A novel liquid oral formulation for 1- Octacosanol an anticancer drug and its stability study

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ABSTRACT

1-Octacosanol is a long chain fatty acid alcohol and a liphophilic waxy compound and hence by nature it is insoluble in water. Therefore, the respective drug active encounters a challenge of active absorption in the clinical settings. Thus, a necessity for the development of water soluble, a bio absorbable and a palatable formulation for 1- Octacosanol was addressed in this study. Apart from this, a secondary objective of presenting this drug product to pediatric and geriatric patients in a palatable manner, in order to increase their compliance. Various galenical feasibility studies were conducted to identify the specific ingredients and its respective proportion to bring out the desired emulsion. The resultant formulation facilitates the increased bioavailability; herewith we disclose a novel liquid oral emulsion formulation. It is a liquid oral emulsion dosage form of oil in water type, containing adjutants like Corn oil, Tween -80, Span-60, Sucralose, Flavour (raspberry), Water (DM), Simethicone, Methyl paraban, Propyl paraban etc. The final product exhibit a milky white emulsion, was further tested for its physical stability and its palatability through numerous experiments

Keywords: Octacosanol, bioavailability, absorption, emulsion, stability

INTRODUCTION

1- Octacosanol also known as n-Octacosanol, Octacosyl alcohol, Cluytyl alcohol, Montany alcohol. It is a straight-chain aliphatic 28-carbon primary fatty alcohol that is common in the epicuticular waxes of plants, including the leaves of many species of Eucalyptus, of most forage and cereal grasses, of Acacia, Trifolium, Pisum and many other legume genera among many others, sometimes as the major wax constituent. Octacosanol also occurs in wheat germ [1].

IUPAC name of 1-Octacosanol is Octacosan-1-ol from and its molecular weight is 410.76 g/mol[2]. It is poorly soluble in water but freely soluble in low molecular-weight alkanes and in chloroform[1]. The pharmacological activity of 1-Octacosanol mainly reduces LDL content in body upto 18% by modifying HMG-CoA Reductase enzyme activity.

Policosanol as a mixture of 1- Octacosanol, Tricontanol and many other long chain fatty acid alcohols has been in use for the treatment of hyperlipidemia[2]. Latest research studies indicates that 1- Octacosanol as a single active were subjected to preliminary study for its potential benefit for patients with Parkinson's disease[1]. However, it has been shown for the first time that 1-Octacosanol possess proapoptic and antiangiogenic activity. The process involved in extracting the desired compound as mentioned by Thippeswamy and Salimath[3]. Since the extraction of this product is a tedious process, the respective compound has been synthesized from 1, 12, dodecanediol and cetyl alcohol [4].

The synthesized compound is waxy and insoluble in water and therefore, the compound has to be suitably formulated. Considering the ease of administration and enhanced bioavailability requirement of the anticancer product, the liquid oral formulation in the form of emulsion has been identified as a dosage form.

The term emulsion is derived from the word emulgeo meaning “to milk”. Milk is example of a natural emulsion. An emulsion is a biphasic liquid preparation having two immiscible liquids, which cannot be dispersed for a long period[3]. Emulsion is thermodynamically unstable and must be stabilized by the addition of emulsifying agent. Emulsified systems range from lotions having comparatively low viscosity to creams which are more viscous. There are two basic types of emulsions, that is, oil in water (O/W) and water in oil (W/O). In addition to these two types, a relatively complex emulsion, called multiple emulsions can also be formulated[5]. An emulsifying agent is added, which form film around the globules, so that a stable emulsion is produced. The size of globules is 0.25 to 25 µm diameters [5]. An emulsion is traditionally defined as a dispersion of droplets of one liquid in another, the two being immiscible [6]. Emulsions are thermodynamically unstable heterogeneous biphasic system. There are certain parameters which if and emulsion follow considered as stable. There are several methods to detect the stability of prepared emulsion. They are rheological assessment, macroscopic examination,

globule size analysis and accelerated stability test [5]. One the other hand, there are lots of sign of physical and chemical instability of emulsion like creaming, cracking, phase inversion, flocculation etc.

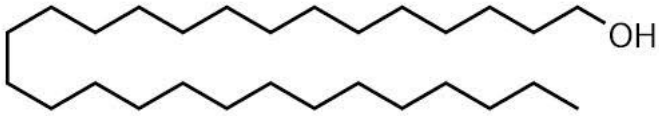

Fig 1: Structure of 1-Octacosanol

MATERIALS AND METHODS

Materials: 1-Octacosanol (synthesized in IISC, Bangalore), Tween -80, Span -20, Span – 60, Soy lecithin, Cremophor, Simethicone, Corn oil, Sucralose, Methyl Paraben, Propyl Paraben, Raspberry flavor, Strawberry flavor, Banana flavor and Water (pharmaceutical grade). Mortar, pestle, Bunsen burner, beakers, glass rod & water bath.

