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Preformulation Studies of Drugs and Excipients for the Formulation of Salmon Fish oil Nanoemulsion Gel for the Treatment of Psoriasis

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ABSTRACT

Psoriasis is a prolonged, immune-mediated inflammatory skin disease. It is characterized by sharply demarcated, red, scaly, coin-sized skin lesions most often on the elbows, hands, knees, scalp and feet. Around 10% of individuals with psoriasis develop arthritis, which may affect the hands, feet, wrists, ankles, neck and lower back. In some cases joints become deformed, causing significant disability. The worldwide prevalence of psoriasis is around 2%, but studies in developed countries have reported higher incidence rates of on average about 4.6%. In India the prevalence of psoriasis fluctuates from 0.44 to 2.8%, it is two times more common in males compared to females, and most of the patients are in their third or fourth decade at the time of presentation. Nearly two thirds of people with psoriasis have a mild form of the disease, Therefore in most of the cases first line treatment approach is topical. Betamethsone dipropionate (BD) loaded in omega-3- fatty acid fish oil nanoemulsion for the healthier absorption of BD in deeper layer of the skin to stop further progression of inflammatory cycle. For the preparation of nanoemulsion different types of preformulation studies require to perform because every drug has its specific intrinsic chemical and physical property which has been consider before development of pharmaceutical formulation. This property provides the framework for drug's combination with pharmaceutical ingredients in the fabrication of dosage form.

Keywords: Preformulation, Betamethasone dipropionate, Nanoemulsion, Psoriasis, HLB surfactants, Salmon fish oil.

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INTRODUCTION

Psoriasis is a prolonged, immune-mediated inflammatory skin disease. It is characterized by sharply demarcated, red, scaly, coin-sized skin lesions most often on the elbows, hands, knees, scalp and feet. Symptoms include irritation, itching, stinging and pain [1-3]. Rarely, the entire surface of skin of the body may be involved; this extensive form of psoriasis can be fatal, as the extreme inflammation and peeling of skin can disrupt the body's ability to regulate temperature and damage the skin's barrier functions [4, 5]. Approximately 10% of individuals with psoriasis develop arthritis, affecting feet, ankles, wrists, neck and lower back [6, 7]. In some individuals joints become deformed, causing substantial disability. Fingernails and toenails may be affected by scaling and crust formation and there may be shedding of nail plates, causing disfigurement [8].

The worldwide occurrence of psoriasis is approximately 2%, however about 4.6% higher prevalence rates have been reported in developed countries [9, 10]. In India the incidence of psoriasis varies from 0.44 to 2.8%, it is two times more common in males compared to females [11]. Almost two thirds of people with psoriasis have a mild form of the disease, Therefore in most of the cases first line treatment approach is topical. Besides drug used for the treatment of psoriasis may produce countable side effect, to overcome this problem physician prefer topical therapy. Topical treatments involve application of medications directly on the skin. They are the main mode of treatment prescribed for most patients with psoriasis. Severe psoriasis needs a variety of other treatments including ultraviolet light or special table [12].

The aim of the treatment of psoriasis is to reduce the inflammation, in the scaly patches of the skin. Topical steroids help to reduce inflammation in the scaly patches of skin. The weaker topical steroids are used on the face and under the arms for short duration treatment protocols [13].The stronger topical steroids are used on the palms and soles for the management of psoriasis. Topical corticosteroids are mostly used for the treatment of plaque-type psoriasis that is most common type of psoriasis [14].

There are numerous topical conventional dosage forms commercially available for the treatment of psoriasis like cream, ointment, lotions etc. The main limitationof these dosages form is the absorption of active moiety to the dermis (deeper layer) of the skin where inflammatory media torsade present [15].

The absorption of drug is also restricted due to hyper-keratinized cell to the epidermis of the psoriatic skin. For removal of this hyper keratinized cell of epidermis salicylic acid is used. Betamethsone dipropionate (BD) loaded in omega-3- fatty acid containing fish oil nanoemulsionis

used. This leads to better absorption of BD in deeper layer of the skin. This causes reduction in progression of inflammatory cycle [16,17].

