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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 insoluble in water. It encounters the challenge of active absorption in clinical settings, thus creating a necessity for the development of water soluble, bio absorbable and palatable formulation of 1-Octacosanol, which is addressed in this study. A secondary objective is to present this drug to pediatric and geriatric patients in a palatable manner in order to improve their compliance to the drug. Various galenical feasibility studies were conducted to identify the specific ingredients and their respective proportions to bring out the desired emulsion. The resultant formulation facilitates increased bioavailability. In this study, we disclose a novel liquid oral emulsion formulation. It is a liquid oral emulsion dosage form of oil in water type containing adjuvants 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, 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]. The IUPAC name of 1-Octacosanol is Octacosan-1-ol 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 the LDL content in the body upto 18% by modifying HMG-CoA Reductase enzyme activity.

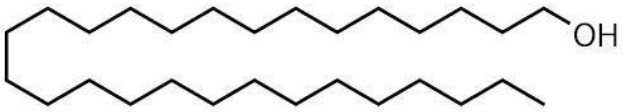

Fig 1: Structure of 1-Octacosanol

Policosanol, as a mixture of 1- octacosanol, tricontanol and many other long chain fatty acid alcohols, has been in use for the treatment of hyperlipedemia[2]. Latest research studies indicate that 1-octacosanol as a single active has been subjected to preliminary studies for its potential benefit for patients with Parkinson's disease. However, it has been shown for the first time that 1-octacosanol possesses proapoptic and antiangiogenic activity. Since the process involved in extracting the desired compound, as mentioned by "Thippeswamy and Salimath3," 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 the enhanced bioavailability requirement of the anticancer product, a liquid oral formulation in the form of emulsion, has been identified as the dosage form. The term emulsion is derived from the word 'emulgeo' meaning 'to milk'. Milk is an 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]. An emulsion is thermodynamically unstable and must be stabilized by the addition of an 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 forms a film around the globules, so that a stable emulsion is produced. The size of the globules is between 0.25 and 25 µm in diameter[5]. An emulsion is traditionally defined as a dispersion of droplets of one liquid in another, the two being immiscible[6]. Emulsions are a thermodynamically unstable heterogeneous biphasic system. There are certain parameters to be followed for the emulsion to be considered stable. There are several methods to detect the stability of the prepared emulsion. They are rheological assessment, macroscopic examination, and globule size analysis and accelerated stability test[5]. On the other hand, there are lots of signs of physical and chemical instability of an emulsion like creaming, cracking, phase inversion, flocculation etc.

MATERIALS AND METHODS

Materials

1-Octacosanol (synthesized in the Organic Chemistry department, Indian Institute of Science, Bangalore), tween-80, span-20, span–60,soylecithin, cremophor, simethicone, corn oil, sucralose, methyl paraben, propyl paraben, raspberry flavour, strawberry flavour, Banana flavour and water (pharmaceutical grade). Mortar, pestle, bunsen burner, beakers, glass rod and water bath.

Table.1: Functional benefits of each ingredient

S.No	Ingredients	Functions
1	1-Octacosanol	Active ingredient
2	Tween – 80	Water based emulsifying agent/Solubilizer/Nonionic surfactant
3	Span - 60	Oil based emulsifying agent/Solubilizer/Nonionic surfactant
4	Soyalecithin	Emulsifier/ Viscosity modifier
5	Simethicone	Emulsion defoamer
6	Corn Oil	Oil base
7	Sucralose	Sweetening agent
8	Raspberry/Bannana/ Strawberry/ Blackcurrent	Flavoring agent

9	Water	Dilution medium
10	Methyl Paraben/Propyl Paraben	Preservatives

GENERAL METHODS OF PRIMARY EMULSION PREPARATION[7&8]

STEP – I

Oil phase ingredients like corn oil and span–20 are taken in a beaker and heated to 65-70°C under a bunsen burner covered with a wired gauze. The components are stirred constantly for a period of 10 to 15 minutes till the mixture attains 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 to 70°C for a span of 10 to 15 minutes till the aqueous solution attains homogeneity (B).

STEP – III

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

EXPERIMENTS

Galenical Feasibility

Trials F1 to F5 were 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. All the formulations from F1 to F4 were not stable formulations; however, primary emulsion was achieved in F5 and the respective formation is probably based on the 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 various trials with the active ingredient 1-Octacosanol.

Table 2: Ingredients of F1- F5.