Table.1.Functional benefits of each ingredient

Ingredient	Functions
1-Octacosanol	Active ingredient
Tween – 80	Water based emulsifying agent/Solubilizer/Nonionic surfactant
Span - 60	Oil based emulsifying agent/Solubilizer/Nonionic surfactant
Soya lecithin	Emulsifier/ Viscosity modifier
Simethicone	Emulsion defoamer
Corn Oil	Oil base
Sucralose	Sweetening agent
Raspberry/Banana/ Strawberry/Blackcurrant	Flavoring agent
Water	Dilution medium
Methyl Paraben/Propyl Paraben	Preservatives

General methods of primary emulsion preparation [7-8]:

Step I: Oil phase ingredients like Corn oil and Span – 20 taken in a beaker heated to 65-70°C under a bunsen burner covered in wired gauze. The components stirred constantly for a period of 10-15 minutes till the mixture attained homogeneity and liquefaction (A).

Step II: While the oil phase is undergoing homogenization with its emulsifier, the water phase and its emulsifiers are heated in another beaker up to 65-70°C for a span of 10 – 15 minutes till the aqueous solution attains homogeneity (B).

Step III: Water phase (A) transferred from the beaker to mortar and triturated with a pestle while the water phase (B) added slowly while it is hot. The pH of the primary emulsion adjusted in the range of 6-7 using sodium hydroxide or hydrochloride.

Galenical feasibility: Trials F1 to F5 conducted to identify the ingredients that could be used to arrive at a primary emulsion. The variations in the concentration of each ingredient were performed based on the HLB values of each ingredient. Each trial was conducted using the general procedure as mentioned above.

Table.2.Ingredients of F1- F5

Ingredients	Experiments/concentration of ingredients					HLB Value
	F1	F2	F3	F4	F5	
1-Octacosanol						
Tween -80	2ml	-	-	-	-	15
Span -20	-	-	-	2ml	3ml	8.6
Soy lecithin	-	2g	-	-	-	4
Cremophor	-	-	5ml	-	-	12
Corn oil	6ml	6ml	6.5ml	6.5ml	5ml	8
Water	12ml	12ml	15ml	15ml	32ml	-

All the formulation from F1 to F4 was not a stable formulation; however, primary emulsion was achieved in F5 and the respective formation is probably based on impact of HLB values of both emulsifier and the oil phase. In this case, both the oil phase as well as the emulsifier has the HLB value of 8.6. Primary emulsions achieved in F5 were put through the various trials with the active ingredient 1- Octacosanol.

Incorporation of 1-Octacosanol in primary emulsion:

Table.3.Formulation – F6

INGREDIENTS	T.WT	P.WT
1-Octacosanol	20mg	20.2mg
Corn oil	2.2ml	2.25ml
Span-20	1.5ml	1.5ml
Sodium Hydroxide solution (0.1M)	1ml	1.05ml
Water	0.5ml	2.5ml

Procedure: Weigh exactly 20mg of 1-Octasosanol and add to beaker containing corn oil, heat the mixture to 65˚C. To this clear solution Span-20 was added and heated further to 65°C-70°C, simultaneously heat 3/4th of water taken in a separate beaker to 65-70˚C on a separate beaker using bunsen burner. Transfer the hot oil phase components to mortar and subsequently, add the hot water little by little into mortar and continue to triturate for a span of 30 minutes. Further, add 1ml of Sodium Hydroxide solution to the mix in the mortar and adjust the pH to 7. The resultant solution was triturated vigorously for another 15 minutes. Transfer the content to volumetric flask make up the volume with water and then stir it immediately. Frothing can be avoided but keeping the stirrer at the bottom of the mixture.