For the preparation of nanoemulsion various types preformulation studies are needed to be performedbecause each drug has its specificintrinsic chemical and physical property which is considered before development of pharmaceutical dosage forms. This property delivers the structural framework for drug's incorporation with pharmaceutical ingredients in the production of dosage form [18].

MATERIALS AND METHOD

Physical characterization

The drug sample was characterized for its authenticity using its monograph [19]. The drug sample was observed for physical appearance, pH, odor, M.P, loss on drying etc. This parameter was matching with reported monograph.

Loss on drying

An accurately weighed (1g) sample was taken in a clean, dried and previously weighed bottle, dried at 105^{o} C for 1 hour. Bottle was weighed again after drying to determine loss on drying.

Partition Coefficient

The partition coefficient was calculated by taking octanol and water, 25 ml each in separating funnel and adding 100 mg drug to the mixture of solvent. The funnel was shaken vigorously and then it was clamped in stand for 24 hrs (shaking in between) to effect partitioning. After 24 hrs sample from each solvent (water and octanol) was taken and diluted and its absorbance was measured[20].

U.V. analysis

For the UV absorption of fish oil 0.05 % oil dissolved in isooctane. Isooctane is used as blank and then examined between 200 nm to 600 nm 22.

DSC analysis

DSC Analysis of betamethasone dipropionate was performed by DSC instrument, Perkin Elmer, Germany. The physical state of the drug was determined by Differential scanning calorimetry (DSC). Samples containing 5 mg of drug was placed in a pan in the instrument and heated from 500C to 3000C at a heating rate of 10^{o}C/ min under inert atmosphere flushed with nitrogen at the rate of 20 ml/ min [23]. For the DSC study of fish oil, Differential Scanning Calorimetry (TA Instruments, New Castle, DE, USA) was used to determine the melting point, enthalpy, and specific heat capacity of salmon fish oil at each purification step, and the melting points and

enthalpies for individual fatty acids were determined. All experiments were conducted in triplicate, and representative DSC thermograms of fish oil were presented.

Melting Point and Enthalpy About 0.5–1 mg of oil sample was kept in a small aluminum sample pan. The pan was then kept on the sample platform of the DSC. An empty aluminum pan was placed on the reference platform. For determination of the melting point a linear heating rate of 5^0C/min over a temperature range of 75 to 125 ^{0}C was used. Liquid nitrogen was used to chill the samples to a lower temperature [24].

FTIR spectral analysis

The FTIR spectral analysis for betamethasone dipropionate was carried out by using KBr pellet technique. (Shimadzu, Japan). FTIR spectrometers are mostly used for measurements in the mid and near IR regions. For the mid-IR region, 2-25µm (5000-400cm-1), the most common source is a silicon carbide element heated to about 1200K. The output is similar to a blackbody. Shorter wavelengths of the near-IR, 1-2.5µm (10000 – 4000cm-1), require a higher temperature source, typically a tungsten-halogen lamp. The long wavelength output of these is limited to about 5µm (2000cm-1) by the absorption of the quartz envelope. For the far-IR, especially at wavelengths beyond 50µm (200cm-1) a mercury discharge lamp gives higher output than a thermal source [25]. For the fish oil FTIR study, Caustic stripping method was used. Moisture was efficiently removed by heating up to 150^0C [26].

Criteria for Excipient Selection

The selected excipients required to be pharmaceutically acceptable and non-immunogenic to the skin. Higher solubility of the drug in the oil phase is important criterion as it assume to be helpful for the nanoemulsion preparation and to maintain the drug in solubilized form [27]. Safety is a major concern in the selection of a surfactant. Large amount of surfactants may cause skin irritation. Therefore the non-ionic surfactants are considered to be less irritant and less sensitizing than ionic surfactants. An important factor for selection of the surfactants is the hydrophilic lipophilic balance (HLB) value to form the o/w nanoemulsion. The HLB value should be greater than 10. The proper ratio of low and high HLB surfactants leads to the formation of more stable nanoemulsion dosage form. The presence of co-surfactant reduces the bending stress of interface and permits the interfacial film sufficiently flexible to take up different curvatures required to prepare nanoemulsion of extensive range of composition.

