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DESIGN, DEVELOPMENT AND EVALUATION OF NANO-PARTICULATE TOPICAL DRUG DELIVERY SYSTEM CONTAINING NON-STEROIDAL ANTI INFLAMMATORY DRUG

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Abstract

The present work was undertaken, for arriving at a sustained release topical formulation of celecoxib with the aim of prolonged skin retention of the drug leading to availability of the active form of the drug in the required level resulting in enhanced therapeutic efficacy through reduced toxicity. The novel formulation chosen for the present study is SLN due to its high and proven profile on research for topical drug delivery. The positives of SLN are it comprise of lipid whereas celecoxib is also lipophilic in nature which may produce a strong bond resulting in a delayed release of the drug; and the skin exposure of the drug can be reduced as the drug is encapsulated in the lipid matrix. The size of SLN can be achieved as decided to retain on skin, because smaller particle size i.e. less than 500 nm, may be absorbed into the skin; SLN can be incorporated into gel in ease: the stability of SLN over other novel delivery system is very high; SLN is a biological preparation which is non toxic in nature.

Keyword: Characterization, In Vitro Release, Preformulation and celecoxib

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1. Introduction :

Historically psoriasis was considered a disease of altered keratinocyte proliferation based on prominent histological and clinical features, such as epidermal thickening and scaling (Figure 1).[1] Currently available treatments for psoriasis involve inhibiting epidermal cell hyperproliferation and suppression of the immune system; though there is no cure for psoriasis.[2] The goal of therapy is to decrease the severity or substantially clear the disease so that it has minimal impact on the patient's daily activities. Various nano-carriers have been developed in an attempt to reversibly modulate the skin barrier and/or to provide novel delivery systems with the interest of targeted drug delivery. In this thesis the technological and biopharmaceutical properties of topical formulations based on Solid Lipid Nanoparticles (SLN) are dealt with. The basic pharmaceutical aspects of how to formulate effective topical and dermatological drugs in nanocarrier like SLN and its gel, that can be released from the vehicle and adhere on the skin to deliver drug at the site of action are experimented and explained in the thesis.

Topical therapies are recommended as initial treatment for mild to moderate amountsof stable plaque psoriasis regardless of disease severity.[3] Phototherapy and systemic agents are generally reserved for patients with more extensive or recalcitrant disease. Several topical therapies currently available include emollients, keratolytic agents (e.g., salicylic acid), coal tar, anthralin, corticosteroids, calcipotriene or celecoxib (CELECOXIB) although all have limitations.[3] Emollients and keratolytic agents have only marginal efficacy and mainly reduce scaling. Coal tars and anthralins have modest efficacy but are limited by their odor and staining. Corticosteroids, the most widely used agents for psoriasis, have a moderate to high degree of efficacy (depending on potency) in the short term and have a high degree of patient acceptance. However, corticosteroids are limited by a relatively high incidence of tachyphylaxis, often making maintenance therapy with these agents ineffective.[4] The use of first-generation retinoids as topical treatment of psoriasis has been attempted. Alltrans retinoic acid (tretinoin)[5,6] and 13-cisretinoic acid (isotretinoin)[7] showed some effectiveness in improving psoriasis, but they were associated with a substantial amount of local irritation. Application of another retinoid celecoxib may cause excessive irritation in the skin of certain sensitive individuals. In some cases there has been necessity to temporarily discontinue therapy, or the dosing has been reduced to a lower concentration (in patients with psoriasis) or to an interval the patient can tolerate, therapy can be resumed, or the drug concentration or frequency of application can be increased as the patient becomes able to tolerate the treatment. CELECOXIB has been available traditionally as gel and cream for the treatment of psoriasis and acne. A fundamental shortcoming of conventional formulations is the provision of topically active agents in relatively high concentrations to the skin with a limited duration of action it gets converted to its active form which is readily biodegradable. Frequency of application should be closely monitored by

careful observation of the clinical therapeutic response and skin tolerance. Efficacy has not been established for less than once daily dosing frequencies.[8] The objective of the present study was to formulate a controlled release celecoxib (CELECOXIB) suspension and gel for dermal delivery using solid lipid nanoparticles (SLN) as lipid nanocarrier. The attempt here is to make a delivery system which can retain on skin intact for a longer duration and delivering the dug in a controlled manner. Novel delivery research for CELECOXIB like, in some patents disclosed CELECOXIB delivered asfoam for topical and in nail polish for nail psoriasis. However, further studies are needed for more dermatological formulations with different characteristics.