Development of stable 1- Octacosanol emulsion – F7 to F11:

Table.4.Experiments for stable 1-octacosanl formulation

Ingredients	Experiments/concentration of ingredients				
	F7	**F8**	**F9**	**F10**	**F11**
1-Octacosanol	**20.2mg**	**20mg**	**20mg**	**50mg**	**50mg**
Tween -80	1ml	0.32ml	0.24ml	0.64ml	0.64ml
Span -20	-	-	-	-	-
Soy lecithin	-	-	-	-	-
Cremophor	-	-	-	-	-
Span - 60	2ml	0.68ml	0.51ml	1.36ml	1.36ml
Corn oil	-	-	-	3.00ml	3.0ml
Soybean Oil	2ml	2ml	1.5ml	-	-
Sodium Hydroxide (0.1M)	1ml	1ml	1ml	-	-
Water	12ml	17.5ml	17.5ml	20.00ml	19.98ml
Flavor	-	-	-	-	2 drops
Sucralose	-	-	-	-	10mg

Procedure for preparation: The procedure for the preparation of formulation F7 to F11 are given as below: Weigh corn oil, span 60, 1-octacosanol (Oil phase) in a beaker, melt it and maintain the temperature at 80°C in an oil bath. Weigh Tween 80 and water in a fresh beaker dissolve sucralose a sweetening agent and heat it to temperature 80°C (Aqueous phase). Then add oil phase to motor little by little and triturate vigorously. Add aqueous mixture to the oil phase and triturate and make the resultant mass homogeneous. Add flavor at the end of the formulation preparation and triturate it well for 15minutes.

Physical stability studies: The above prepared formulation F7 – F11 were kept aside in a respective sample bottles, adequately labeled and observed for the span of two weeks for its compatibility with each ingredients in the product.

Observation: These test products exhibited varying degree of instability in the form of layer separation to settling of the component. Apparently, the formulation without the flavoring agents have maintained reasonable

level of stability and this led to the pursuit of suitable flavoring agent that is compatible with all other functional ingredients

Identification of a suitable flavoring agent: The formulation was prepared using banana, raspberry, strawberry, black current etc. The details of the ingredients and its proportions were mentioned in the below given chart. Procedures of the preparation of the each formulation are as per the general procedure sited above

Table.5.Experiments for a suitable flavoring agent

Ingredients	Experiments/concentration of ingredients		
	F12	**F13**	**F14**
1-Octacosanol	**0.020g**	**0.045g**	**0.051g**
Tween -80	0.64g	0.64g	0.64g
Span -20	-	-	-
Soy lecithin	-	-	-
Cremophor	-	-	-
Span - 60	1.36g	1.36g	1.36g
Corn oil	3g	3g	3g
Soybean Oil	-	-	-
Sodium Hydroxide (0.1M)	-	-	-
Water	0.5	0.5	0.5
Flavor	Banana(2 drops)	Strawberry (2 drops)	Raspberry(2 drops)
Sucralose	0.015g	0.010g	0.010g
Inference	Separated within a week	Separated within two (2) weeks	Stable emulsion as compared to the earlier formulation. However, found to be dull on storage

Observation: Apparently, the formulation made out of each flavors shown the impact on the stability of the final product and therefore, the method of product preparation was considered as one of the variable factor that could have an impact on the stability. The following experiments were carried out accordingly.

New process (Formulation F15): Aim: To prepare 1-octacosanol emulsion as batch F15, by changing method of trituration

Table.6.New Process

INGREDIENTS	%WT	T.WT	P.WT
1-Octacosanol	0.2%	0.05g	0.048g
Corn oil	12.%	3g	3.002g
Tween -80	2.56%	0.64g	0.6402g
Span-60	5.44%	1.36 g	1.3605g
Sucralose	0.04%	0.01g	0.0101g
Flavour(rasp)	0.05%	12.5mg	2drops
Water	QS	QS	QS

Procedure: Weigh corn oil, span -60, 1-Octacosanol in one beaker followed by melting the same through heating at 60-65°C. Then weigh water, Tween 80, sucralose and heat them at 65-70°C. Add oil phase to mortar and triturate well by adding little by little water phase for 1 hr.

Observation: The emulsion was found to be stable; however, the process of making emulsion was tedious because of excess froth.

Inclusion of antifoaming agent (F16): Aim: To prepare 1- Octacosanol emulsion by adding anti foaming agent (Simethione) and black current flavor.

Table.7.Inclusion of antifoaming agent

INGREDIENTS	%WT	T.WT	P.WT
1-Octacosanol	0.2%	0.05g	0.048g
Corn oil	12.%	3g	3.001g
Tween -80	2.56%	0.64g	0.64g
Span-60	5.44%	1.36 g	1.36g
Sucralose	0.05%	0.01g	0.011g
Flavor(black current)	0.05%	0.0125g	2drops
Simethicone	0.01%	0.0025g	0.003g
Water	QS	QS	QS

Procedure:

Oil phase: Weigh 1-Octacosanol, Corn oil, Span-60 in a beaker melt it at temperature 65-70°C in a beaker.