RESULTS AND DISCUSSION

Characterization of Betamethasone dipropionate

Physical Properties

Nature: Amorphous.

Colour:Whitish Yellow

Odour:Odourless.

Melting point: 176.40C (Melting point apparatus),

Solubility: Freely soluble (methanol), soluble (ethanol, chloroform, dichloromethane) and practically insoluble in water, phosphate buffer (pH 6.8) and acetate buffer (pH 5.5).

Loss on drying

Weight of empty bottle (A) = 14.7800 g.

Weight of bottle and drug (B) = 15.7800 g.

Weight of drug before drying (D=B-A) = 1.000 g.

Weight of bottle with drug after drying (C) = 15.776g.

Weight of drug after drying (E=C-A) = 0.996g.

Therefore, Loss on drying = (D-E / D) x 100

= 0.40%

IDENTIFICATION TESTS

Partition Coefficient

Partition Coefficient is the measure of drug lipophilicity and an indication of its ability to cross cell membrane. It is defined as the ratio of unionized drug distributed between organic and aqueous phases at equilibrium.

$$Po/w = (Coct / Cwater) \text{ at equilibrium}$$

$$Po/w = 3.39$$

Table 1: Apparent partition coefficient of betamethasone dipropionatein octanol/water

Total amount of drug (µg)	Amount in organic phase (µg)	Amount in aqueous phase (µg)	Partition coefficient (± SD)
100	80.91	23.81	3.39 ± 0.56

The partition coefficient of betamethasone dipropionate was determind and found to be 3.39(Reported 3.82).This suggests that the drug is lipophilic because drug have partition coefficient more than 1 are supposed to lipophilic (Table 1).

Drug Identification Test

Characterization of Betamethasone dipropionate

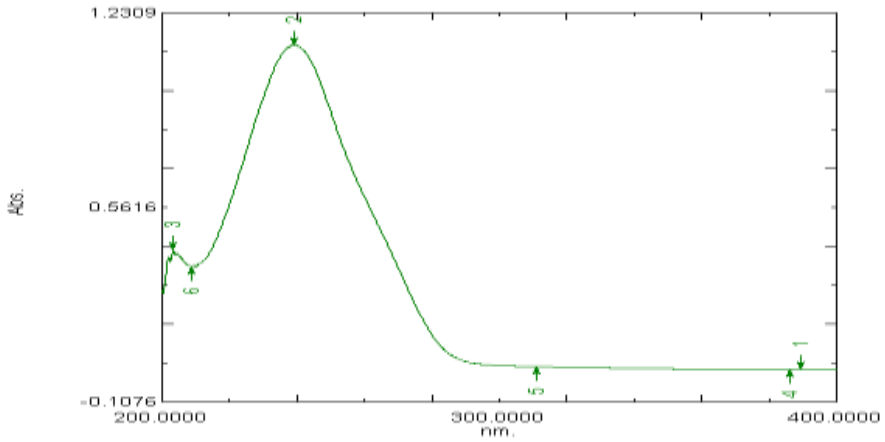


Figure 1: U.V absorption spectra of betamethasone dipropionatein methanol

The U.V absorption spectra of betamethasone dipropionatein methanol showed peak at 240 nm (Figure 1).