Celecoxib

Plaque psoriasis, the most common variant, is characterized by welldefined erythematous scaly plaques, with or without associated nail disease and arthritis. In most patients, the disease is localized and amenable to topical therapy. For the treatment of psoriasis, Retinoids are widely used.

Celecoxib belongs to a novel, nonisomerizable class of retinoic acid receptor (RAR)- specific retinoids, the acetylenic retinoids, and is the first topical retinoid developed for the treatment of psoriasis. Celecoxib (6-[(3,4-Dihydro-4,4-di-methyl-2H-1-benzothiopyran- 6- yl)ethynyl]-3-pyridinecarboxylic acid ethyl ester) is a white solid third-generation retinoid approved for the treatment of psoriasis and acne vulgaris.[187] Celecoxib is a pro-drug of tazarotenic acid, a receptor-selective retinoid, which has shown efficacy in the treatment of these disorders. In the treatment of acne vulgaris, it has greater comedolytic activity than the currently available topical retinoids. In psoriasis, celecoxib normalizes keratinocyte differentiation, reverses keratinocyte hyperproliferation and has better anti-inflammatory effects than any of the currently available topical retinoids. It is most commonly used as combination therapy with a topical corticosteroid or phototherapy in localized plaque psoriasis, or with an antibiotic in acne in order to enhance efficacy and tolerability.[188] Adjunctive use of a mid or high-potency steroid result in greater reductions of overall disease severity, plaque elevation and adverse events. In mice, celecoxib blocks ornithine decarboxylase enzyme activity, which is associated with cell proliferation and hyperplasia. In cell culture, it suppresses markers of epidermal inflammation and inhibits cornification of the keratinocytes.[189] In the treatment of psoriasis, celecoxib is believed to be a mediator of cell differentiation and proliferation.

Celecoxib is the first topical retinoid approved by the Food and Drug Administration (FDA) for the treatment of psoriasis, acne and photoaging. Side effects like burning, itching, and skin irritation are relatively common, and patients should avoid sun exposure.

2. Material and Methods

Celecoxib was received in ex gratia from Glenmark Pharmaceuticals, Mumbai.

®

Compritol 888 ATO was received as gift sample from Orchid Chemicals & Pharmaceuticals, Chennai. Lutrol F 68 was received as gift from orchid chemicals & pharmaceuticals, chennai.

Tween 80 is the trade name of polysorbate 80 and was a gift from Plethico Pharmaceuticals,Indore.

All other chemicals and reagents were of analytical grade and were used without further purification.

Drug Profile

The drug profile of celecoxib is given in detail in chapter appendix. The structural image is given in figure 17 in appendix. The drug profile comprises of the description,[240] drug's molecular weight, IUPAC name[241] chemical name, taxonomy, available formulation, trade name, predicted properties,[242] pharmacology,[239] indication, pharmacodynamics, mechanism of drug action, absorption, protein binding, route of elimination, half life, toxicity, pharmacology in different models,[242] pharmacokinetics,[243] adverse reactions,[243] dosage and administration, use, precaution for using this medicine.[243]

GB) is a market product from Gattefossé gmbh based on glycerol esters of behenic acid (C22). It is composed of glycerol tribehenate (28-32%), glycerol dibehenate (52-54%) and glycerol monobehenate (12-18%). A breif description is given in appendix.

Surfactants Profile

The surfactants used in the formulation were, Poloxamer[247] as shown in figure 18 is the molecular formula of Poloxamer 188, the other surfactant used was Polysorbate 80[248] as shown in figure 19 in appendix is the Chemical structure of Tween® 80. A breif description is given in Appendix.

Viscosity-increasing agent

Carbomer 940[249] as shown in figure 20 in appendix is the chemical structure of Acrylic acid monomer unit in carbomer resin. A breif description is given in Appendix.

Other ingredients

Water used in the experiment was obtained after reverse osmosis from milli Q, a description

is given in Appendix. All other chemicals and reagents were of analytical grade and were used without further purification.

Preformulation Studies

Physiochemical properties

The physiochemical properties of the pure drug, was studied.[250] The organoleptic parameters analyzed where, its physical state, normal form and colour. The solubility and the melting point of celecoxib were also tested. The melting point was tested in Biochem melting point apparatus. The pure drug was further characterized with DSC for to find the purity, infrared Fourier transform spectroscopy (IR) to confirm the compound structure are intact, X-ray powder diffraction (PXRD) to find the crystalinity and 1H NMR was performed to find the purity of the compound.

