV. Korsmeyer Model: The release rates from controlled release polymeric matrices can be described by the equation proposed by korsmeyer *et al*.

$Q = K_m t^n$

Where

Q - Percentage of drug released at time‘t’

K_m - Kinetic constant incorporating structural and geometric characteristics of the tablets

n - Diffusional exponent indicative of the release mechanism

The results of *in-vitro* drug release profile obtained for all the formulations were plotted in modes of data treatment as follows:

i. Cumulative percent drug released versus time (zero-order kinetic model)

ii. Log cumulative percent drug remaining versus time (First-order kinetic model)

iii. Cumulative percent drug released versus square root of time (Higuchi's model)

iv. Log cumulative percent drug released versus log time (Korsmeyer equation)

Table 7: Kinetic Treatment Of Drug Release Data Of Various Formulations F1 To F9

Formulation Code	Zero order model	First order model	Higuchi's model	Korsmeyer-Peppa's model
	R^2			
F1	0.9949	0.8840	0.9668	0.9636
F2	0.9918	0.8785	0.9707	0.9552
F3	0.9861	0.8471	0.9675	0.9422
F4	0.9809	0.8370	0.9457	0.9307
F5	0.9905	0.8739	0.9510	0.9516
F6	0.9820	0.8442	0.9257	0.9329
F7	0.9930	0.8690	0.9797	0.9581
F8	0.9937	0.8750	0.9559	0.9640
F9	0.9949	0.8788	0.9736	0.9643

Stability studies

Stability studies were carried out for optimized batch (F7) of DHA tablet. The tablets were packed in aluminium foil placed in airtight container and kept at 4°C in refrigerator, 40°C and 60°C for 60 days. At the interval of 15 days, the tablets were withdrawn and evaluated for physical properties and *in-vitro* drug release.

RESULTS AND DISCUSSION

Evaluation of DHA tablet

A] Precompression study:

Formulated powder ready for compression containing drug and various excipients were subjected for pre-compression parameters such as Angle of repose, Bulk density, Tapped density, Carr's consolidation index, and Hausner's ratio to study the flow properties of powder to achieve uniformity of tablet weight. The evaluated parameters were within acceptable range for all the 9 formulations**. (Table no. 3)** Hence, all the formulations exhibited good flow properties.

B] Post-compression Parameters:

The tablets prepared by direct compression technique were subjected to preliminary characterization such as appearance, hardness, thickness, % weight variation, friability and drug content. The evaluated parameters were within acceptable range for all the 9 formulations. **(Table no. 4)** Results of post-compression parameters of formulations indicated that the prepared tablets were found to be uniform in weight, drug content and possess good mechanical strength with sufficient hardness.

C] *In vitro* dissolution studies:

The *in-vitro* release study was carried out on all the batches using USP XXII dissolution test apparatus-II at 50 rpm, 900 ml of buffer pH 1.2 used as dissolution media. **(Table no. 5 and 6)** From the above results it was observed that the lactose in combination with microcrystalline cellulose showed better drug release as compared with starch and dicalcium phosphate in combination with microcrystalline cellulose.

D] Drug Release Kinetics:

The *in vitro* drug release data was subjected to goodness of fit test by linear regression analysis according to zero order, first order kinetic equations, Higuchi and Korsmeyer models to ascertain the mechanism of drug release**.(Table no. 7)** When the regression coefficient 'R^2' value of zero order and first order plots were compared, it was observed that the 'R^2' values of zero order were in the range of 0.98 to 0.99 whereas the 'R^2' values of first order plots were found to be in the range of 0.83 to 0.88 indicating drug release from all the formulations were found to follow zero order kinetics. The good fit of the dissolution profiles of all the formulations were follow zero order model since 'R^2' values were nearer to unity.

E] Stability study:

The optimized formulation F7 was selected for stability studies..Stability studies of the drug formulations were performed to ascertain whether the drug undergoes any degradation during its shelf life. From the stability study data, it was concluded that the drug is stable in the optimized formulation for the study period.

CONCLUSION

The conventional tablets of Docosahexaenoic acid were formulated by using different excipients like microcrystalline cellulose, lactose and Di-calcium phosphate by direct compression technique. All the prepared tablets were found to be good without chipping, capping and sticking. The drug content was uniform (96.76 ± 0.26 to 99.89 ± 0.41) and well within the accepted limits with low values of standard deviation indicating uniform distribution of drug within the tablets of same batch. The *in vitro* dissolution profiles of all the prepared tablets of Docosahexaenoic acid were found to immediate drug release of Docosahexaenoic acid from most of the formulations was found to follow zero order kinetics (0.97 to 0.99) Optimized batch of tablet (F7) found to be stable for 60 days.

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