Differential scanning calorimetry

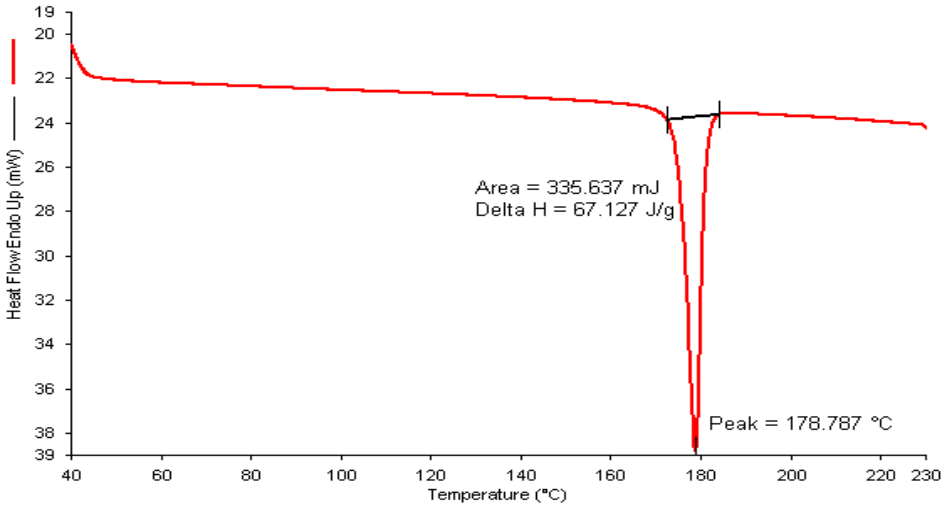
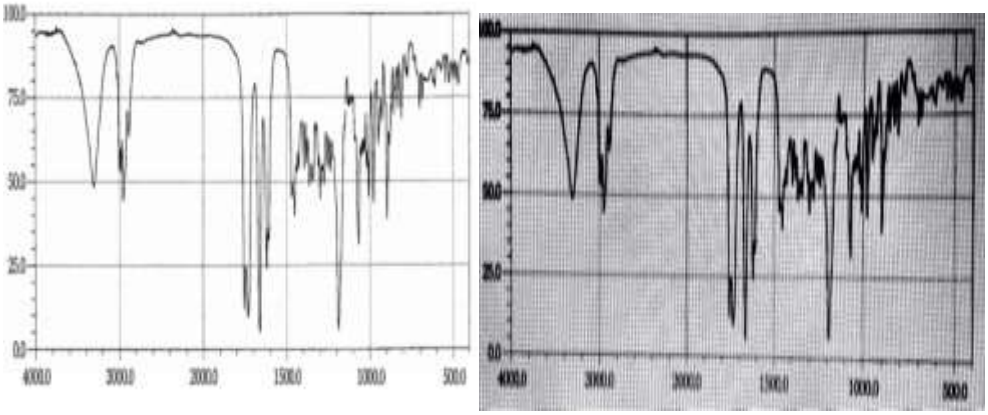


Figure 2: DSC thermogram of betamethasone dipropionate

The DSC thermogram of betamethasone dipropionate shows that endothermic energy was 67.127 J/g and the area of the peak was 335.63 mJ and melting point found to be 178.780C which was in between the 1750C to 1800C (reported value), Shown in (Figure 2).

FT-IR spectral analysis



(a) Standard **(b) Sample**

Figure 3: FT-IR spectra of betamethasone dipropionate.

Table 2: Characteristic peak of betamethasone dipropionate

Functional groups	Peak(Wave number cm^{-1})
Aromatic ketonic group	1729
Hydroxyl group	3584
Ester group	1608
Another ketonic group at 17-position	1662
Methylene steaching	2990

The FT-IR spectra of betamethasone dipropionate showed in (Figure 6) and characteristic peak given in the (Table 2) and it matched exactly with the standard drug. So, drug was authentic.

Characterization of salicylic acid

Physical characterization

The drug sample was characterized for its authenticity using its monograph .The drug sample was observed for physical appearance, pH, odor, M.P, loss on drying etc. These parameter were matching with reported monograph.

Loss on drying:

An accurately weighed (1g) sample was taken in a clean, dried and previously weighed bottle, dried at 105o C for 1 hour. Bottle was weighed again after drying to determine loss on drying.

Partition Coefficient

The partition coefficient was calculated by taking octanol and water, 25 ml each in separating funnel and adding 100 mg drug to the mixture of solvent. The funnel was shaken vigorously and then it was clamped in stand for 24 hrs (shaking in between) to effect partitioning. After 24 hrs sample from each solvent (water and octanol) was taken and diluted and its absorbance was measured.

U.V. analysis

Dissolved 10 mg of salicylic acid in ethanol and make up the volume to 100 ml with methanol. Diluted 5 ml of the above solution with methanol to 100ml. Examined between 200 nm to 600 nm.

DSC Analysis

DSC analysis of salicylic acid was performed by DSC instrument, Perkin Elmer, Germany. The physical state of the drug was determined by Differential Scanning Calorimetry (DSC). Samples containing 5 mg of drug was placed in a pan in the instrument and heated from 500^{o}C to 3000^{o}C at a heating rate of 10^{o}C/ min under inert atmosphere flushed with nitrogen at the rate of 20 ml/ min.