The physiochemical properties of the lipid, glyceryl behenate were studied. The organoleptic parameters analyzed physical state, normal form and colour. The melting point of lipid was found using Biochem melting point apparatus. Glyceryl Behenate was further characterized with DSC, infrared Fourier transform spectroscopy (IR) and X-ray powder diffraction (PXRD).

Compatibility study

The physical mixture after the storage time was examined for organoleptic character.[251] To confirm there is no reaction and the drug is intact it was characterized with the following tests, thermal techniques was used for evaluating possible incompatibility quickly through comparison of thermal curves of pure substances with curve obtained from a 1:1 mixture. The celecoxib and lipid mixture was further characterized for compatibility with infrared Fourier transform spectroscopy (IR), and X-ray powder diffraction (PXRD).

Preparation of physical mixtures

The compatibility study (interaction) was performed by preparing a physical mixture of celecoxib and glyceryl behenate of equal weights (1:1 mass/ mass). The physical mixtures were prepared through simple mixing, in an amber glass vial, and kept for storage at 25°C (±2°C) and relative humidity (RH) of 60% (±5%) for 3 months at and submitted for analysis with DSC, infrared Fourier transform spectroscopy (IR), and X-ray powder diffraction (PXRD). The same sample was prepared for stability testing and the organoleptic observations are reported.

Preformulation studies

Physiochemical properties and characterization of celecoxib

Physiochemical properties

The Physiochemical properties and the organoleptic characters of celecoxib (CELECOXIB) are given in the table 13 below.

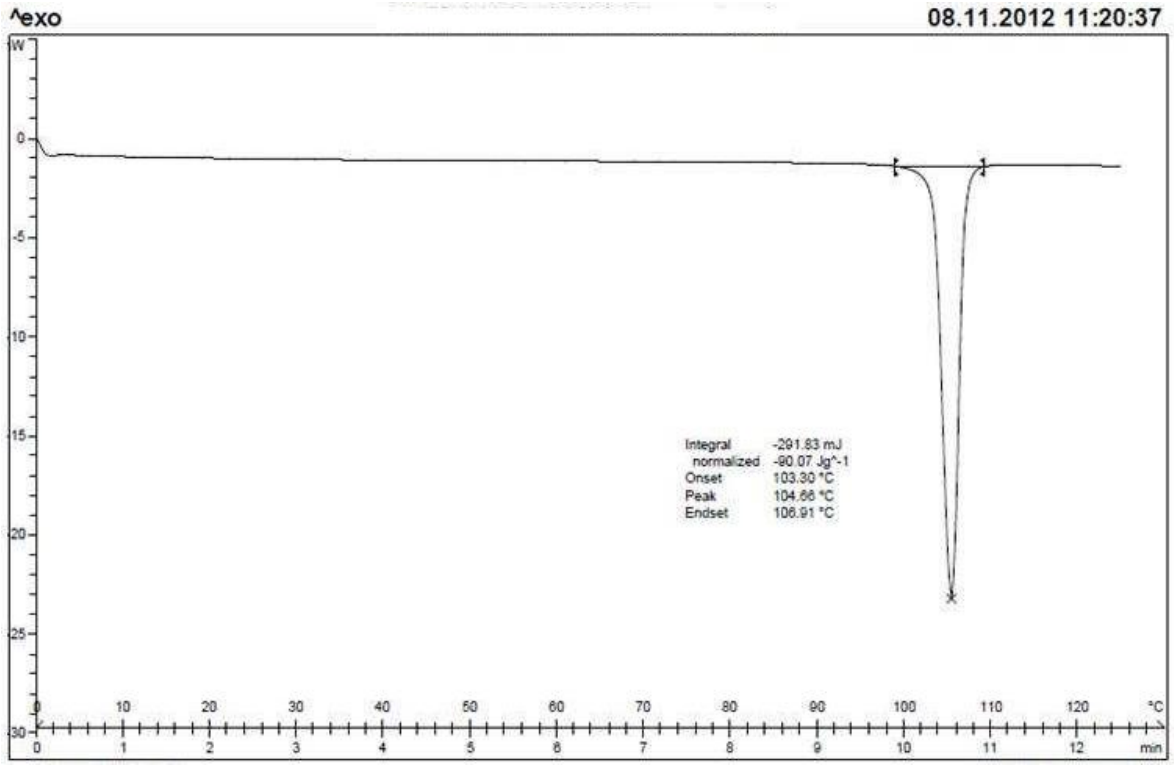
Table. Physiochemical properties of celecoxib

S. No.	Character	Observation
1	State	Solid
2	Form	Powder
3	Colour	Very light Yellow
4	Melting Point	102 - 105°C

Characterization of Celecoxib

The DSC curves obtained for CELECOXIB are shown in the figure 23. Differential scanning calorimetry resulch clearly states the presence of single compound by a sharp endothermic event. The onset of the peak was at 103.30°C, peak was detected at 104.66°C, the enthalpy DHfusion is -90.07 Jg^{-1} and the integral is -293.81 mJ.