S.NO	INGREDIENTS	EXPERIMENTS/CONCENTRATION OF INGREDIENTS					HLB Value
		F1	F2	F3	F4	F5	
1	**1-Octacosanol**						
2	**Tween -80**	2ml	-	-	-	-	15
3	**Span -20**	-	-	-	2ml	3ml	8.6
4	**Soy lecithin**	-	2g	-	-	-	4
5	**Cremophor**	-	-	5ml	-	-	12
6	**Corn oil**	6ml	6ml	6.5ml	6.5ml	5ml	8
7	**Water**	12ml	12ml	15ml	15ml	32ml	-

Incorporation of 1-octacosanol in primary emulsion

Table 3: Formulation F6

S.NO	INGREDIENTS	T.WT	P.WT
1	**1-Octacosanol**	20mg	20.2mg
2	**Corn oil**	2.2ml	2.25ml
3	**Span-20**	1.5ml	1.5ml
4	**Sodium Hydroxide solution (0.1M)**	1ml	1.05ml
5	**Water**	0.5ml	2.5ml

PROCEDURE

20mg of 1-Octasosanol was weighed and added to the beaker containing corn oil and the mixture was heated to 65°C. To this clear solution, Span-20 was added and heated further to 65°C-70°C Simultaneously 3/4th of the total water taken was heated to 65- 70°C on a separate beaker using Bunsen burner. The hot oil phase components were transferred to mortar, and subsequently, hot water was added little by little into the mortar and the trituration was continued for a span of 30 minutes. Further, 1 ml of Sodium Hydroxide solution was added to the mix in the mortar and the pH was adjusted to 7. The resultant solution was triturated vigorously for another 15 minutes. The content was transferred to a volumetric flask and the volume was made up with water and then stirred immediately. Care was taken to avoid frothing by 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

S.NO	Ingredients	EXPERIMENTS/CONCENTRATION OF INGREDIENTS				
		F7	F8	F9	F10	F11
1	1-Octacosanol	20.2mg	20mg	20mg	50mg	50mg
2	Tween -80	1ml	0.32ml	0.24ml	0.64ml	0.64ml
3	Span -20	-	-	-	-	-
4	Soy lecithin	-	-	-	-	-
5	Cremophor	-	-	-	-	-
6	Span - 60	2ml	0.68ml	0.51ml	1.36ml	1.36ml
7	Corn oil	-	-	-	3.00ml	3.0ml
8	Soybean Oil	2ml	2ml	1.5ml	-	-
9	Sodium Hydroxide (0.1M)	1ml	1ml	1ml	-	-
10	Water	12ml	17.5ml	17.5ml	20.00ml	19.98ml
11	Flavor	-	-	-	-	2 drops
12	Sucralose	-	-	-	-	10mg

Procedure for preparation

The procedure for the preparation of formulation F7 to F11 is given below: Weighed corn oil, span 60 and 1-Octacosanol (Oil phase) were taken in a beaker. These components were in an oil bath while maintaining the temperature at 80°C. Subsequently, Tween 80 was weighed and taken in a beaker. Water was added and sucralose, a sweetening agent, was dissolved in it. This was heated to a temperature of 80°C (Aqueous phase). Then, oil phase was added to motor little by little and triturated vigorously. The aqueous mixture was added to the oil phase and triturated and the resultant mass was made homogeneous. Flavor was added at the end of the formulation preparation and triturated well for a span of 15 minutes.

Physical stability studies

The above prepared formulations **F7 – F11** were kept aside in respective sample bottles, adequately labeled and observed for a span of two weeks for its compatibility with each ingredient in the product.

Observation

These test products exhibited varying degrees of instability in the form of layer separation to settling of the component. Apparently, the formulation without the flavoring agents has 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 FLAVOURING AGENT

The formulation was prepared using banana, raspberry, strawberry, black currant etc. The below-mentioned details of the ingredients and their proportions are mentioned in the chart Procedures of the preparation of the each formulation are as per the general procedure cited above.