FTIR spectral analysis

The FTIR spectral analysis for salicylic acid was carried out by using KBr pellet technique. (Shimadzu, Japan).

Physical Properties

Nature: Amorphous.

Colour: White

Odour: Odourless.

Melting point: 155.60C (Melting point apparatus),

Solubility: Freely soluble (ethanol), soluble (methanol, chloroform, dichloromethane) and slightly soluble in water.

Loss on drying

Weight of empty bottle (A) = 13.2100 g.

Weight of bottle and drug (B) = 14.2100 g.

Weight of drug before drying (D=B-A) = 1.000 g.

Weight of bottle with drug after drying (C) = 14.2038 g.

Weight of drug after drying (E=C-A) = 0.9955 g.

Therefore, Loss on drying = (D-E / D) x 100

= 0.45%

Identification Tests

Partition Coefficient

Partition Coefficient is the measure of drug lipophilicity and an indication of its ability to cross cell membrane. It is defined as the ratio of unionized drug distributed between organic and aqueous phases at equilibrium.

Po/w = (Coct / Cwater) at equilibrium

Po/w = 2.09

Table 3: Apparent partition coefficient of salicylic acidin octanol/water

Total amount of drug (mg)	Amount in organic phase (mg)	Amount in aqueous phase (mg)	Partition coefficient(± SD)
100	40.08	19.17	2.09 ± o.456

The partition coefficient salicylic acid of was determined and found to be 2.09 (Reported 2.26). This suggests that the drug is lipophilic because drug have partition coefficient more than 1 are supposed to lipophilic (Table 3).

Drug identification test

Characterization of salicylic acid by UV method

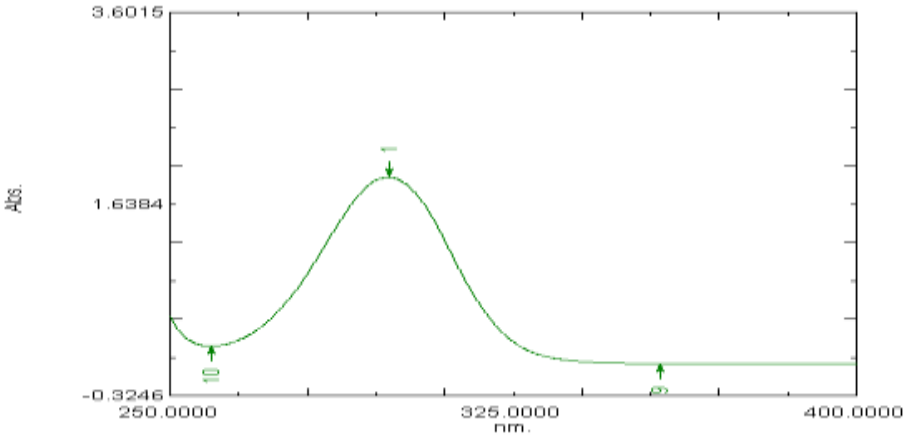


Figure 4: UV scan of salicylic acid in phosphate buffer (pH 7.4) solution (297 nm)

The U.V absorption spectra of salicylic acid in methanol showed peak at 297 nm (Figure 4).