Figure. DSC curve of celecoxib



Fourier Transform Infrared Spectroscopy

The FTIR spectrum of celecoxib obtained is in given in the figure 24. The important peak characteristics of CELECOXIB are as in table 14 below which proves the structural intactness of the molecule.

Table. FTIR spectral data of celecoxib

S. No.	Wavelength cm^{-1}	Functional Group
1	Aromatic Ring	3418.71 cm^{-1}
2	C triple bond C	2850.57 cm^{-1}
3	C double bond	1719.67 cm^{-1}
4	Aromatic C=C bond	
5	C double bond S	1468.86 cm^{-1}
6	C = N bond	1416.81 cm^{-1}
7	Ester	1381.27 cm^{-1}

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Powder XRD of celecoxib

The powder XRD spectrum of celecoxib showed more number of sharp peaks at 2 θ scattered at 21.6, 29.1, 43.66, 52.1, 37.8, 24.8, 18.2 for the pure drug. The resulch indicatethat CELECOXIB is crystalline in nature. The figure 25 is the spectrum and observation is given in table 15.

Table. XRD spectral data of celecoxib

S. No.	Angle 2-Theta	d-value
1	21.6	4.105
2	29.1	3.066
3	43.66	2.071
4	52.1	1.752
5	37.8	2.371

Nuclear magnetic resonance (1H NMR) spectroscopy of protons

H_1NMR spectra of celecoxib are as given in figure 26. The interpretation for chemicalshift, integrity, shift type are given in the table 16.

Table. H_1NMR spectral data of celecoxib

S. No.	Integration	Chemical Shift	Proton from	Shift type
1	6.12	1.36	CH3-C-CH3	Singlet
2	3.11	1.966	CH3-C-CH3	Singlet
3	2.04	3.076	C-CH3	Triplet
4	2.03	4.433	C-O-CH2	Quadrupl e
5	1.00	7.084	Aromatic ring	
6	0.99	7.25	Aromatic ring	-
7	2.02	7.636	Aromatic ring	-