Aqueous phase: Weigh Water, Tween -80, Sucralose and dissolve, heated to 65-70°C. Transfer oil phase to mortar by adding little by little water phase & triturate it vigorously for 40minutes, then Simethicone an antifoaming agent added, followed by triturate for 30 minutes. The resultant product was adequately supplied with flavor and continued stirring further for 10 minutes in a magnetic stirrer.

Report: The emulsion formed is good, milky white with pleasant flavor

Evaluation of 1-Octacosanol emulsion:

Dye Solubility Test[9]: In this test an emulsion is mixed with a water soluble dye (amaranth) and observed under the microscope. If the continuous phase appears red, it means that the emulsion is o/w type as water is in the external phase and the dye will dissolve in it to give color.

If the scattered globules appear red and continuous phase colorless, then it is w/o type. Similarly if an oil soluble dye (Scarlet red C or Sudan III) is added to an emulsion and the continuous phase appears red, then it is w/o emulsion.

Observation: F15 tested for its identity and found to be o/w type, since the amaranth dye was found to be soluble in water and emitted red color under microscope

Accelerated stability testing: Formulation F 15 was evaluated under storage condition according to ICH guidelines. The samples were collected at regular intervals and evaluated for the physical stability of emulsion.

Table.8.Stability testing

Study	Storage condition	Evaluation period
Accelerated	40°C ±2°C /75 % RH ±5%RH	3 Months
Room Temperature	25°C ±2°C /60 % RH ±5%RH	6 Months
Refrigerator	5°C ±3°C	3 Months

DISCUSSION

Numerous trials were conducted in order to ensure a biocompatible, physically stable primary emulsion. Apparently, F1 emulsion got separated within a few minutes of the formation. In the case of F2, emulsion was formed but it is too thick and identified that the same is difficult to handle. On the other hand, in F3 emulsion was formed using cremophor but the stability was only momentary. F4 emulsion was formed, but it was too thick and of the view that the addition of higher water quantity could dilute the same.

In view of the above inferences from formulation F1 to F4, explored the possibility of forming a primary emulsion by integrating the HLB (Hydrophilic Lipophilic Balance) values of the oil and oil phase emulsifier. In F5 emulsion, both Span -20 an emulsifier and Corn oil an oil phase possess the HLB value of 8.6, through the primary preparation process the same was found to be reasonably stable emulsion.

Incorporation of the active into the primary emulsion was carried out; in our case, F5 was chosen as the base formula and the same method was used to create F6. The experiment indicated the instability of the emulsion system in the presence of 1-Octacosanol. The pH of the emulsion was adjusted to 7.01. In the case of F7, the emulsion was separated out due to imbalance of emulsifier & oil. F8, the Emulsion formed with frothing. F9, the emulsion is separating within 5 hours of the formation. The emulsion with soybean oil was found to

be very thick; hence it is inferred that corn oil is better option than soybean oil. F10, the emulsion was separating out with some settling within two weeks of its formation. F11, The emulsion was formed but it was dull and frothy.

F12, the emulsion was formed but dull in color and the same separated out within a week. For F13 formulation, the flavor was not compatible with other ingredients of the emulsion and moreover the emulsion was dull. On storage for the span of two weeks the emulsion was found to be settling.F14 emulsion was good, milky white, but bit of frothy and mild flavored. This result in comparison to the earlier formulation was found to be stable. However, on storage the same was found to be dull in color. F15 too was found to be frothy.

The emulsion formed out of F16 is good, milky white with pleasant flavor. Found to be stable and palatable.

From the dye test performed for the formulation F16, continuous phase appears red; it confirms that oil in water type of emulsion. Subjecting the formulation F16 into accelerated, room temperature and refrigerator condition at various humidity conditions for the span of 3 months and its inherent stability indicates the product is stable. There was a complete absence of cracking, creaming, phase inversion, etc.

CONCLUSION

In view of the above experiments and its inference, it is apparent that the synthesized version of 1-Octacosanol can be made into emulsion, a liquid oral dosage. On evaluation, it has become evident that 1-octacosanol is stable physically, oil in water emulsion. Stable at higher temperature as well as at lower temperature. The physically stable emulsion can be effectively used to enhance the bioavailability of the active in the clinical settings. Moreover, the product can be portrayed as unique in its nature due to its novelty in the usage of synthetic active and in addition a bio-available liquid oral dosage form. Thus, this study provides a useful novel formulation for the pediatric and geriatric patients and other broad spectrum of patients for its stability, efficacy and ease of administration.

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