DSC analysis of salicylic acid

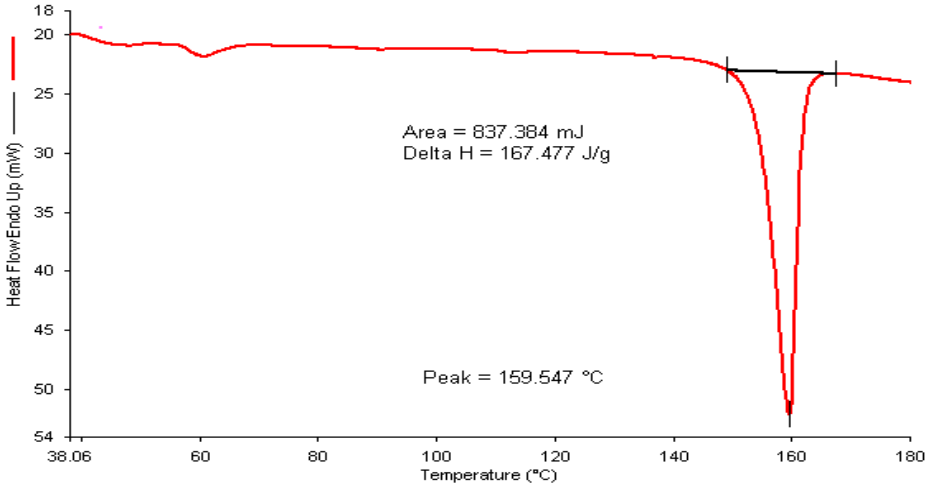
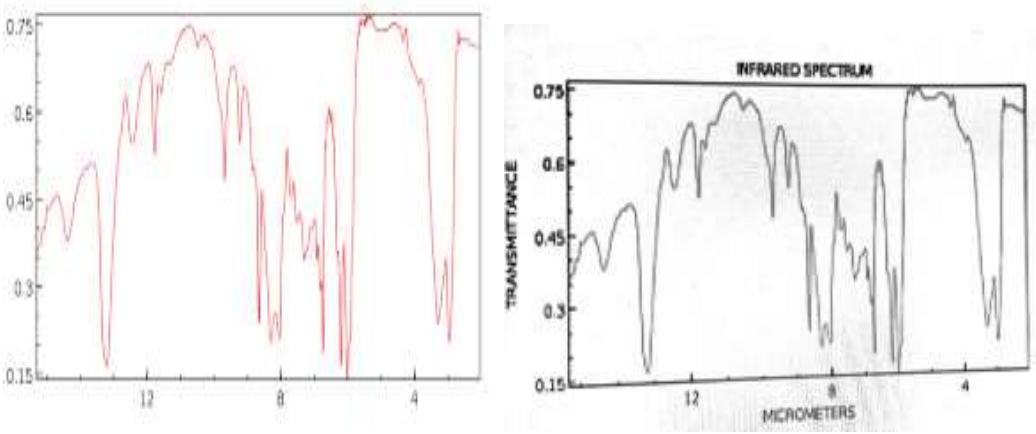


Figure 5: DSC thermogram of salicylic acid

The DSC thermogram of betamethasone dipropionate showed in (Figure 5) that endothermic energy was 167.47 J/g and the area of the peak was 887.384 mJ and melting point found to be 159.540C which was in between the 1550C to 1600C (reported value).

FT-IR spectral analysis



(c) Standard **(d) Sample**

Figure 6: FT-IR spectra of salicylic acid

Table 4: Characteristic peak of salicylic acid

Functional groups	Peak (Wave number cm-^{1})
Aromatic –CH stretching	3070
-OH functional group with hydrogen bonding	3600
-C=O stretching	1665
-OH functional group within –COOH group	1442

The FT-IR spectra of betamethasone dipropionate shown in (Figure 6) and characteristic peak given in the table and it matched exactly with the standard drug. So, drug was authentic (Table 4).

Characterization of Salmon fish oil

Physical Properties

Nature: Thick liquid.

Colour: dark yellow to brown

Odour: Petroleum.

Boiling point: >250 °C.

Solubility: Slightly soluble in water. Freely soluble in isooctane.