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Celecoxib

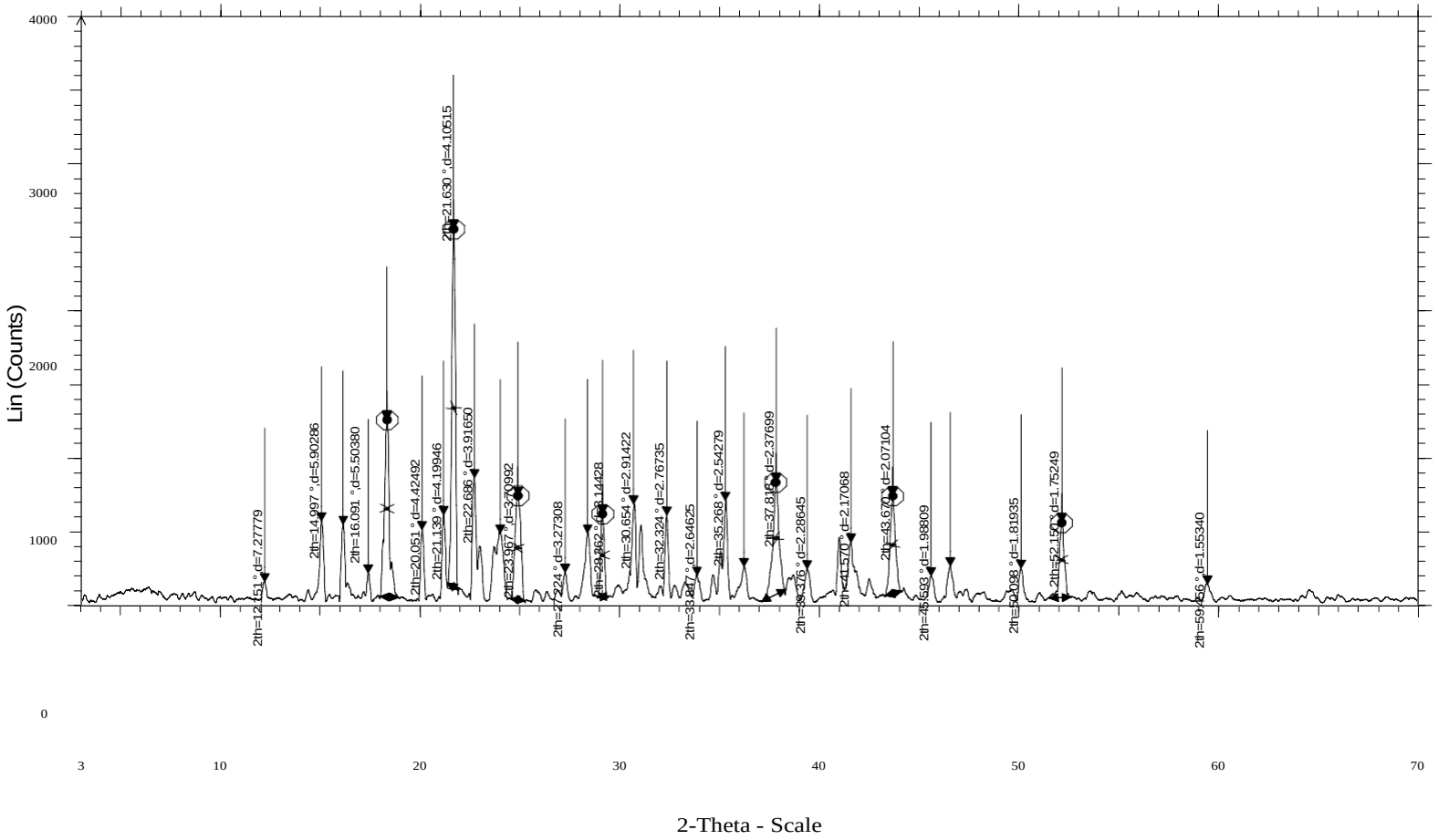

Figure 25. X-ray diffractometry spectrum o tazarotene

File: SAIFXR121019C-01(Celecoxib).raw - Step: 0.020 °- Step time: 31.2 s - WL1: 1.540

Operations: Smooth 0.150 | Background 0.380,0.000 | Import

1) Obs. Max: 21.636 °- FWHM: 0.204 °- Raw Area: 1 8.63 Cps x deg. - Net Area: 16.33 2) Obs. Max: 29.103 °- FWHM: 0.208 °- Raw Area: 4 .760 Cps x deg. - Net Area: 3.769 3) Obs. Max: 43.662 °- FWHM: 0.281 °- Raw Area: 8 .563 Cps x deg. - Net Area: 6.615 4) Obs. Max: 52.145 °- FWHM: 0.270 °- Raw Area: 6 .458 Cps x deg. - Net Area: 4.817 5) Obs. Max: 37.815 °- FWHM: 0.284 °- Raw Area: 1 1.25 Cps x deg. - Net Area: 8.833 6) Obs. Max: 24.862 °- FWHM: 0.344 °- Raw Area: 8 .569 Cps x deg. - Net Area: 7.826 7) Obs. Max: 18.289 °- FWHM: 0.229 °- Raw Area: 1 2.64 Cps x deg. - Net Area: 11.18