Table 5: Experiments for a suitable flavoring agent

S.NO	Ingredients	EXPERIMENTS/CONCENTRATION OF INGREDIENTS		
		F12	**F13**	**F14**
1	**1-Octacosanol**	**0.020g**	**0.045g**	**0.051g**
2	**Tween -80**	0.64g	0.64g	0.64g
3	**Span -20**	-	-	-
4	**Soy lecithin**	-	-	-
5	**Cremophor**	-	-	-
6	**Span - 60**	1.36g	1.36g	1.36g
7	**Corn oil**	3g	3g	3g
8	**Soybean Oil**	-	-	-
9	**Sodium Hydroxide (0.1M)**	-	-	-
10	**Water**	0.5	0.5	0.5
11	**Flavor**	BANNANA(2 drops)	STRAWBERRY (2 drops)	RASPBERRY(2 drops)
12	**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 of the flavors shows the impact on the stability of the final product, and therefore, the method of product preparation is 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

S.NO	INGREDIENTS	%WT	T.WT	P.WT
1	**1-Octacosanol**	0.2%	0.05g	0.048g
2	**Corn oil**	12.%	3g	3.002g
3	**Tween -80**	2.56%	0.64g	0.6402g
4	**Span-60**	5.44%	1.36 g	1.3605g
5	**Sucralose**	0.04%	0.01g	0.0101g

6	**Flavour(rasp)**	0.05%	12.5mg	2drops
7	**Water**	QS	QS	QS

Procedure

Weighed corn oil, span-60 and 1-Octacosanol were taken in a beaker and melted by heating them between 60 and 65°C. Then water, tween 80 and sucralose were weighed and heated to 65-70°C. Oil phase was added to mortar and triturated well by adding water little by little for the duration of one hour.

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 the adding anti foaming agent (Simethione) and black currant flavor.

Procedure oil phase

1-Octacosanol, corn oil and Span-60 were weighed in a beaker and melted at a temperature of 65-70°C.

Aqueous phase

Water, Tween-80 and sucralose were weighed in a beaker; and subsequently dissolved and heated to 65-70°C. Oil phase was transferred to the mortar by gentle addition, little by little, into the water phase and triturated vigorously for 40 minutes Then Simethicone, an antifoaming agent was added. The resultant product was triturated for 30 minutes. Adequate flavour was added and the product was continuously stirred in a magnetic stirrer for another ten minutes.

Report

The emulsion formed is good and milky white with pleasant flavour.

Table 7: Inclusion of antifoaming agent

S.NO	INGREDIENTS	%WT	T.WT	P.WT
1	**1-Octacosanol**	0.2%	0.05g	0.048g
2	**Corn oil**	12.%	3g	3.001g
3	**Tween -80**	2.56%	0.64g	0.64g
4	**Span-60**	5.44%	1.36 g	1.36g
5	**Sucralose**	0.05%	0.01g	0.011g
6	**Flavor(black current)**	0.05%	0.0125g	2drops
7	**Simethicone**	0.01%	0.0025g	0.003g
8	**Water**	QS	QS	QS

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 the 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 was 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 the 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 the 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, the F1 emulsion got separated within a few minutes of the formation. In the case of F2, the emulsion was formed, but it was too thick and difficult to handle. On the other hand, the F3 emulsion was formed using cremophor, but the stability was only momentary. In the F4 formulation, the emulsion was formed, but it was too thick and it was felt that the addition of higher water quantity could dilute the same. In view of the above inferences from formulation F1 to F4, the possibility of forming a primary emulsion by integrating the HLB (Hydrophilic Lipophilic Balance) values of the oil and oil phase emulsifier was explored. In theF5 emulsion, both Span-20, an emulsifier and corn oil, an oil phase possess the HLB value of 8.6, and through the primary preparation process, it was found to be a reasonably stable emulsion. Incorporation of the active into the primary emulsion was carried out and in this 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 to7.01. In the case of F7, the emulsion was separated out due to imbalance of emulsifier and oil. The F8 emulsion formed frothing. In the F9, the emulsion separated within five hours of the formation. The emulsion with soybean oil was found to be very thick; hence, it is inferred that corn oil is the better option when compared to soybean oil. In the F10 formulation, the emulsion was separating out with some settling within two weeks of its formation. In the F11 formulation, the emulsion was formed, but it was dull and frothy. In the F12, the emulsion was formed, but dull in colour and the same separated within a week. In the F13 formulation, the flavor was not compatible with other ingredients of the emulsion; and moreover, the emulsion was dull on storage, and with a span of two weeks, the emulsion was found to be settling. In the F14 formulation, the emulsion was good, milky and white, but a bit 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 colour. F15 too was found to be frothy. The emulsion formed out of F16 was good, milky white with a pleasant flavor and found to be stable and palatable. From the dye test performed for formulation F16, the continuous phase appeared red, which confirms the 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 three months indicate that 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 an emulsion, a liquid oral dosage. On evaluation, it has become evident that 1-Octacosanol is a physically stable, oil in water emulsion, stable at higher temperature as well as at higher 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 pediatric and geriatric patients and other broad spectrum of patients for its stability, efficacy and ease of administration.

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