Identification test of Salmon fish oil

Characterization of Salmon fish oil by UV method

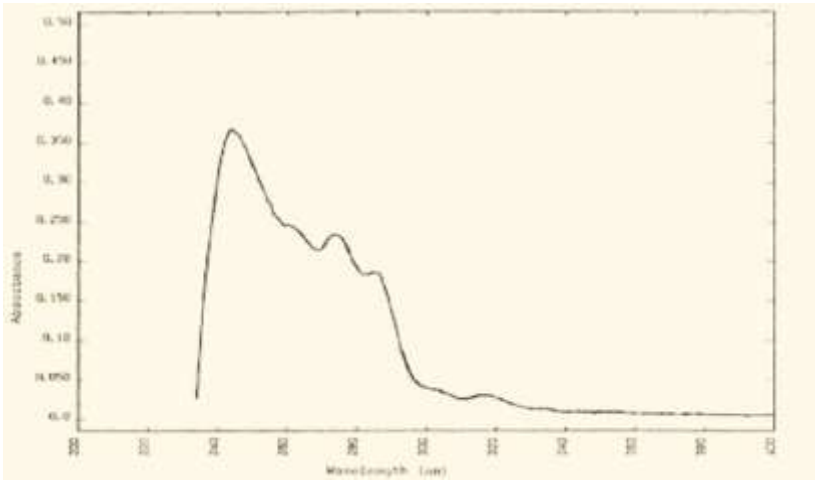


Figure 7: UV spectrum of fish oil 0.05 % oil in isooctane showing maximum absorption at 254 nm.

Differential Scanning Calorimetry (DSC)

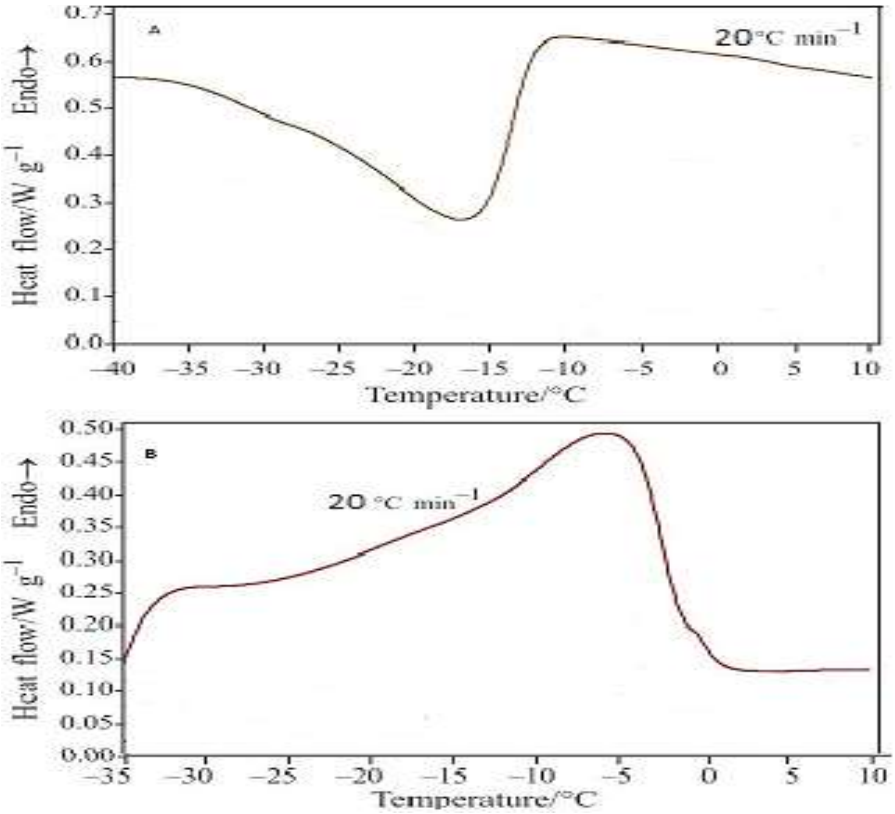


Figure 8: DSC curves (a – cooling; b – melting) taken on fish oil.

Table 5: Transition temperature and enthalpy during cooling and melting of salmon fish oil

Scanning rate/^{0} C min.$^{-1}$	Cooling		Melting	
	T_{max} /^{0}C	ΔH/ Jg^{-1}	T_{max} /^{0}C	ΔH/ Jg^{-1}
	-8.24	-11.68	-8.10	23.77