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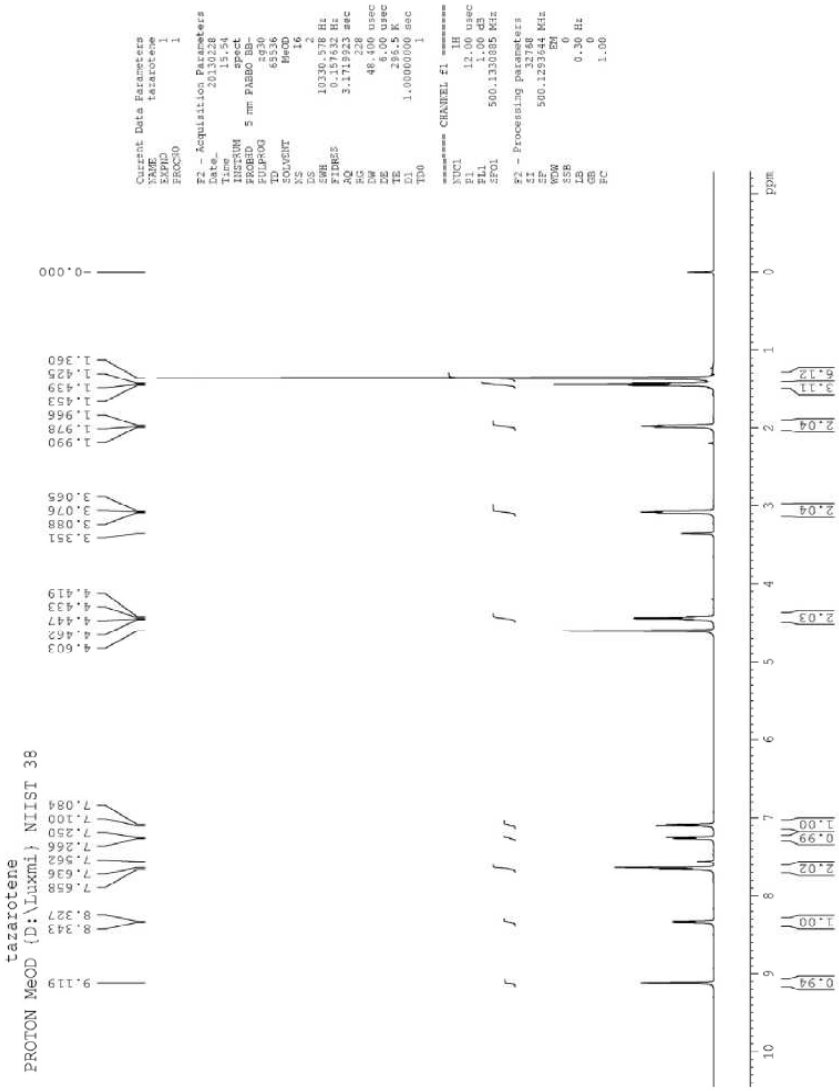
Figure. H1NMR spectrum of celecoxib



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Physiochemical properties of compritol® 888 ATO

The physiochemical property, organoleptic character of compritol® 888 ATO are as tabled below table 17.

Table. Physiochemical properties of compritol® 888 ATO

S. No.	Character	Observation
1	State	Solid Powder
2	Form	White
3	Colour	69 - 72°C
4	Melting Point	

Characterization of Compritol® 888 ATO

The DSC curves obtained for compritol® 888 ATO are shown in Figure 27. Differential scanning calorimetry resulch clearly states the presence of single compound by a sharp endothermic event. The onset of the peak was at 69.03°C, peak was detected at 72.22°C, endset of the peak was 76.0°C, the enthalpy DHfusion is -129.12 Jg^{-1} and the integral
is -360.25 mJ.

Fourier Transform Infrared Spectroscopy

The FTIR spectrum **of compritol® 888 ATO** obtained is in figure 28. The important peak characteristics of CELECOXIB are as table 18 below.

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Characterization of celecoxib and compritol® 888 ATO

The individual chemicals used in the preparation were celecoxib as drug and compritol® 888 ATO as lipid, other ingredients were surfactants poloxamer 188 and tween80, cryoprotectant trehalose and analytical reagents. The drug and lipid were characterized for its physiochemical properties like state, form, colour and melting point. Tazarotent is solid, powder, light yellow in colour and 102-105°C and compritol® 888 ATO is solid, powder, white in colour and having melting point of 69-72°C.

The drug and the lipid were analytically characterized for its intact nature by DSC, FTIR, XRD and H1 NMR and found the compounds were in their pure form. For characterization of celecoxib the crystallinity and purity of the compound were determined by the single peak in DSC (figure 23) which had the onset of the peak was at 103.30°C, peak was detected at 104.66°C, the enthalpy DHfusion is -90.07 Jg^{-1} and the integral is -293.81 mJ.