T-Temperature, ΔH*-change in enthalpy,* Jg^{-1}*-Joules per gram.*

FTIR study of fish oil

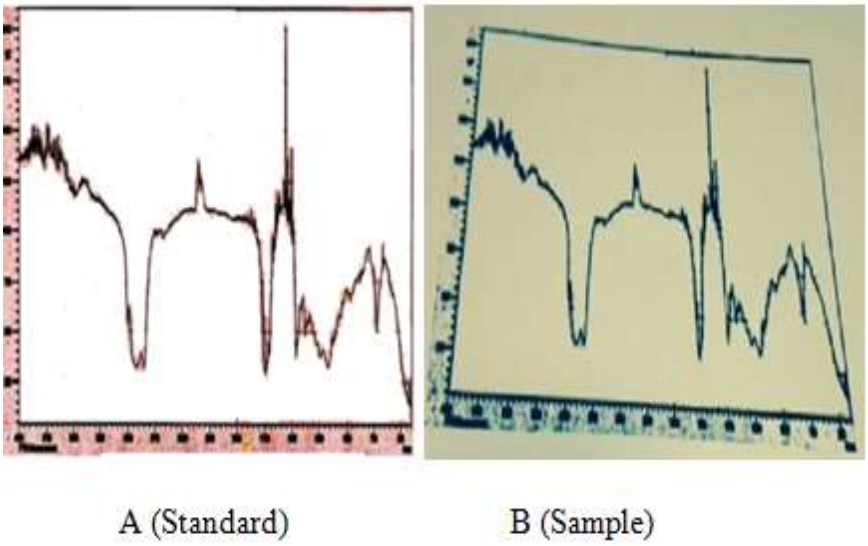


Figure 9: FTIR spectra of Fish Oil, A (Standard) & B (Sample)

Absorption in the range of 3600-3300 cm^{-1} was found indicating the nonappearance of moisture in the fish oil. Further, there is presence of ester absorption in the range of 1750-1730 cm^{-1} in all the spectra recorded. Aliphatic C-H. Stretch of higher fatty acid ester was found from 2925-2850 cm^{-1} and that of processing. Further, there is presence of ester absorption in the range of 1750-1730 cm^{-1} in all the spectra recorded. Aliphatic C-H stretch of higher fatty acid ester was found from 2925-2850 cm^{-1} and that of =C-H stretching absorption in the range of 3190-3050 cm^{-1}, respectively.

SCREENING OF EXCIPIENTS

Development of nanoemulsion systems loading of drug per formulation is a very critical designing factor for poorly soluble drugs that depends upon thedrug solubility in oil phase. Solubility of BD in Salmon fish oil was 18.95 mg/mL which is very good for topical delivery since the dose of BD is very low.

Nanomulsification of oil is very difficult due to the presence of other fatty acid in Salmon fish oil. The miscibility of oil with surfactant and co-surfactant is important for the preparation of good nanoemulsion region in ternary phase diagram. Therefore, the miscibility of oil was performed with different surfactants and co-surfactants (Table 6).

Table 6: Miscibility of Fish oil with surfactants and co-surfactants.

Miscibility of Salmon fish oil				
S.No	**Withsurfactant(1:1)**	**Observation**	**With co-surfactant(1:1)**	**Observation**
1	Tween 20	Turbid	Ethanol	Turbid
2	Tween 80	Clear	Transcutol P	Clear
3	Lecithin	Turbid	PEG 200	Turbid
4	Unitop 100	Turbid	Pleurololeique	Turbid

Another important condition is the selection of surfactant with suitable HLB value. Hydrophilic surfactant and co-surfactant are considered to prefer the interface and to lower the necessary energy to form the nanoemulsion, consequently improving the stability. For example, the required HLB value to form o/w nanoemulsion is greater than 10. So proper selection of surfactant and co-surfactant with appropriate HLB value is required [28].

The miscibility of Salmon fish oil was observed highest with Tween 80 in case of surfactant and Transcutol P in case of co-surfactant with 1:1 ratio. Apart from this, Tween 80 has high HLB value which can impart good emulsification to the salmon fish oil. Transcutol P is very good solubilizing agent which can impart better penetration strength to the lipophilic drug such as BD by increasing the solubility of the drug in the lipophilic domain of the stratum corneum. So, for the development of pseudoternary phase diagram Salmon fish oil was selected as an oil phase, Tween 80 as surfactant and Transcutol P as a co-surfactant.

CONCLUSION

On the basis of the above preformulation studies of drugs and excipients, it is confirmed that the selected drugs and excipients were appropriate and authentic for further studies.

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