The FTIR (figure 24) was confirmed with the spectrum showing all the characteristic functional groups of celecoxib like aromatic ring at 3418.71 cm^{-1}, C double bond C at 1719.67 cm^{-1}, [263] Aromatic C=C bond at 1468.86 cm^{-1}, C double bond S at 1416.81 cm^{-1}, C = N bond at 1381.27 cm^{-1}, Ester at 1301.27 cm^{-1} and Mono substituted benzene at 721.46 cm^{-1}. Further the crystallinity was characterized by powder XRD, and was found to be crystalline in nature. [264] The H1 NMR was performed and the characteristic shift of the protons were observed and the purity of the drug was confirmed.[265]

Lipid was selected based on reported data indicating that compritol® 888 ATO ATO (glyceryl behenate) generates SLN with smaller size and with good stability with GRAS status.[266] Compritol® 888 ATO ATOis very well tolerated on skin application, it is widely used for the stability, intermediate crystallinity compared to other lipids due to its chemical combination. Further, SLNs loaded with the lipophilic drug like retinoids showed optimum physicochemical characteristics on topical application.[106] SLN prepared with fast crystallizing lipids glycerides with long chain fatty acids and surfactants are not disturbing the crystallization process of lipid even after melting. For characterization of compritol® 888 ATO , DSC was taken which had onset of the peak at 69.03°C, peak was detected at 72.22°C, endset of the peak was 76.0°C, the enthalpy DHfusion is -129.12 Jg^{-1} and the integral is -360.25 mJ. The FTIR confirmed with the spectrum showing all the characteristic functional groups of celecoxib like C-H stretching at 2850.57 cm^{-1}, C = O (carbonyl) stretching at 1739.29 cm^{-1}. This confirmed the intactness of the compound. Further the crystallinity was characterized by powder XRD, and was found to be 52.11% crystalline in nature.[264]

Compatibility of celecoxib and lipid

Compatibility studies for celecoxib and compritol® 888 ATO were carried out by DSC, FTIR,

XRD and H1 NMR. It was performed to confirm the crystallinity, intact form of drug, or any other physicochemical interactions between these components of the formulation through the comparison of thermal curves, FTIR, XRD and H1 NMR spectraum obtained for pure drug with the curve obtained from a 1:1 physical mixture.

Differential Scanning Calorimetry

The thermal profiles of the mixtures of celecoxib and compritol® 888 ATO show single peak. The melting point of celecoxib was 104.66^{0}C and that of compritol® 888 ATO was 72.22^{0}C, but together they formed the melt peak at 72.9^{0}C. This may be due to the drug solublizing effect of molten lipid and the existence of the drug in the lipid matrix at molecular level. The curve demonstrates the absence of any interaction or change in their form. Generally, DSC examination suggested that the lipids in SLN were in less ordered crystalline arrangement than the corresponding bulk lipid. These results were confirmed by X-ray diffraction studies.

Besides the physico-chemical features of the drug, the structure of the lipid influences the drug incorporation capacity of SLN as drug delivery system. For instance, lipids allowing for a limited space in the formed crystal lattice may result in expulsion of the drug from the lipid matrix during crystallization.[267] Drug expulsion is reduced when mixed lipids (mono and diglycerides) are used as they allow for more space in the crystal lattice formed upon crystallization. The absence of the peak corresponding to temperature indicates the presence of drug in dissolved state in lipid.

Furthermore, the lipid polarity,[268] the interaction of the drug with the lipid structure as well as the use of high temperature in production and high surfactant concentration might influence the drug loading and the loading profile.[269] In brief, the internal structure of the drug-loaded lipid particles is a function of the formulation ingredients and the production conditions.[269]

Fourier transform Infrared spectroscopy

The IR spectra interpretation for the mixture of drug and lipid match with the characters of the individual peaks of celecoxib and the compritol® 888 ATO , which confirm the absence of drug, lipid chemical interaction in solid state. The peaks observed were for aromatic ring at 3418.69 cm^{-1}, C double bond C at 1720.35 cm^{-1}, Aromatic C=C bond at 1469.84 cm^{-1}, C double bond S at 1418.08 cm^{-1}, C = N bond at 1388.32 cm^{-1}, Ester at 1301.11 cm^{-1} and Mono substituted benzene at 723.38 cm^{-1} of drug and C-H stretching at 2850.39 cm^{-1}, C = O (carbonyl) stretching at 1720.35 cm^{-1} of compritol® 888 ATO .

Differential Scanning Calirometry (DSC)

The thermal analyses of drug and lipid in 1:1 concentration was carried out for a DSC 822e, Mettler Toledo, Germany, using aluminium pans. The values of enthalpies, onset of peak and peak values are tabled. DSC analysis was performed using 5 mg of different samples which were placed in hermetically sealed pans. A heating rate of 10°C /min was employed after 1 min stabilization at 25°C and the temperature range 0-130°C was scanned. Inert atmosphere was maintained by purging nitrogen at a flow rate of 20ml/min.[252]

Fourier Transform Infrared spectroscopy

FT-IR was carried out by diffusing the samples in anhydrous KBr. The instrument used was IRAffinity-1, Shimadzu, Japan. Each sample was run to yield spectra in the 400 - 4000 cm^{-1} interval with a 2 cm^{-1} resolution. The spectra shown result from subtraction of the background contribution with a spectrum of dry KBr obtained with the same preparation protocol and acquisition parameters. [226]

X-rays powder diffraction (PXRD)

X-rays powder diffraction (PXRD) was performed in AXS D8, Bruker Advance, Germany. The CuK .radiation was obtained with a bent-graphite monochromator.

H1 NMR

H1 NMR was performed to obtain a proton shift spectrum which will confirm the structural modification of celecoxib in the physical mixture of drug and lipid.

Solubility study of Celecoxib

The highly lipophilic celecoxib was tested for solubility with a wide range of solvents. It was very clear by literature search the least solubility of TZR in water. The other solvents studied were, chloroform, dichloro methane, ethanol, dimethyl sulphoxide, methanol and acetonitrile. Test sample was taken in a test tube in weighed quantity and the measured quantity of the solvent was added. Also these tests were performed at room temperature. These determinations were performed in duplicate.

References

1. http://wordinfo.info/unit/3348/s:a%20disease%20of%20the%20skin%20in%20which%20raised
2. Mansbridge JN, Knapp AM. Changes in keratinocyte maturation during woundhealing. J Invest Dermatol 1987; 89: 253-63.
3. http://www.patient.co.uk/doctor/chronic-plaque-psoriasis.
4. Krueger G, Koo J, Lebwohl M, Menter A, Stern RS, Rolstad T. The impact of psoriasis on quality of life: results of a 1998 National Psoriasis Foundation patient- membership survey. Arch Dermatol 2001; 137: 280–284.
5. Lew W, Bowcock AM, Krueger JG.Psoriasis vulgaris: cutaneous lymphoid tissue supports T-cell activation and

'Type 1' inflammatory gene expression. Trends Immunol 2004;25: 295–305.

6. Nickoloff BJ, Nestle FO. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. J Clin Invest 2004;113: 1664–1675.
7. Gudjonsson JE, Johnston A, Sigmundsdottir H, Valdimarsson H. Immunopathogenic mechanisms in psoriasis. Clin Exp Immunol 2004; 135: 1–8.
8. Griffiths, CE. T-cell-targeted biologicals for psoriasis. Curr Drug Targets Inflamm. Allergy 2004; 3: 157–161.
9. Gurny R, Teubner A. Dermal and transdermal drug delivery - New insights and perspectives, Ed, 1993, Taylor & Francis Group.
10. Quinn AG, Biology of the skin and dermatological disease, The Medicine Publishing Company Ltd, Basic Science, Medicine 2004; 32:12, 1-3
11. Phillips WG, Breathnach SM. Psoriasis: immune indicators and treatment. Clin Immunother 1994; 1: 157-67.
12. Boehncke WH, Dressel D, Zollner TM, Kaufmann R, Pulling the trigger on psoriasis. Nature 1996; 379: 777.
13. Konstantinova NV, Duong D-MT, Remenyik E, Hazarika E Chuang A, Duvic M. IL-8 is induced in skin equivalents and is highest in those derived from psoriatic keratinocytes. J Invest Dermatol 1996;107:613-21.
14. Greaves MW, Weinstein GD. Treatment of psoriasis. N Engl J Med 1995;337: 581-8.
15. Liem WH, McCullough JL, Weinstein GD. Effectiveness of topical therapy for psoriasis: results of a national survey. Curls 1995; 55: 306-10.
16. Frost R, Weinstein GD. Topical administration of vitamin A acid for ichthyosiform dermatoses and psoriasis. JAMA 1969; 207:1863-8.
17. Fredriksson T. Antipsoriatic activity of retinoic acid (vitamin A acid). Dermatologica 1971; 142:133 -6.
18. Bischoff R, De Jong EM, Rulo HF, Sendagorta E, Czarnetzki BM, Van de Kerkhof PC. Topical application of 13-cis-retinoic acid in the treatment of chronic plaque psoriasis. Clin Exp Dermatol 1992; 17: 9- 12.
19. Davidson A, Diamond B. Autoimmune diseases. N Engl J Med. 2001; 345: 340–350.Nestle FO, Turka LA, Nickoloff BJ. Characterization of dermal dendritic cells in psoriasis. Autostimulation of T lymphocytes and induction of Th1 type cytokines.J Clin Invest1994; 94: 202–209.